Histol Histopathol (1995) 10: 603-609

Histology and Histopathology

Immunohistochemical analysis of adrenal proliferation and corticosterone expression in experimental adrenal regeneration

R. Sarría[1], J. Losada[2] and J.L. Bueno-López[1]

[1]Department of Neuroscience and [2]Department of Surgery, School of Medicine and Dentistry, Basque Country University, Spain

Summary. The proliferative activity, the organization and the corticosterone expression of adrenocortical cells in an experimental adrenal regeneration process after the transplantation of neonatal adrenal glands to adult hosts was investigated.

Three days after transplantation, the medullar and the innermost adrenocortical cells of the neonatal adrenal glands showed degenerative and necrotic changes due to the lack of vascular supply. The remaining outermost adrenocortical cells did not display any PCNA immunoreaction. The first PCNA expression, pointing out the beginning of the proliferative cycle, was observed in a 45.4% of the adrenocortical cells, one week after transplantation. After three weeks, several regenerated adrenocortical nodules with a bigger size than the one observed in the previous periods were seen. In these nodules, while the outermost adrenocortical cells were disposed in parallel to the capsule or in rounded groups, the bulk of the regenerated mass width was composed of cells forming longitudinal cords. PCNA immunoreaction was almost exclusively restricted to subcapsular cells (62.5%) and to cells of the outermost portion of the cords (32.5%), the global percentage of PCNA immunopositive cells being 18.4%. Twelve weeks after transplantation, regenerated adrenocortical cells were arranged in three layers: glomerulosa, fasciculata and reticularis. Only 1.85% of the adrenocortical cells were PCNA immunopositive.

Although in the early stages of the regeneration process, all the adrenocortical cells, both proliferating and non proliferating cells expressed corticosterone, a restriction of this immunoreactivity to the zonae fasciculata and reticularis was observed when cell zonation was apparent.

Key words: Adrenal, Transplantation, Regeneration, PCNA, Corticosterone

Offprint requests to: Dr. Rafael Sarría Aróstegui, Departamento de Neurociencias, Sección de Anatomía, Facultad de Medicina y Odontología, Universidad del País Vasco/Euskal Herriko Unibersitatea, E-48940 Leioa, Vizcaya, Spain

Introduction

In the rat, the adrenal cortex is an endocrine tissue with a great regenerative capacity (Belloni et al., 1991). After adrenal transplantation, a necrotic process that affects the medulla and the greater part of the cortex takes place. Adrenocortical regeneration process completely replaces degenerated tissue in a variable period of time that depends on transplantation site and techniques (Matsumoto, 1990). The absence of changes in the number and ultrastructure of capsular cells and the proliferative activity observed by means of autoradiographic studies in the remaining adrenocortical cells after transplantation, suggests that regenerated adrenocortical tissue arises from glandular cells attached to the connective capsule (Vendeira et al., 1992).

Proliferating cell nuclear antigen (PCNA) is a 36 kD nuclear protein that plays a fundamental role in DNA replication, as an auxiliary protein of DNA polimerase delta (Bravo et al., 1987). Immunocytochemically, it has been shown that in the cell cycle, PCNA increases through G1, peaks in S phase, decreases through G2 and reaches undetectable levels in M phase and quiescent cells (Bravo and Macdonald-Bravo, 1985). This particular distribution through the cell cycle makes the use of an antibody against PCNA as a marker for proliferating cells possible (Morris and Mathews, 1989) and permits the study of adrenocortical proliferation in adrenal transplantation models. In the adrenal cortex of normal rats, corticosterone is formed from 11-deoxycorticosterone by 11 beta-hydroxilase. Using immunohistochemical and in situ hybridization techniques, a specific distribution of 11 beta-hydroxylase and its mRNA in the zonae fasciculata and reticularis has been reported (Oghisima et al., 1992; Ho and Vinson, 1993). On the other hand, using anti-corticosterone serum, corticosterone-positive cells appeared exclusively situated in the zona fasciculata of normal adrenal cortex (Thaete et al., 1990).

In the present work, using anti-PCNA and anti-corticosterone antibodies we examined the proliferative activity, the organization and the corticosterone

expression of adrenocortical cells in regenerated adrenocortical nodules in an adrenal transplantation model in which neonatal adrenal glands are transferred to adult hosts.

