J. Anat. (1982), **135**, 2, pp. 245–253
With 11 *figures*
Printed in Great Britain

Ultrastructural observations on the regeneration of adrenocortical autotransplants in the rat spleen†

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(*Accepted* 5 *October* 1981)

INTRODUCTION

It is well known that adrenal autotransplants regenerate and restore their normal form in about 1–2 months (see Geiringer, 1954, for review). However, the ultrastructural changes associated with regeneration have received little attention. Penney, Patt & Dixon (1963) described the ultrastructural features of adrenal halves implanted in the rat trapezius and latissimus dorsi muscles, but unfortunately only osmium tetroxide fixation was employed.

It, therefore, seemed worth while carefully to examine the fine structure of small adrenocortical grafts after various times from their autotransplantation in the spleen.

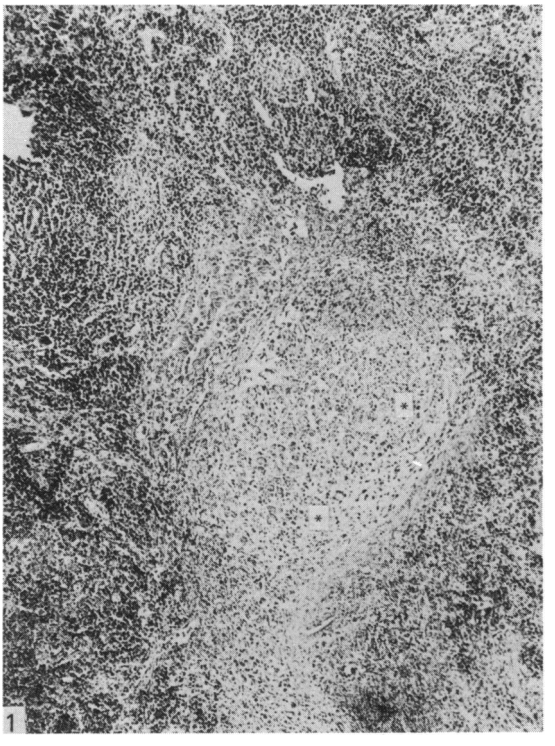
MATERIALS AND METHODS

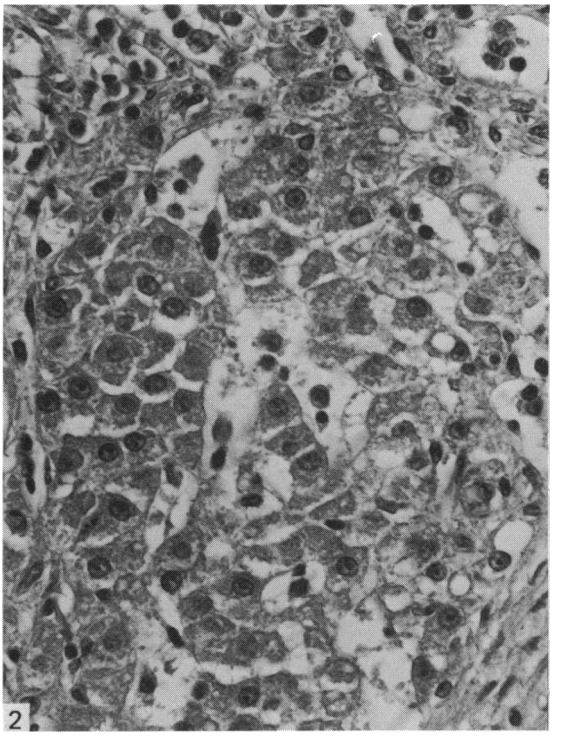
Thirty male albino rats of the Wistar strain weighing about 200 g were laparotomized and bilaterally adrenalectomized. The right gland was discarded. The left gland was cleaned of all connecting fat and sliced in half, when the zona medullaris and the inner portion of the cortex were removed under the dissecting microscope. The outer cortex was then chopped in fragments of about 1 mm in diameter. Four pockets were made along the dorsal surface of the spleen, opposite to the hilus. One adrenal fragment was inserted in each pocket, which was then closed by a 8–0 prolene stitch.

