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The origin and differentiation of adrenocortical cells in rats with portacaval shunt. A structural and ultrastructural study.

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Summary. The formation of adrenocortical cells in the rat was studied by light and electron microscopy in an experimental model, namely portacaval shunt (P.C.S.), in which strong hyperplasia of the cells of the capsular region occurs. The results of this study indicate that in physiological conditions at the level of the adrenal gland capsule some epithelial cells, morphologically distinguishable as dark and clear cells, are found which can be interpreted as precursors of adrenocortical cells.

From observations of intermediate forms between capsular precursors and mature adrenocortical cells, which are found in high numbers following P.C.S., it seems that the dark precursors give rise to cells of the zona glomerulosa and the clear precursors evolve into cells of the zona intermedia, which are to be considered as the starting point for the formation of cells of the zona fasciculata.

Key words: Adrenocortical cells, Differentiation, P.C.S.

Introduction

Since the end of the last century, there have been many studies on the formation of adrenocortical cells during physiological renewal in mammals.

Earlier studies (Gottschau, 1883; Celestino da Costa, 1951) proposed that adrenocortical cells originated at the periphery of the gland from blasts located in the zona capsularis (Zwemer et al., 1938; Salmon and Zwemer, 1941; Wotton and Zwemer, 1943; Gruenwald and Konikow, 1944; Jayne, 1953; Bachmann, 1954) and that they subsequently migrated centripetally in the gland.

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According to more recent research, however, the young and mitotically active cells seem to be concentrated preferentially in the zona intermedia, lying between the zona glomerulosa and zona fasciculata (Nussdorfer, 1986) and from here, they would migrate into both the zona glomerulosa and zona fasciculata.

Experimental studies on regeneration of the adrenocortical gland after enucleation or transplant clearly showed that the cells from the capsule and residues of the zona glomerulosa remaining attached to it, are able to proliferate and differentiate into a histologically and functionally normal cortex (Long, 1975). However, it has not been definetely established whether the new cortex originates exclusively from cells of the zona glomerulosa which remain attached to the capsule, as some authors proposed (Greep and Deane, 1949; Brenner et al., 1953; Chester Jones and Spalding, 1954; Nickerson et al., 1969; Belloni et al., 1982; Taki and Nickerson, 1985) or whether fibroblastic-like capsular cells can also differentiate into cortical parenchyma (Backer and Baillif, 1939; Ingle and Higgins, 1938; Turner, 1939; Butcher, 1948).

Since in the rat bearing an end to side portacaval shunt (P.C.S.), for reasons unknown at present, a strong hyperplasia of the capsular zone cells is obtained, we aim in this study to investigate ultrastructurally whether there are precursor cells of the adrenocortical parenchyma among the hyperplastic cells of the capsule and if possible, to define the various steps of their differentiation into mature adrenocortical cells.

Materials and methods

Seventeen male albino adult rats of the Wistar strain, weighing between 250 and 300 gr. were used in the present study. Ten rats underwent end-to-side portacaval shunts under ethyl-ether anaesthesia (Lee and Fischer, 1961). They were sacrifyed by decapitation and the adrenal glands were removed from 7 animals one month after and from 3 animals two months after

surgery. Seven age-matched untreated rats were used as controls. Throughout the period of the experiment, the rats were housed under controlled conditions of temperature, humidity and lighting and fed ad libitum with a standard diet.

Small tissue fragments of the adrenal glands were fixed by immersion in cold glutaraldehyde 4% in 0.1 M cacodylate buffer, pH 7.4, at room temperature, and postfixed in 1% $0s0_4$ in 0.1 M phosphate buffer, pH 7.4, at 4°C. The specimens were dehydrated in graded acetone series, passed through propylene oxide and embedded in Epon 812 (Luft, 1961).

Semithin sections, 1-2 μm. thick, were stained with toluidine blue-Na tetraborate, and observed under the light microscope.

Ultrathin sections were stained with uranyl acetate and either lead citrate or alkaline bismuth subnitrate (Riva, 1974) and examined under a Siemens Elmiskop 102 electron microscope at 80 kV.

Results

Control rats

Light microscopy showed that the superficial portion of the adrenal gland is bounded by a capsule, the thickness of which is not uniform. The capsule was made up of cells and connective ground substance containing bundles of collagen fibres. There were two cell types (Fig. 1). The first were deeply stained fibroblast-like cells (dark cells) with fusiform cellular body and long, thin cytoplasmic processes, oval nucleus and fairly dense chromatin. The second cell type, found where the capsule was thickest, was made up of poorly stained elements (clear cells) present in lower numbers and intermingled with the dark cells. These clear cells had a globular cell body and shorter, stumpy cytoplasmic processes, and an oval nucleus with dispersed chromatin. Whereas the dark cells were only found at the capsular level, the clear cells were also occasionally found among the cells of the zona glomerulosa, up to its boundaries with the zona fasciculata.

Electron microscopy demonstrated in the capsule that besides blood vessels and truly connective components - consisting of typical fibroblasts and intercellular matrix- there are also quite numerous non-connective cells. The non-connective nature of these cells was indicated by the presence of a surrounding basal lamina and the more or less extensive close epithelial contacts provided with intercellular junctions. These cells formed a loose cellular network in which the individual cells were preferentially placed in layers parallel to the gland surface, and were separated by intercellular connective substance rich in collagen fibres. These cells could also be distinguished ultrastructurally into two cell types, corresponding to the dark and clear cells seen under the light microscope.