Materials and methods

Animals

Twenty female Sprague-Dawley rats weighing 170-200 g, housed under standard conditions: solar light cycle, temperature 22±2 ºC, relative humidity 50±5%, free access to food (balanced diet from Panlab) and water, were used to transplant adrenal tissue. Initially, we proceeded in all the animals to isolate an everted small intestine segment (ESIS). Then, after the removal of the adrenal glands, each animal was transplanted with six neonatal adrenal glands into the ESIS, which was immediately placed under the skin of the abdominal wall. For all the surgical procedures, rats were anaesthetized with 40 mg/Kg Kethamine, im (Parke Davis) and with 3.9 ml/Kg Urethane 20%, ip (Fluka). Donor adrenals were obtained, under ether anaesthesia, from decapitated rats of either sex (1-2 days old), cleaned of adhering fat and placed in sterile saline isotonic solution at room temperature. Transplantation was performed immediately after the bilateral adrenalectomy. 3 days and 1, 3, and 12 weeks later, animals were sacrificed, under ether anaesthesia, by decapitation. Transplanted rats were carefully inspected for possible adrenal regeneration *in situ*.

Sixteen Sprague Dawley rats sacrificed 3 days and 1, 3 and 12 weeks after birth served as control animals.

Processing of samples

Control adrenals and the ESISs with the grafts were carefully removed, fixed in Bouin's liquid, embedded in paraffin and serially cut at 7 µm. ESISs were processed according to the ABC immunocytochemical method in order to identify the PCNA and the corticosterone expression in regenerated adrenocortical nodules. Control adrenals were stained with Martin's trichrome stain.

Immunocytochemical study

Following inhibition of endogenous peroxidase and pretreatment with goat normal serum at a dilution of 1:30, pituitary, anti-proliferating cell nuclear antigen (PCNA), clone PC10 mouse serum (Boehringer 1486 772), at a dilution of 1:200 and anticorticosterone rabbit serum (kindly provided by Dr. L.G. Thaete, Colorado State University) at a dilution of 1:100, were employed as primary antibodies, the incubation being carried out at 4 ºC overnight. The second sera employed were horse biotinylated anti-mouse immunoglobulins (Vector Lab PK-4002) and goat biotinylated anti-rabbit immunoglobulins (Vector Lab PK-4001) at a dilution of 1:100 for 60 minutes at room temperature. Finally, sections were incubated with the ABC reagent for 60 minutes at room temperature (Vector Lab PK-4001). The reaction was visualized using a 0.05% solution of 3,3'-diaminobenzidine in TRIS-HCl (0.05 M) buffer (pH 7.5) containing hydrogen peroxide at 0.01% for 5 minutes at room temperature. Sections were counterstained, dehydrated, cleared in xylene and mounted with DPX (Serva). TBS was used as the buffer for the washing fluids and for diluting the antisera employed (0.05 M TRIS buffer in NaCl, pH 7.5). For testing the specificity of the antibodies, immunosuppression tests were carried out using the purified antigens and the disappearance of the immunoreactivities was observed. As a negative control, the primary antibodies were substituted by washing buffer and normal rabbit and mouse serums.

Morphometry

To obtain the percentage of PCNA reactive cells in the regenerated adrenocortical nodules, we counted stained and unstained cells in four fields on each of four sections studied per animal (10000 cells/experimental group) in a manner similar to that employed by Childs et al. (1983).

Statistics

Comparison between percentages of PCNA-reactive cells measured in the regenerated adrenocortical nodules at different periods after transplantation were analyzed according to Statistic Z (0.1). Values of $p<0.05$ were considered statistically significant.

Results

Three days after the transplantation of neonatal adrenal glands to adult hosts, several transplanted adrenals with a size of 1.5x1 mm^2 were found to be present in the ESISs. They were well demarcated from the structures situated in the lumen of the ESISs, surrounded by a connective capsule and composed in their outer portion by adrenocortical cells. The inner part of the transplants showed necrotic changes, due to the lack of an adequate vascular supply to the medullar cells and to the inner cortical layers. Although remaining adrenocortical cells were situated beneath the capsule, they were not arranged in rounded groups as the glomerulosa cells of control adrenals. At this time after transplantation, there was no positive PCNA reaction in any of the adrenocortical cells presented in the transplanted glands, suggesting that the onset of the adrenal regeneration process had not yet begun. However, all the remaining adrenocortical cells showed a weak but generalized corticosterone expression. Capsular cells and necrotic tissue did not show reaction for corticosterone (Fig. 1).