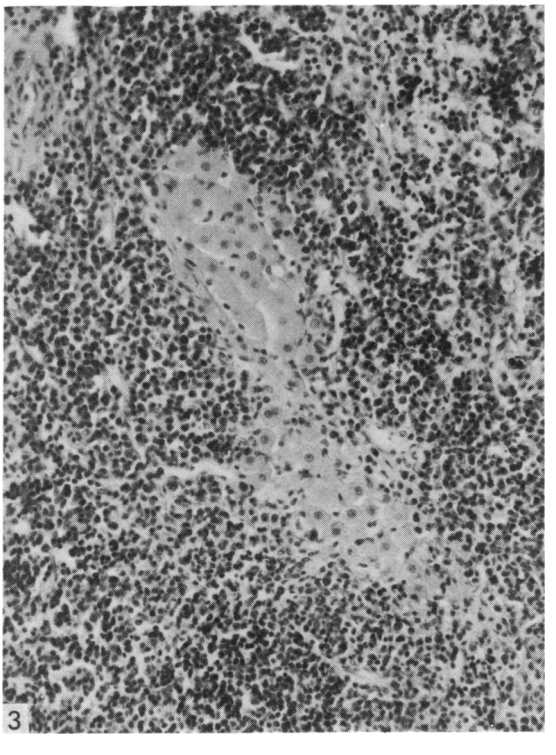
For a period of one week after the operation animals were given a drinking solution of 1 % saline, after which tap water was again supplied. All the rats were maintained on purina rat-mouse chow during the entire experimental period and groups of animals were killed by cervical dislocation after 3, 7, 15, 30 or 36 days from the operation.

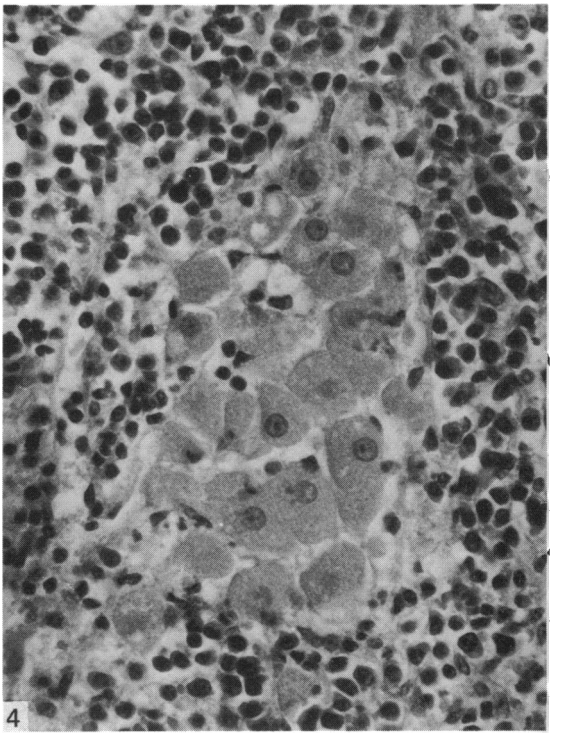
Spleens were pre-fixed *in situ* with 3 % glutaraldehyde in 0·1 M cacodylate buffer (Dal Lago & Lucke, 1973) and were then excised and halved along a cutting plane perpendicular to the hilus. One half-spleen was fixed in 10 % buffered formalin, embedded in paraffin and serially cut at 7 μm. From the other half, spleen parenchyma containing adrenal grafts (landmarks were prolene stitches) was excised and cut into fragments of about 0·5 mm in diameter, which were fixed in 3 % glutaraldehyde in 0·1 M cacodylate buffer (pH 7·2), post-fixed in 1 % OsO_4 in 0·1 M phosphate buffer (pH 7·1) and embedded in an epoxy resin. Thick sections were made with

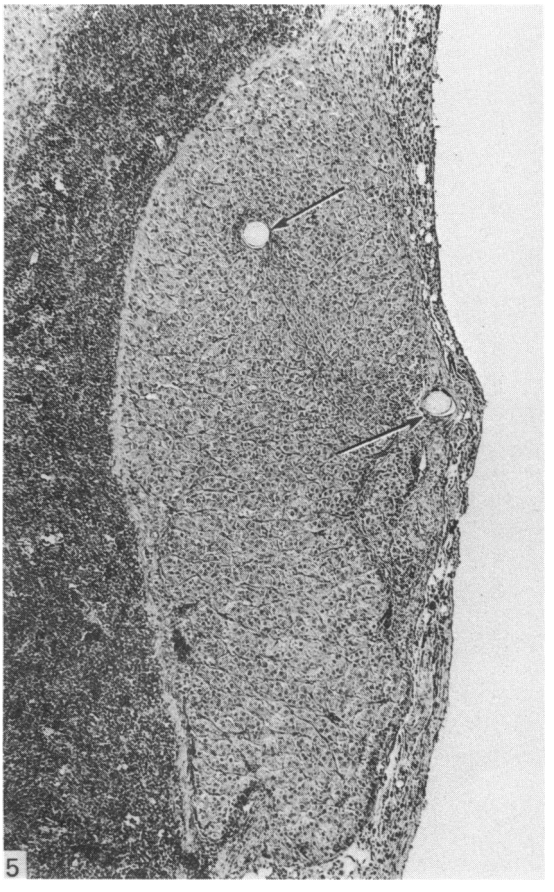
† Reprint requests to Professor Nussdorfer.