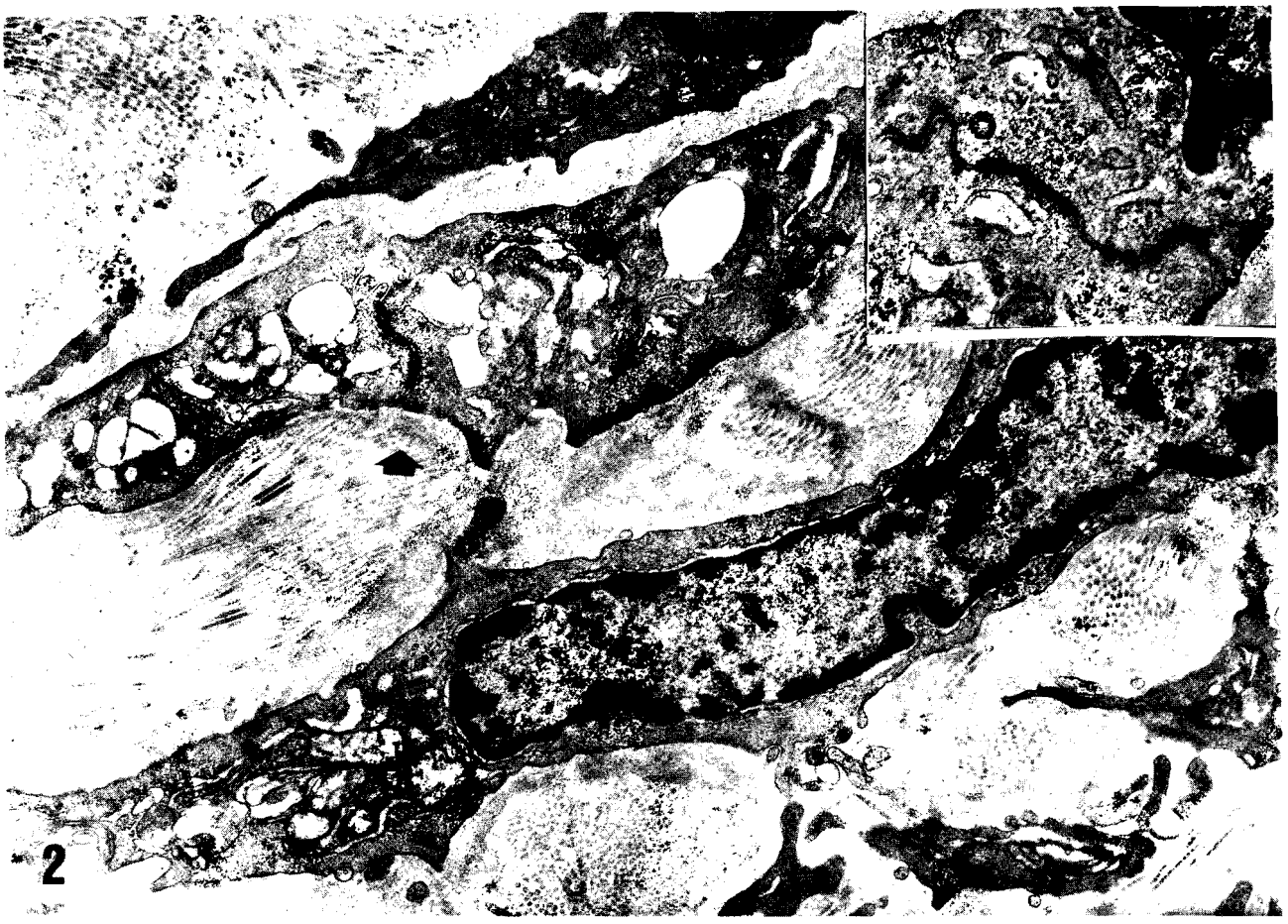
The dark cells (Fig. 2) had flattened nuclei with slightly undulated contours, large chromatin masses mainly located against the nuclear envelope, and an inconspicuous nucleolus. The cytoplasm showed an electrondense matrix, some cisternae of rough endoplasmic reticulum with dilated lumen, rather numerous free polyribosomes, a small Golgi apparatus, oval mitochondria with a clear matrix and a few short laminar cristae. The organelles were mainly located in the innermost part of the cytoplasm, whereas the periphery contained numerous cytofilaments attached to the plasmalemma by means of extensive dense plaques, both in the junctional areas (Fig. 2 insert) and in the remaining portions.

The clear cells (Fig. 3) had oval nuclei with quite regular contours, small chromatin masses and an inconspicuous nucleolus. The electron-lucent cytoplasm presented a more abundant rough endoplasmic reticulum with small cisternae with a narrow lumen, numerous free

Fig. 1. Control rat. In the capsule two distinct cell types can be seen, namely dark and clear cells. The former are more numerous than the latter. Semi-thin section stained with toluidine blue-NA-tetraborate. × 2,500

Fig. 2. Control rat. Ultrastructural features of dark cells in the capsule. Note the enveloping basal lamina, the abundant cytofilaments and the intercellular junctions (arrow and insert). E.M. × 15,000. Insert × 21,000

Fig. 3. Control rat. Ultrastructural features of clear cells in the capsule. Note the enveloping basal lamina, the extensive epithelial contacts and the rich complement of organelles. E.M. × 10,000. Intercellular junctions are also present (insert). × 25,000