One week after adrenal transplantation, transplanted neonatal adrenals with a size of 2x1.5 mm^2 still persisted

in the ESISs. They were mainly formed by adrenocortical cells, although a considerable amount of necrotic tissue still existed in the central portion of the transplants (Fig. 2a). Glandular cells were surrounded by a connective capsule, with a variable thickness, made up of flattened spindle cells. Beneath the capsule, adrenocortical cells were disposed in 2 to 4 rows oriented parallel to it, many of them being PCNA immunoreactive (Fig. 2b). The rest of the cells, some of which showed nuclei stained with anti-PCNA serum, had morphological features of fasciculata cells but were not clearly disposed in the classical radial arrangement characteristic of these cells in the control adrenals. In addition, some capsular cell nuclei were also PCNA immunopositive. The morphometrical study revealed that 45.4% of the adrenocortical cells of these transplants showed nuclei stained with anti PCNA serum. This expression was present in 59% of the adrenocortical disposed in rows beneath the capsule and in a 41.7% of the remaining cells. A weak corticosterone immunostaining was present in all adrenocortical cells, proliferating and non proliferating cells (Fig. 3).

Three weeks after transplantation, regenerated adrenocortical nodules were completely composed of adrenocortical cells. No necrotic tissue was found. Due to the proliferative activity, regenerated nodules were quite bigger (3x2.5 mm^2) than the ones observed in the previous periods. Nodules appeared well demarcated from the adjacent structures and surrounded by a connective capsule with great thickness differences. Cell organization beneath the capsule was very complex. In some regions, cells appeared disposed in parallel to the capsule while in others rounded cell groups were present. Glandular cells disposed in longitudinal columns were also observed detaching from the deep surface of the connective capsule. The bulk of the regenerated transplants was formed by large cells with a spherical nucleus arranged in longitudinal cords, one or two cells thick, separated by connective tissue. This organization was more marked in the proximity of the capsule, disappearing inwards. In these nodules, most of the PCNA-reactive cells were present just beneath the capsule and in the outer portion of the longitudinal columns (Fig. 4a,b). Occasionally, immunopositive cells were also observed in the inner part of the columns. The morphometrical study showed that in these nodules, adrenocortical PCNA immunoreactive cells (18.4%) were significatively decreased ($p<0.001$) in comparison with the ones measured 1 week after transplantation (45.4%). This PCNA immunoreactivity was almost exclusively present in the cells localized beneath the capsule (62.5%) and in the outermost portion of the longitudinal columns (32.5%), the percentage of immunoreactive cells in the innermost portion of the

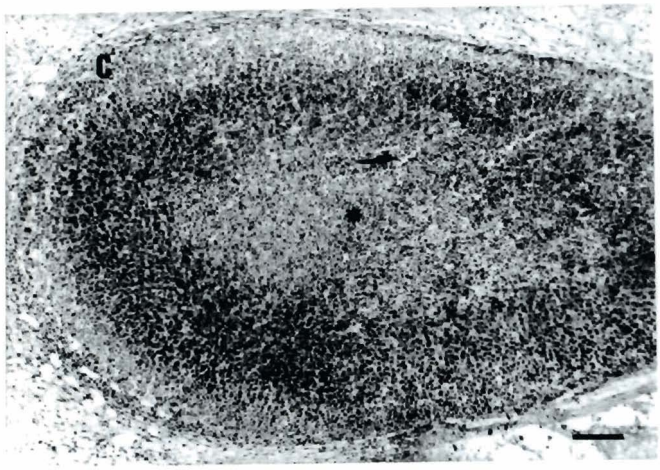

Fig. 1. Three days after transplantation, adrenals display a peripherical rim of adrenocortical cells surrounded by a connective capsule (c) and a large central necrosis area (*). Adrenocortical cells show a weak positive reaction for corticosterone. Bar=100 µm.