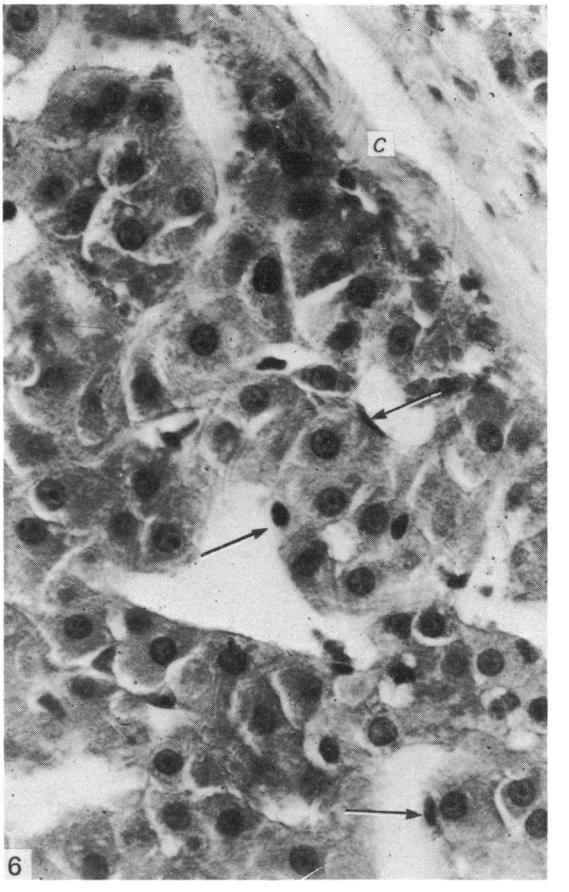


Fig. 5. Regenerated adrenal autograft 1 month after implantation in the rat spleen. The graft is completely demarcated from the spleen tissue by an obvious connective tissue capsule and its parenchymal cells display an evident cordonal arrangement. The arrows indicate two prolene stitches. × 68.

Fig. 6. Same specimen as shown in Fig. 5. Parenchymal cells are arranged in short cords intermingled with large capillaries. The arrows indicate the nuclei of endothelial cells. *C*, connective tissue capsule surrounding the graft. × 430.

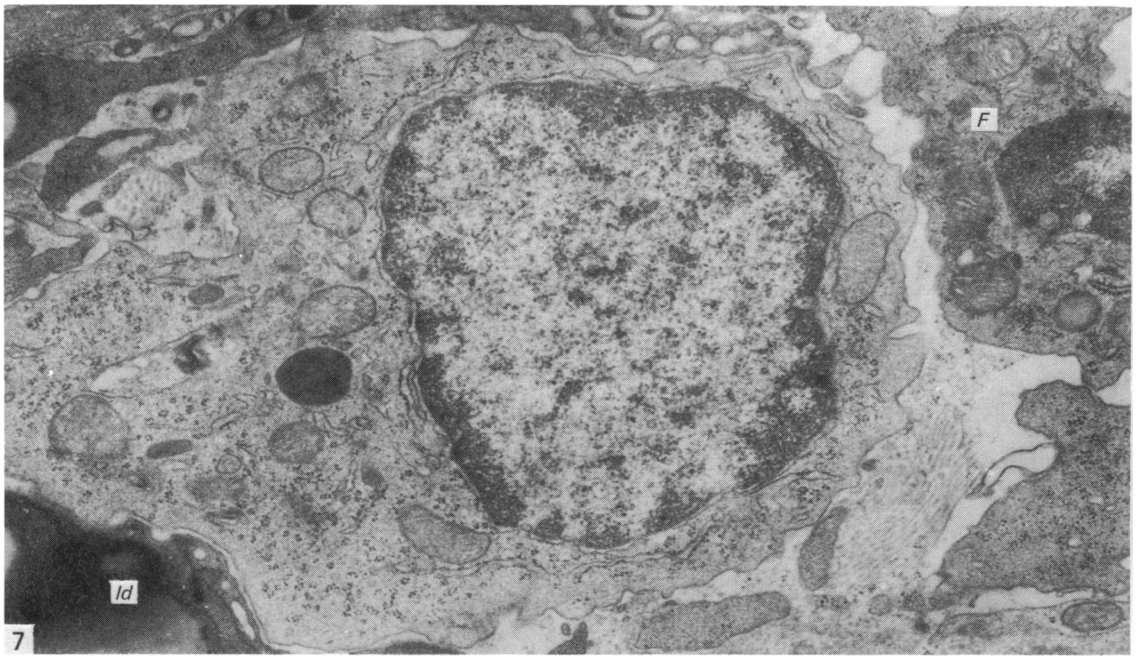
LKB III ultramicrotomes and observed under the light microscope to select adrenal graft tissue. Thin sections were counterstained with lead-hydroxide (Karnovsky, 1961) and examined in a Hitachi HS-9 electron microscope.