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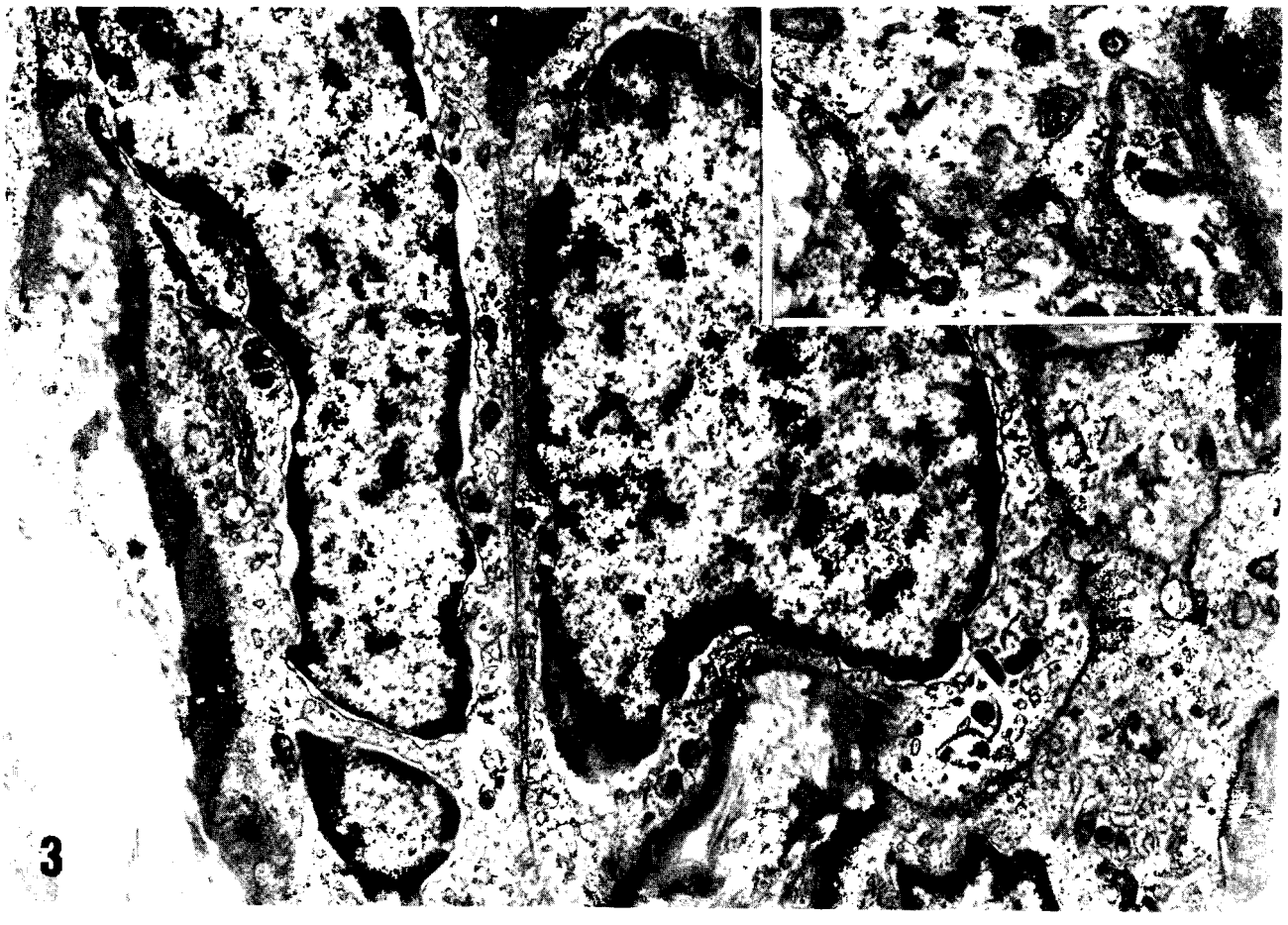

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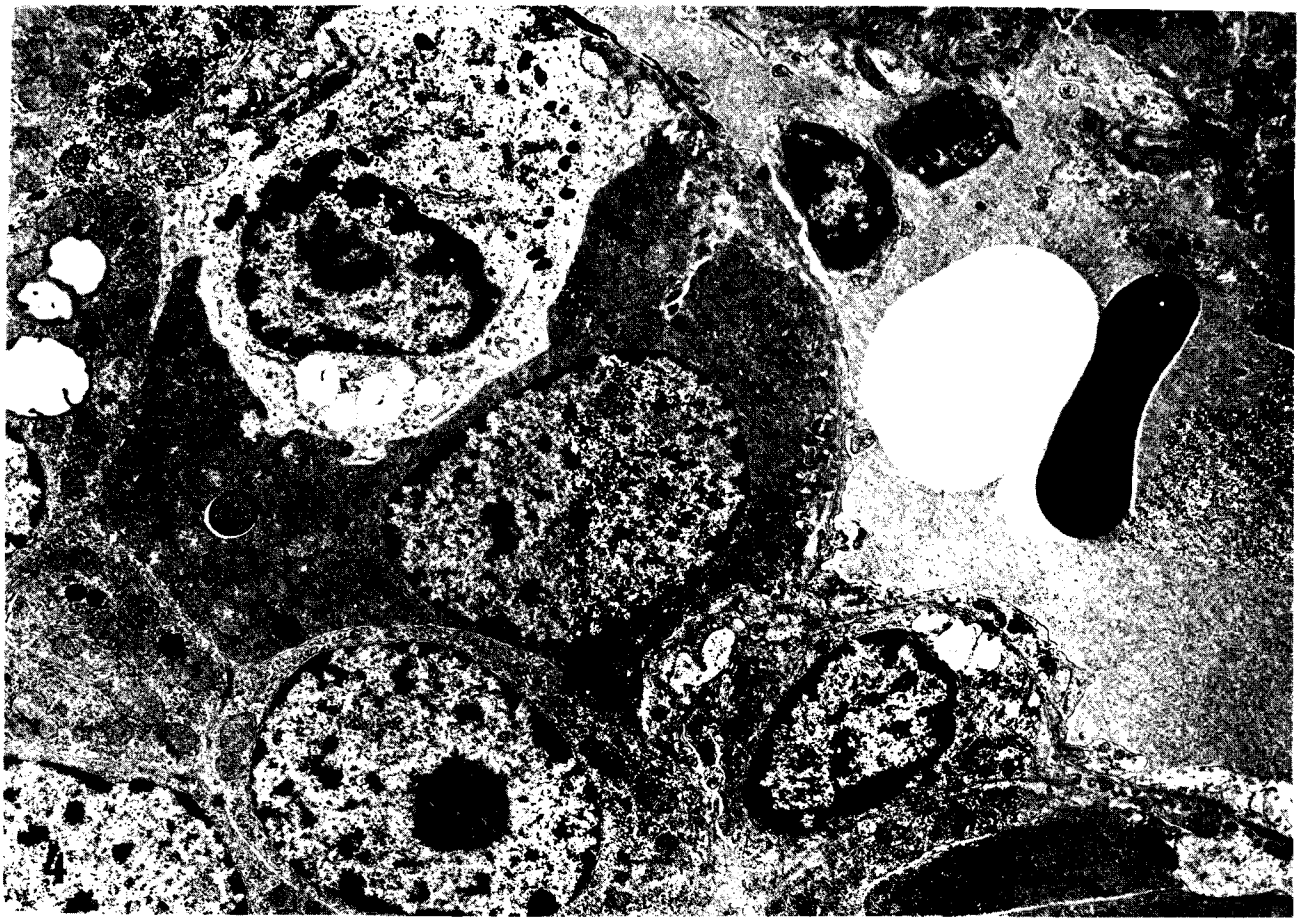