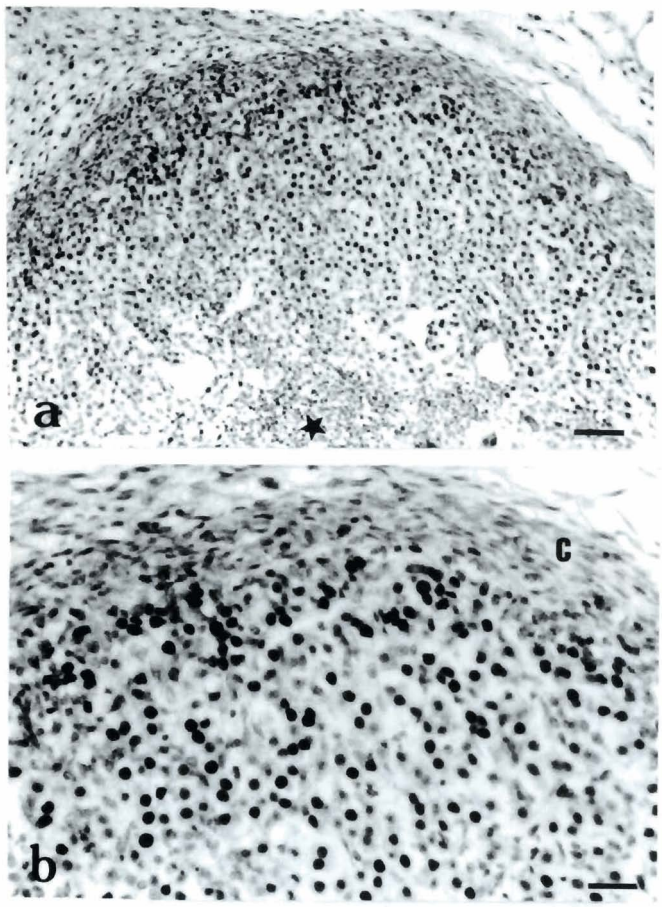

Fig. 2. a. The first PCNA-positive reaction is observed in adrenal transplants, seven days after transplantation. Note that they are almost exclusively composed of adrenocortical cells, although a small necrotic area still persistent in the central portion (black star). Bar= 100 µm. **b.** A higher magnification of the same transplant showing that many small pheripherical adrenocortical cells disposed in rows situated in parallel to the capsule (c) are PCNA positive. A conspicuous PCNA reaction is also observed in the innermost adrenocortical cells that appear irregularly arranged. Bar=200 µm.

columns being very scarce (1.5%). Corticosterone expression was found to be present in all the regenerated adrenocortical cells, the immunostaining intensity being stronger than in the glandular cells of the above mentioned regenerated transplants (Fig. 5).

Twelve weeks after transplantation, regenerated nodules were much more voluminous than the implanted neonatal adrenals (4x3.5 mm^2). In all the regenerated transplants, adrenocortical cells were clearly disposed in three layers: glomerulosa, fasciculata and reticularis. Glomerulosa cells were arranged in loops, some of their nuclei showing a faint PCNA immunoreaction (Fig. 6). Most of the regenerated tissue width was composed of cells disposed in radially straight columns separated by vascular vessels running along connective tracts. A conspicuous zona reticularis, composed of smaller cells than those of the fasciculata was present occupying the central part of these nodules. Occasionally, fasciculata and reticularis cells showed nuclei stained with PCNA serum. The morphometrical study revealed that in these regenerated nodules, only 1.8% of the adrenocortical cells were PCNA immunopositive; the percentage significatively decreased ($p<0.001$) in comparison with the ones measured one and three weeks after transplantation. This immunoreactivity was more prominent in the glomerulosa cells (18.5%), the immunopositive fasciculata and reticularis cells being very scarce (0.81% and 0.63% respectively). Adrenocortical cell zonation was accompanied by a restriction of the corticosterone expression to the fasciculata and reticularis cells (Fig. 7).

Discussion

Numerous studies have provided evidence that adrenocortical tissue has a great regeneration capacity either after adrenal enucleation or after adrenal transplantation. Adrenocortical regeneration has been mainly studied in adrenal enucleation models in which the medulla and the greater part of the adrenal cortex are extracted from the glands that conserve their capsule and the neural and vascular connexions (Taki and Nickerson, 1985; Estivariz et al., 1988). However, in our model, adrenals are transferred to hosts without conserving their vascular connexions, a fact that determines that previous to regeneration, a revascularization process of the transplants has to take place.

Since the beginning of the adrenal transplants and enucleations, attention has been focused on the elucidation of which of the remaining adrenal cells, capsular cells or glandular cells adhere to the capsule, proliferate and regenerate the adrenocortical tissue. Initially, the quantification of mitotic figures was the method used to assess the adrenal growth (Zieleniewski and Nowakowska-Jankiewicz, 1986). However, this method was not of great use to evaluate adrenal growth fraction since metaphase phase is only a short phase of

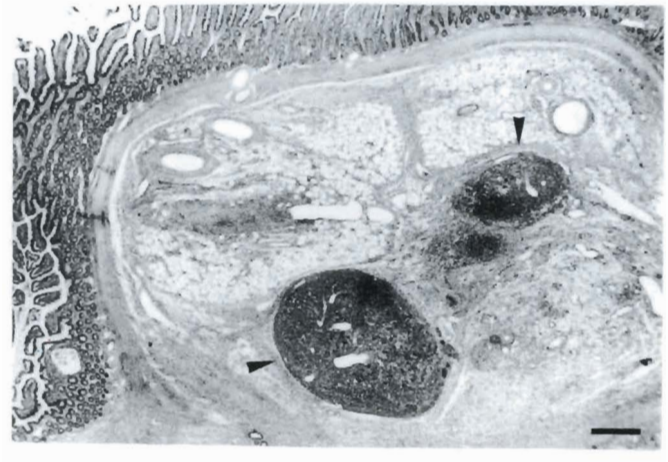

Fig. 3. Seven days post-transplantation. Transplanted adrenals are observed in the lumen of the ESISs displaying a positive reaction for corticosterone (arrow heads). Observe that the epithelium of the ESISs is also immunopositive for corticosterone. Bar=400 µm.

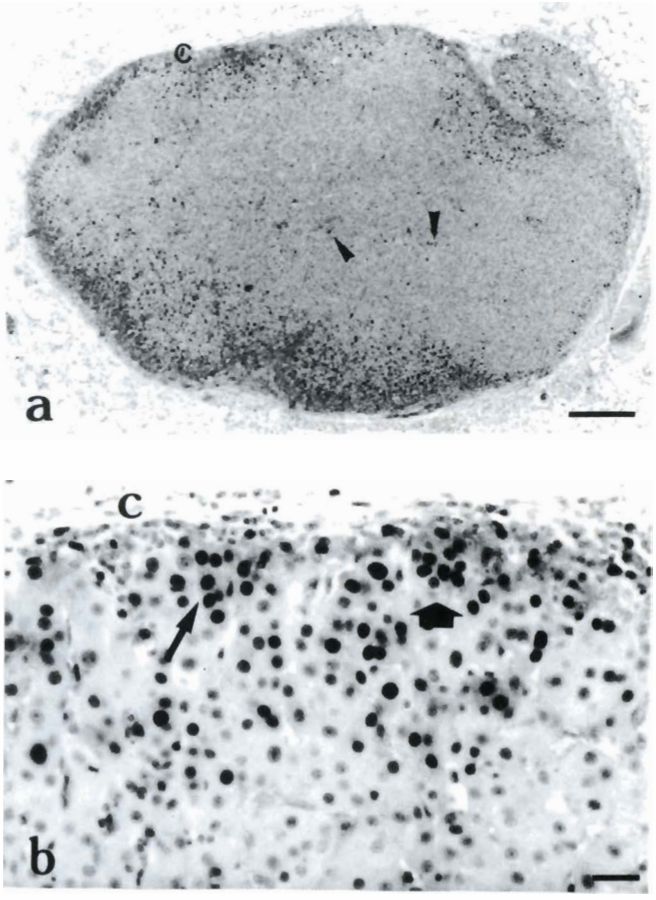

Fig. 4. Three weeks post-transplantation. **a.** PCNA-positive reaction is observed in many adrenocortical cells localized beneath the capsule (c). Note that only isolated nuclei of centrally localized adrenocortical cells in the regenerated nodules are positive for PCNA (arrow heads). Bar=200 µm. **b.** Magnification of the same transplant. Beneath the capsule (c), adrenocortical PCNA-positive cells are arranged in rounded groups (wide arrow) or in longitudinal cords (arrow). Bar=25 µm.

the cell cycle. Autoradiographic techniques, in which cells are labelled with ^{3}H-thymidine during the S-phase of the cell cycle, have also been extensively employed for this purpose (Galand and Degraef, 1989). At present, diverse monoclonal antibodies directed against cell cycle antigens are used as immunocytochemical markers for proliferating cells (Hall and Woods, 1990). The proliferating cell nuclear antigen (PCNA) is expressed only in cells committed to DNA synthesis and is immunocytochemically detectable only during late G1 and S phases. PCNA is the most specific marker of the cell cycle for detecting proliferating cells and can be employed in paraffin-embedded tissues (Louis et al., 1991). In comparison with autoradiographic techniques, the PCNA immunostaining that we have employed in our experimental model to evaluate which cells are responsible for the adrenocortical regeneration process, is faster and simpler to perform. In addition radioactive material is avoided.