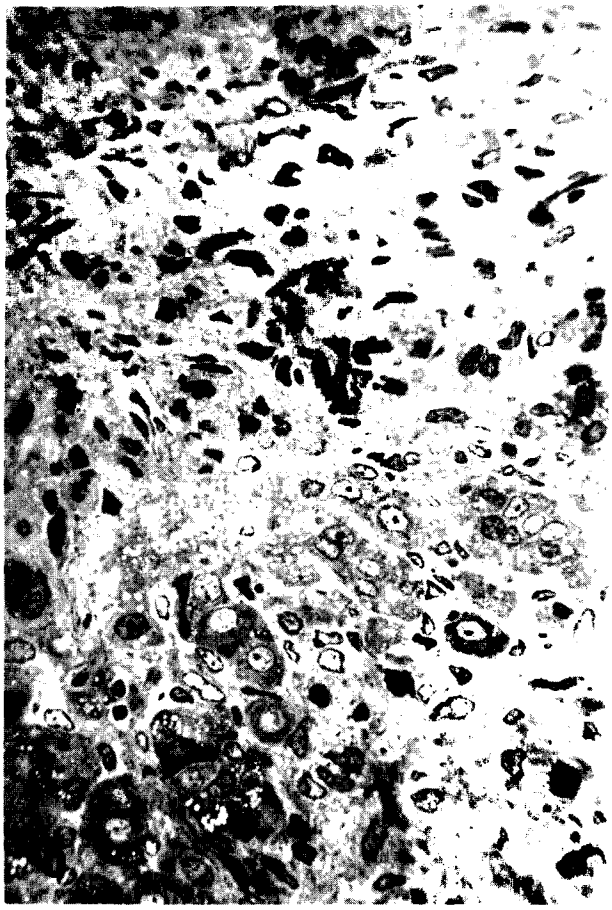
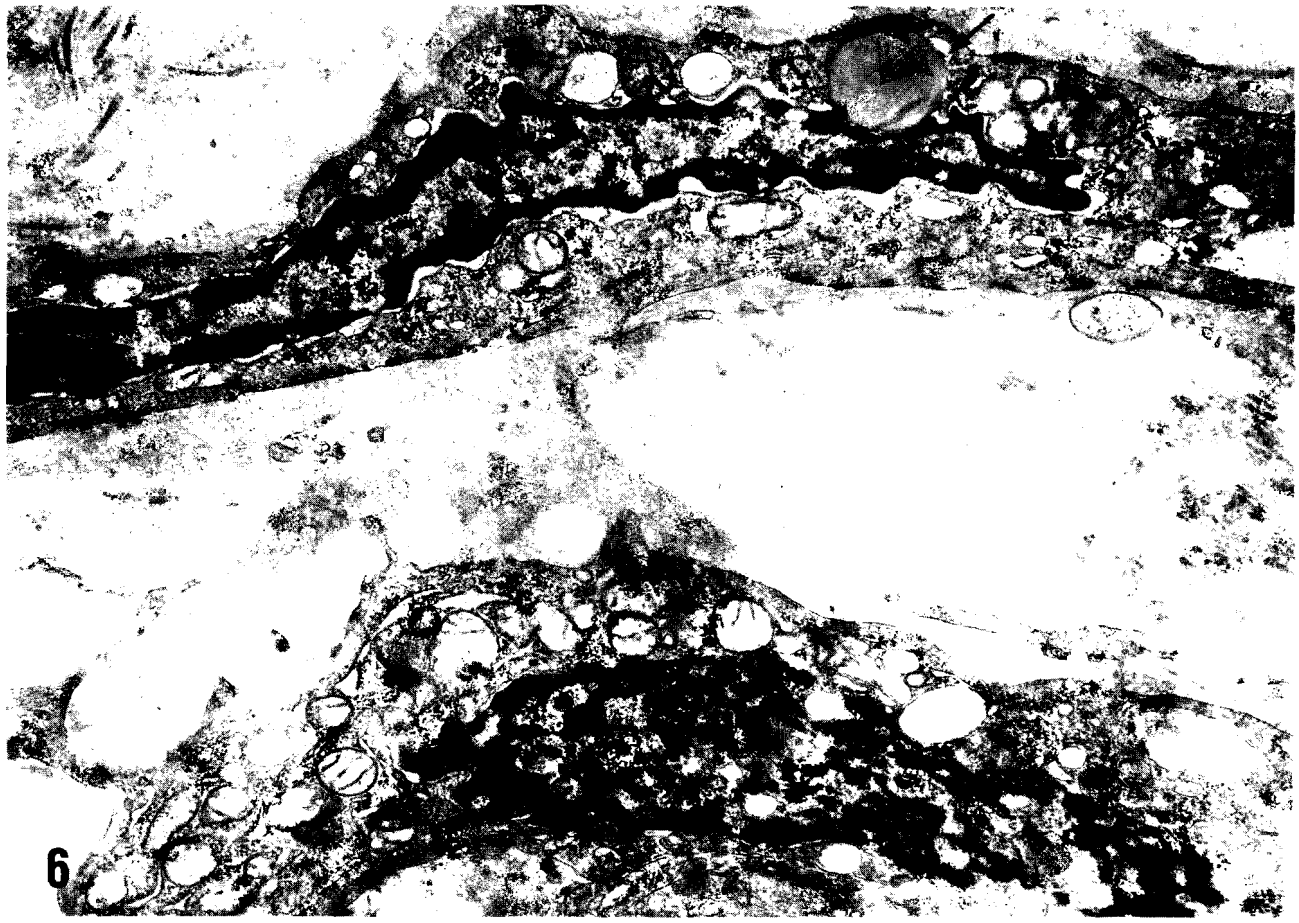