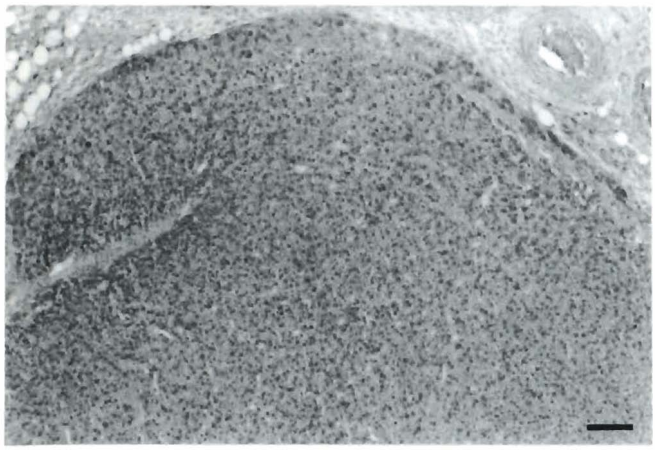

Fig. 5. 3 weeks after transplantation. All the adrenocortical cells of the regenerated transplants express corticosterone. Bar=100 µm.

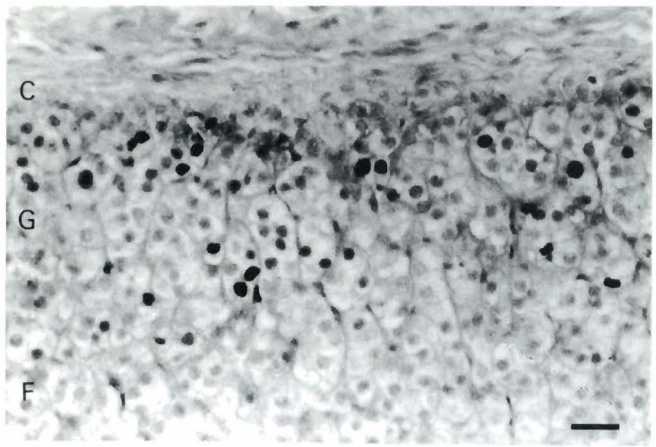

Fig. 6. 12 weeks post-transplantation. Only some glomerulosa cells of the regenerated transplants are PCNA immunopositive. C: capsule; F: fasciculata; G: glomerulosa. Bar= 25 µm.

In our work, no PCNA-reactive cells were observed in the adrenals 3 days after adrenal transplantation. However, 7 days after transplantation, a conspicuous number of capsular and glandular cells were PCNA positive. These findings are consistent with the result obtained in a study by Vendeira et al. (1992) carried out with autoradiographic techniques in which they found an intense capsular and glandular cell labelling 1 week after autotransplantation of adrenal tissue. In adrenal enucleation models, it has been reported that the proliferative activity of the remaining adrenocortical cells begins at day 3 (Taki and Nickerosn, 1985). This shorter interval, in comparison with the one observed in transplantation models, could be related with the above mentioned conservation of vascular links in enucleation models that makes a quicker nourishment of the remaining adrenal cells possible.

Although, in the present study, both capsular cells and glandular cells attached to the connective capsule expressed PCNA, the absence of structural modifications in capsular cells instead of their proliferation activity, clearly indicates that the regenerated adrenocortical tissue arises from glandular cells. This regeneration process, that completely replaces the degenerated adrenocortical tissue serves to ratify the «cell migration theory» (Stachowiak et al., 1990) based on the occurrence of cell division in the outer cortex and cell death in the zona reticularis. The «zona theory» based on the happening of DNA synthesis and in a structural and functional autonomy of each cortical zone, has also been employed to explain adrenal cortex growth and differentiation. This theory hardly agrees with the observed regeneration process of our model, since the innermost adrenocortical cells are completely affected by a necrotic process after transplantation. However, when regeneration process is completed, some fasciculata and reticularis cells are PCNA positive, a fact

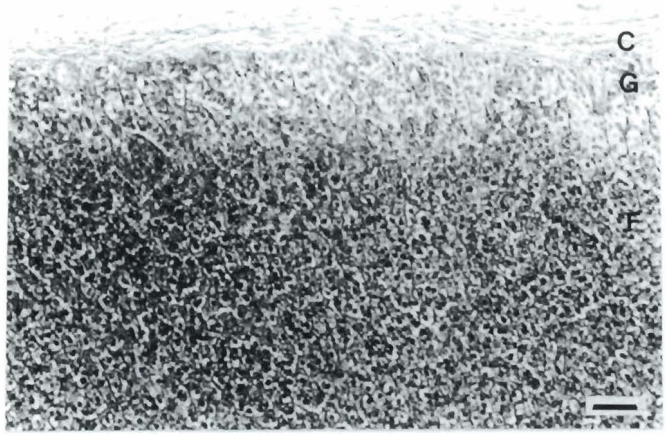

Fig. 7. The complete restoration of a normal cortex architecture, 12 weeks after transplantation, is accompanied by a restriction of the corticosterone expression to fasciculata (F) and reticularis cell, the glomerulosa cells (G) being corticosterone immunonegative. C: capsule. Bar=100 µm.

that points to the occurrence of some proliferative activity in these layers. The proliferative activity observed in the capsular cells could be associated with the formation of connective tissue that initially separates the viable cells from the necrotic tissue. In addition, the newly-formed connective tracts favour an adequate revascularization process of the transplants, since blood vessels run along these tracts.

The sequence of changes in the organization of regenerated adrenocortical cells in the transplants resembles the differentiation process that experiences the adrenal cortical tissue in newborn animals. Similarly to the cell zonation observed in the regenerated adrenocortical nodules twelve weeks after transplantation, a clear adrenocortical cell zonation is only observed in normal adult rats (Mitchell, 1948).

In the normal rat adrenal cortex, corticosterone is synthesized from 11-deoxycorticosterone by an 11-beta hydroxylase. Using immunohistochemical and in-situ hybridization techniques, 11-beta hydroxylase and its mRNA have been localized in the zonae fasciculata and reticularis, but not in the zona glomerulosa (Ogishima et al., 1992; Ho and Vinson, 1993). These specific localizations have been related with the sites of glucocorticoid production (Yabu et al., 1991). However, corticosterone expression has only been observed in the fasciculata cells of normal adrenal cortex (Thaete et al., 1990).

In our experimental model, we have observed that in the early stages of regeneration, when a clear cell zonation was not apparent, all the adrenocortical cells of the transplants were immunopositive to corticosterone. The weak corticosterone expression observed could be related to a deficiency in the activity of the 11-beta hydroxylase during the three weeks post-transplantation (Bergon et al., 1974). The appearance of an adrenocortical cell zonation, twelve weeks after transplantation, was accompanied by a restriction of the corticosterone expression to the fasciculata and reticularis layers. Corticosterone expression by zona reticularis cells could indicate that although a complete morphological differentiation process has taken place in the adrenal cortex, functional differentiation is not yet well established. The above mentioned deficiency in the activity of the 11-beta hydroxylase in the adrenals following transplantations is accompanied by reduced plasmatic concentrations of corticosterone (Vendeira et al., 1992). Restoration of the basal plasmatic concentrations of corticosterone differs in the different experimental models. While Okamoto et al. (1992) have reported that 2 weeks after adrenal autotransplantation, basal corticosterone was similar to that of sham-operated animals. Belloni et al. (1990) have observed that 4 months after adrenal autotransplantation to musculus gracilis, basal corticosterone blood concentration was 40% lower in adrenal autotransplanted animals than in sham-operated animals.

In conclusion, we have observed that after neonatal adrenal gland transplantation, a necrotic process that affects the medulla and the innermost adrenocortical cells takes place. The proliferative activity of the remaining outermost adrenocortical cells, as indicated by the PCNA reaction, completely restore normal cortex architecture. Although a generalized corticosterone expression is observed in all the adrenocortical cells in the early stages of regeneration, adrenocortical cell zonation is accompanied by a restriction of the corticosterone expression to fasciculata and reticularis cells.

Acknowledgements. We are grateful to Mr. Zacarias Alvarez for his technical assistance.

References

Belloni A.S., Neri G., Musajo F.J., Andreis P.G., Mazocchi G., Boscaro M., D'Agostino D., Rebuffat P., Boshier D.P., Gottardo G. and Nussdorfer G.G. (1990). Investigations on the morphology and function of adrenocortical tissue regenerated from gland capsular fragments autotransplanted in the musculus gracilis of the rat. Endocrinology 126, 3251-3262.

Belloni A.S., Neri G., Andreis P.G., Musajo F.G., Boscaro M., Mazzocchi G. and Nussdorfer G.G. (1991). Effects of prolonged sodium restriction on the morphology and function of rat adrenocortical autotransplants. Cell Tissue Res. 265, 35-41.