Fig. 4. Control rat. A developing clear cell is seen inside the zona glomerulosa. The organellular complement is like that of the clear cells of the capsule apart the larger Golgi apparatus. E.M. × 7,500

Fig. 5. Rat with P.C.S. since 1 month. The dark cells in the capsule are much more numerous than in the control rats. Semi-thin section stained with toluidine blue-NA-tetraborate. × 3,100



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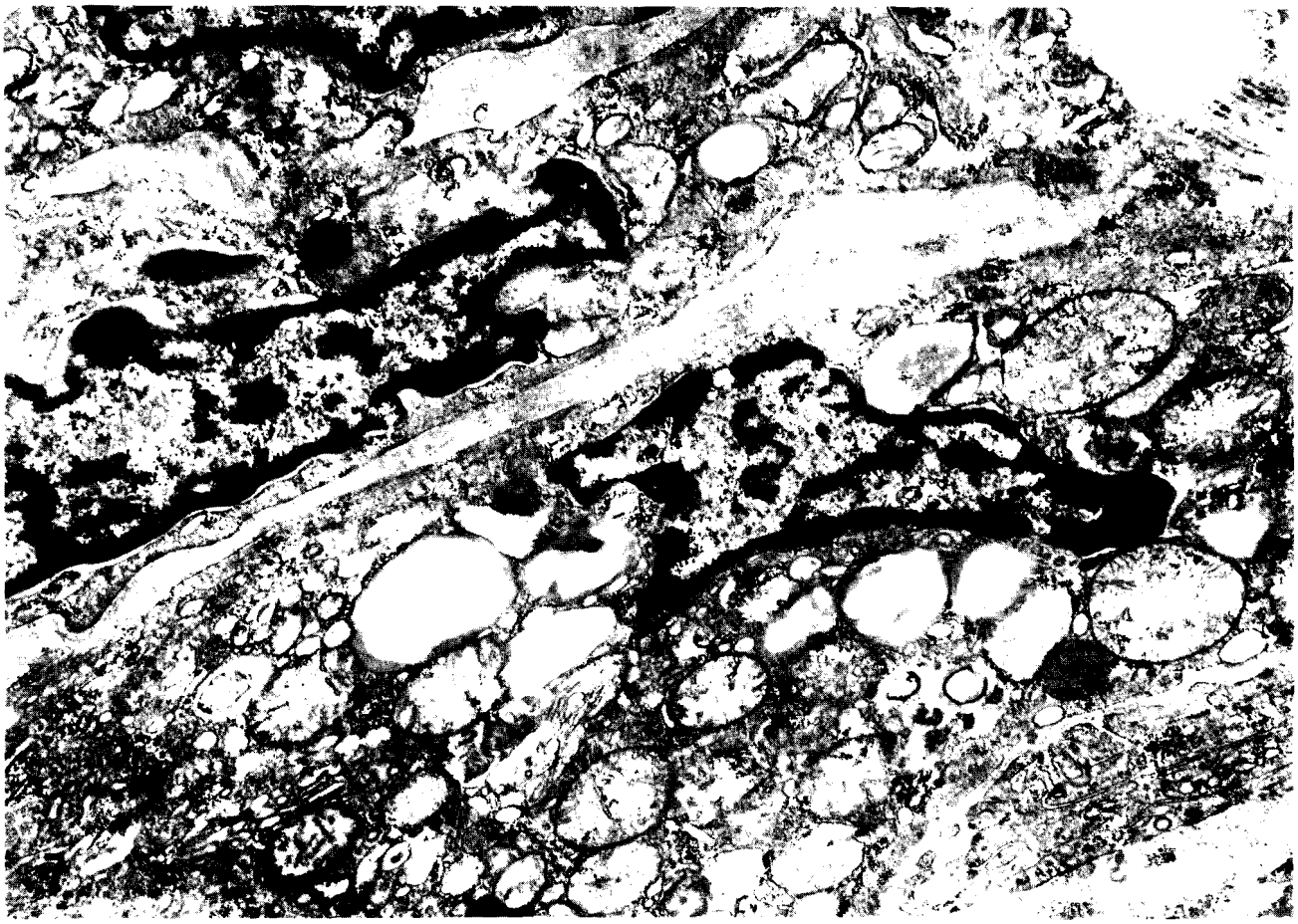

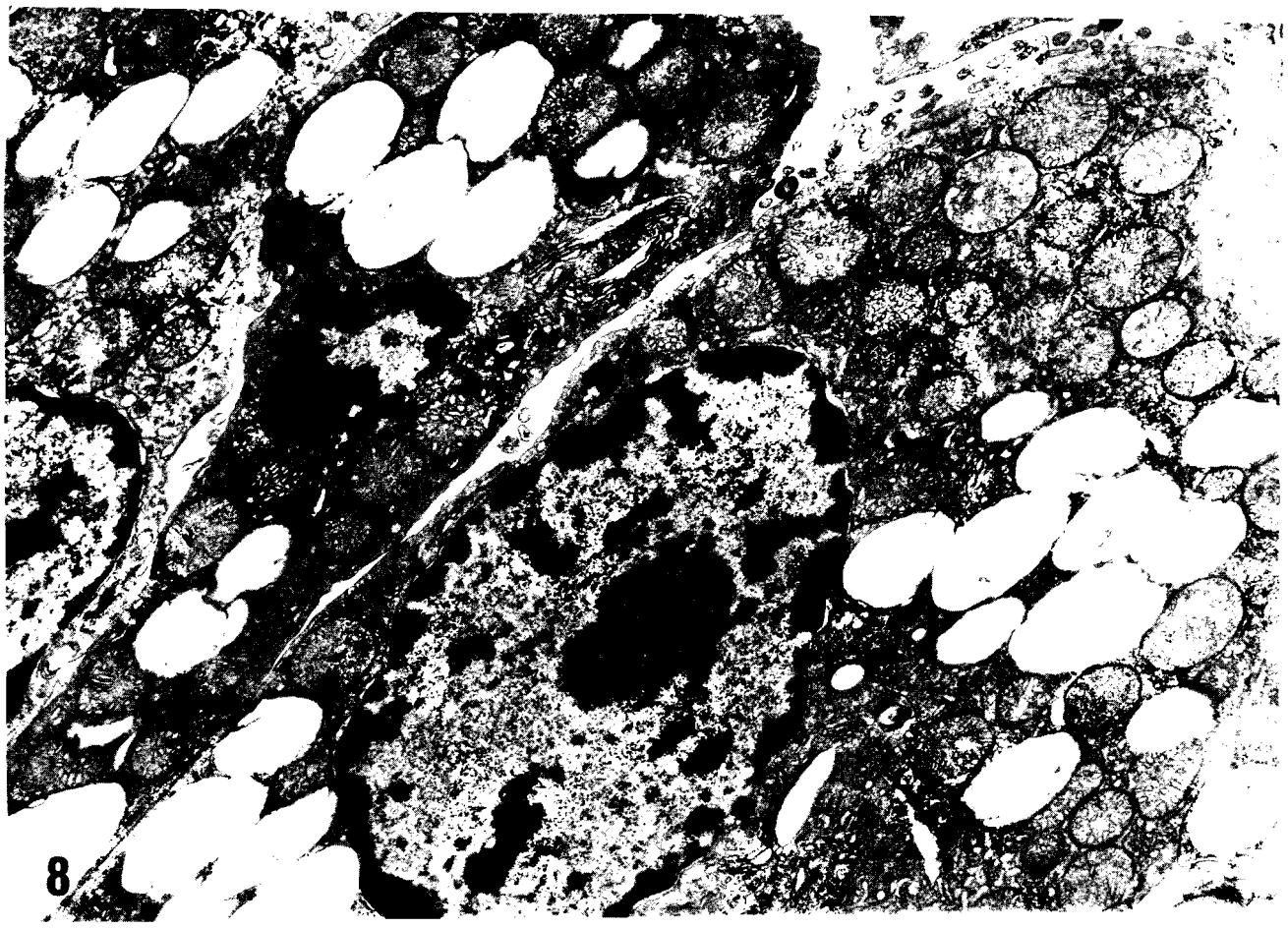

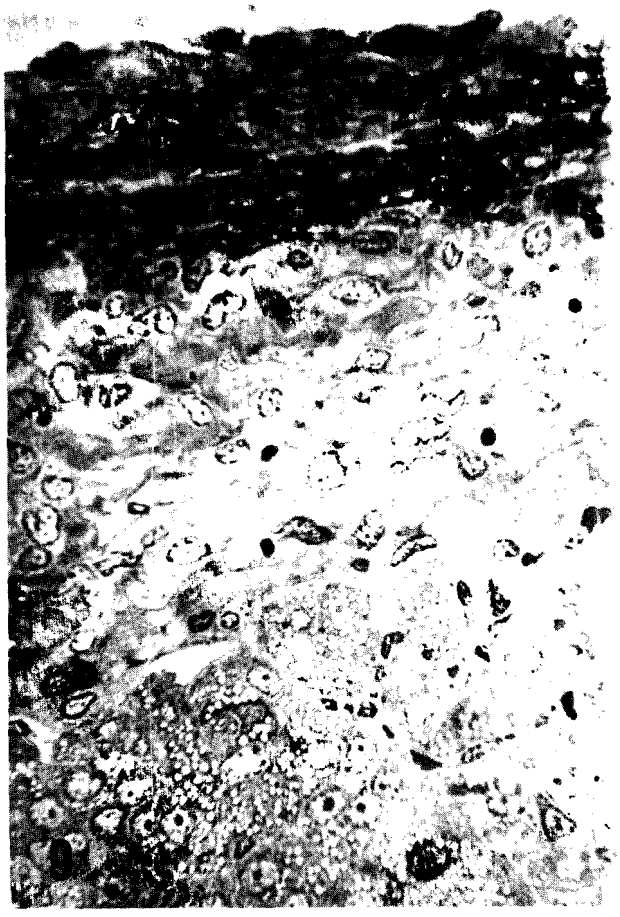

Fig. 6. Rat with P.C.S. since 1 month. Developing dark cells of the capsula showing increased number of organelles, enlarged mitochondria and a liposome (arrow). E.M. × 20,000

Fig. 7. Rat with P.C.S. since 1 month. Developing dark cells of the capsula in a further stage of differentiation compared with those of Fig. 6. Note the increase in cytoplasmic mass and in the number of organelles, especially smooth endoplasmic reticulum. Mitochondria show a mixture of laminar and tubular cristae, and liposomes are abundant. E.M. × 20,000