Bergon L.L., Gallant S. and Brownie A. (1974). Cholesterol side-chain cleavage activity and levels of high-spin cytochrome P-450 in adrenal regeneration hypertension (ARH). Endocrinology 94, 336-345.

Bravo R. and Macdonald-Bravo H. (1985). Changes in the nuclear distribution of cyclin (PCNA) but not its synthesis, depend on DNA replication. EMBO J. 4, 655-661.

Bravo R., Frank R., Blundell P.A. and Macdonald-Bravo H. (1987). Cyclin/PCNA is the auxiliary protein of DNA polimerase delta. Nature 326, 515-517.

Childs G.V., Hyde C. and Naor Z.V.VI. (1983). Morphometric analysis of thyrotropes in developing and cycling rats: Studies of intact pituitaries and cell fractions separated by centrifugal elutriation. Endocrinology 113, 1601-1607.

Estivariz F.E., Morano M.L., Carino M., Jackson S. and Lowry P.J. (1988). Adrenal regeneration in the rat is mediated by mitogenic N-terminal pro-opiomelanocortin peptides generated by changes in precursor processing in the anterior pituitary. J. Endocrinol. 116, 207-216.

Galand P. and Degraef C. (1989). cyclin/PCNA immunostaining as an alternative to tritiated thymidine pulse labelling for marking S phase cells in paraffin sections from animal and human tissues. Cell. Tissue Kinet. 22, 383-392.

Hall P.A. and Woods A.L. (1990). Immunohistochemical markers of cell proliferation: achievements, problems and prospects. Cell. Tissue Kinet. 23, 505-522.

Ho M.M. and Vinson G.P. (1993). 11 beta hydroxylase gene expression in the rat adrenal cortex. J. Endocrinol. 139, 301-306.

Louis D.N., Edgerton S., Thor A.D. and Hedley-Whyte E.T. (1991). Proliferating cell nuclear antigen and Ki-67 immunohistochemistry in brain tumors: a comparative study. Acta Neuropathol. 81, 675-679.

Matsumoto T. (1990). Autotransplantation of the adrenal cortex into the

spleen of dogs. Jpn. J. Surg. 20, 307-315.

Morris G.F. and Mathews M.B. (1989). Regulation of proliferating cell nuclear antigen during the cell cycle. J. Cell Biol. 264, 13856-13864.

Mitchell R.M. (1948). Histological changes and mitotic activity in the rat adrenal during postnatal development. Anat. Rec. 101, 161-186.

Ogishima T., Suzuki H., Hata J-I., Mitani F. and Ishimura Y. (1992). Zone-specific expression of aldosterone synthase cytochrome P-450 and cytochrome P-450 11 beta in rat adrenal cortex: histochemical basis for the functional zonation. Endocrinology 130, 2971-2977.

Okamoto T., Fujimoto Y., Obara T., Ito Y. and Kodama T. (1992). Experimental study on adrenal autografts in rats to preserve normal adrenocortical function after bilateral adrenalectomy. Eur. Surg. Res. 24, 112-118.

Stachowiak A., Nussdorfer G.G. and Malendowicz L.K. (1990). Proliferation and distribution of adrenocortical cells in the gland of ACTH or dexamethasone treated rats. Histol. Histopathol. 5, 25-29.

Taki T.M. and Nickerson P.A. (1985). Differentiation and proliferation of adrenocortical cells during the early stages of regeneration. Lab. Invest. 53, 91-100.

Thaete L.G., Nesbitt M.N. and Malkinson A. (1990). Genetic analysis of the distribution of corticosterone-containing cells in mouse adrenal cortex. P.S.E.B.M. 194, 97-102.

Vendeira P., Magalhães M.M. and Magalhães M.C. (1992). Autotransplantation of the adrenal cortex: A morphological and autoradiographic study. Anat. Rec. 232, 262-272.

Yabu M., Senda T., Nonaka Y., Matsukawa N., Okamoto M. and Fujita H. (1991). Localization of the gene transcripts of 11 beta-hydroxylase and aldosterone synthase in the rat adrenal cortex by in situ hybridization. Histochemistry 96, 391-394.

Zieleniewski J. and Nowakowska-Jankiewicz B. (1986). Studies of mitotic activity in course of adrenal cortex regeneration after enucleation. Acta. Med. Pol. 27, 93-96.

Accepted February 20, 1995