Fig. 8. Rat with P.C.S. since 1 month. Mature cells in the outermost part of the zona glomerulosa. Note the close similarities with the cells in Fig. 7, especially the dark cytoplasmic matrix, the mitochondrial structure and the features of nuclear chromatin. E.M. × 15,000

Fig. 9. Rat with P.C.S. since 2 months. The clear cells in the capsule are very numerous especially in the deeper portion. Semi-thin section stained with toluidine blue-NA-tetraborate. × 2,100

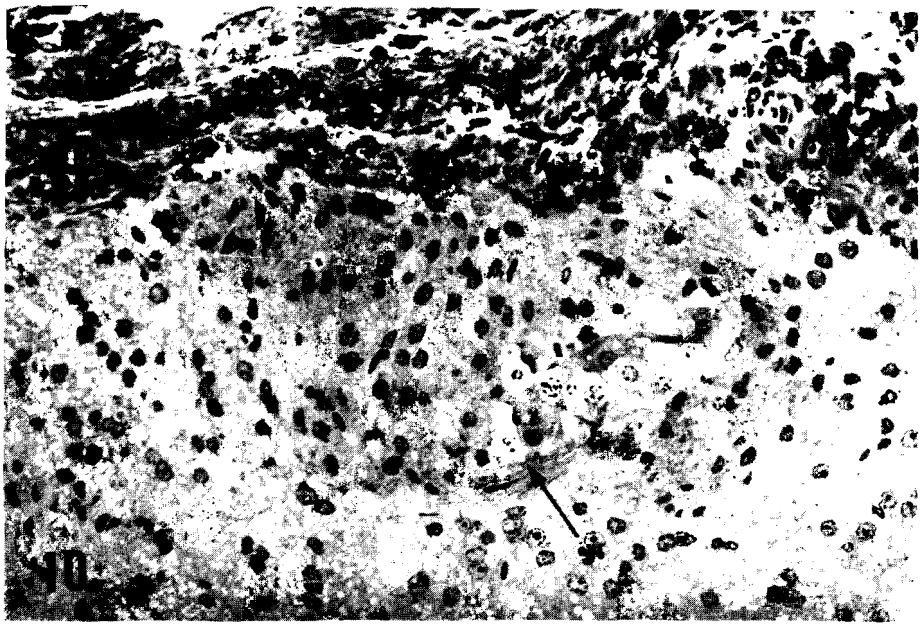

Fig. 10. Rat with P.C.S. since 2 months. Clear cells of the capsule forming a large nodule protruding into the zona glomerulosa. Semi-thin section stained with toluidine blue-NA tetraborate. × 1,000

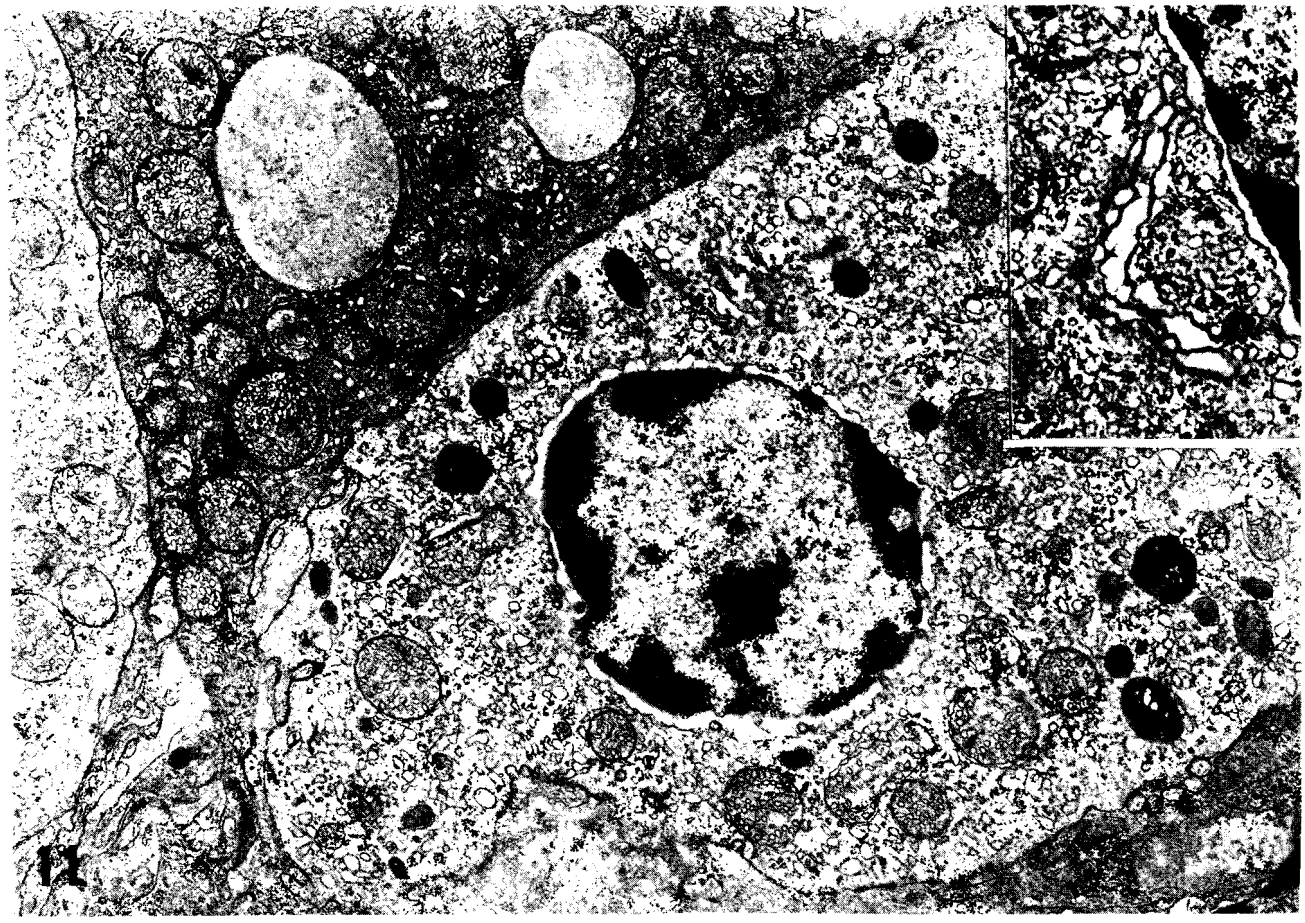

Fig. 11. Rat with P.C.S. since 2 months. Ultrastructural appearance of a clear cell corresponding to that indicated with an arrow in Fig. 10. Note the similarity of this cell with the clear developing cell of the capsule apart from the more abundant smooth endoplasmic reticulum, the mitochondria with tubuloconvolute cristae and the larger Golgi apparatus. × 12,500. insert × 16,000

polyribosomes, small Golgi apparatus and very thin and elongated mitochondria with dense matrix. The organelles were widely spread throughout the cytoplasm apart from a thin peripheral rim, where few cytofilaments were present converging on the plasmalemma at the level of small dense plaques. The cytoplasmic processes in these cells were shorter and thicker than in the dark cells and the epithelial contacts with neighbouring clear cells occurred for large extensions of the cell body. At the points of contact, it was also possible to find junctional complexes (Fig. 3 insert).

The clear cells, as well as in the capsule, could also be found in contact with the external surface of the zona glomerulosa and here they were intermingled with typical zona glomerulosa cells. In this case, however, the clear cells, although maintaining the characteristics described above, had a more extensive Golgi apparatus (Fig. 4).

Rats with Portacaval Shunt

Light microscopy revealed an increase in the thickness of the zona capsularis. This was mainly due to an increase in the amount of the fibroblast-like cells, both dark and clear.

One month after surgery, the dark cells were much more prevalent in the capsule (Fig. 5).

Electron microscopy revealed that the dark cells were morphologically heterogeneous. In fact, in the outermost portions of the capsule, these had characteristics similar to those observed in the control rats, whereas near the zona glomerulosa these cells showed several changes: an increase in size, an oval or polyhedral shape, a decrease in the number of polyribosomes and cisternae of the rough endoplasmic reticulum, and increase in the smooth endoplasmic reticulum and in the extent of the Golgi apparatus, large oval mitochondria with cristae which are laminar or a mixture of laminar and tubular, and the appearance of liposomes (Figs. 6, 7).

Cells with dense chromatin and electron-dense cytoplasmic matrix, like those previously described, were also found inside the zona glomerulosa, and liposomes and organelles similar to those of mature zona glomerulosa cells could be seen, including mitochondria with tubular cristae (Fig. 8).

The few clear cells found in the capsule showed similar characteristics to those observed in the control rats.

Two months after surgery, at light microscopy, in one of the three animals examined, the picture did not differ greatly from that observed at one month after surgery. In the two remainings rats, on the contrary, there was a conspicuous increase in the amount of the clear cells in large areas of the deeper face of the capsule, continuous with the zona glomerulosa (Fig. 9).

In some areas, nodular masses of clear cells protruded deeply into the zona glomerulosa, reaching the zona fasciculata (Fig. 10).

Electron microscopy revealed that the dark cells had similar features to their counterparts in the control rat.

The clear cells of the capsular zone were similar to those described in the control rats. On the contrary, the clear cells located in proximity to or in the zona glomerulosa and nodular masses, although still having a electron-lucent cytoplasmic matrix, showed an increase of the smooth endoplasmic reticulum and extension of the Golgi apparatus, large oval or spherical mitochondria, with clear matrix and tubular convoluted cristae. In these cells, there were also secondary lisosomes, and liposomes were occasionally found (Fig. 11 and insert).

The clear cells in the nodular formations growing down in the zona glomerulosa were intermingled with cells with features typical of cells of the zona intermedia, apart from a more electron-lucent cytoplasmic matrix and fewer mitochondria.

Discussion

The results of this study indicate that in the adrenal gland capsule, in physiological conditions, there are epithelial cells which are thought to be precursors of adrenocortical cells. These cells can be divided morphologically into two types: dark and clear precursors.

From cytological analysis of the intermediate types between capsular precursors and mature adrenocortical cells, it seems that in conditions of enhanced formation of adrenocortical cells, as occurs in rats with P.C.S., the dark precursors give rise to the cells of the zona glomerulosa, whereas the clear precursors, developing into cells with the known attributes of the zona intermedia cells (Nussdorfer, 1986), seem to be the starting point for the formation of cells of the zona fasciculata.

Our findings, therefore, are in agreement with the theory suggesting the existence of a capsular blastema (Zwemer et al., 1938; Salmon and Zwemer, 1941; Gruenwald, 1942; Wotton and Zwemer, 1943; Gruenwald and Konikow, 1944; Jayne, 1953; Bachmann, 1954) and with the migratory theory during the histogenesis of the adrenal gland cortex (Nussdorfer, 1986). However, our findings, especially those relevant to the modifications observed at one and two months after portacaval anastomosis, indicate that the pathway followed by the precursors in the course of their differentiation into mature adrenocortical cells and their migration towards the interior of the gland is not only one, but two distinct pathways. There is a shorter way, which stops in the zona glomerulosa and leads the dark capsular precursors through definite differentiative steps to the mature zona glomerulosa cells. In the other, longer way, the clear capsular precursors give rise to the zona intermedia cells and from these probably evolve into the mature cells of the zona fasciculata.

From our findings, it is unlikely that the cells of the zona intermedia can generate cells of the zona glomerulosa.

Thus, our data cannot support the hypothesis previously put forward (Nussdorfer, 1986) that zona glomerulosa cells can transform into zona fasciculata cells, but it does seem possible that there are two distinct and separate lines of differentiation for each of these cell types.

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