Fig. 1. At the seventh day after implantation in the rat spleen, adrenal autografts show large areas of necrosis (*). × 68.

Fig. 2. Same specimen as shown in Fig. 1. The graft cells possess dark nuclei and atrophic cytoplasm. × 430.

Fig. 3. Regenerating adrenal autograft 15 days after implantation in the rat spleen. Due to the intense degeneration during the first week of transplantation, the graft is very small, but its cells are all viable. Adrenal tissue is not well demarcated from the surrounding spleen parenchyma. × 172.

Fig. 4. Same specimen as shown in Fig. 3. Note the evident hypertrophy of the parenchymal cells. × 430.



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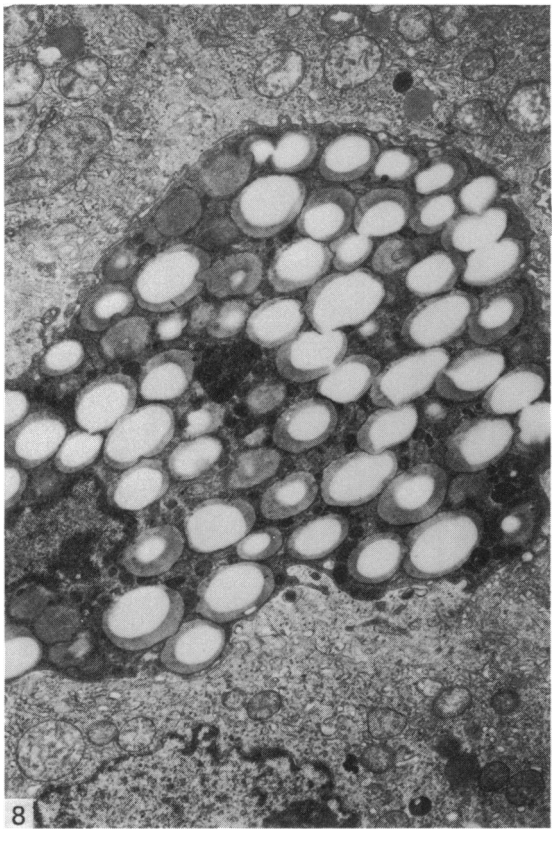

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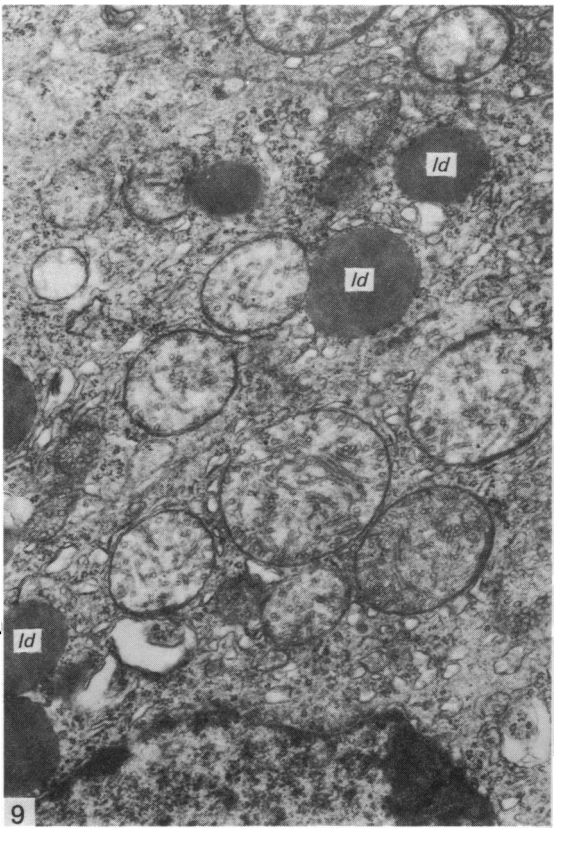

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RESULTS

Up to the seventh day after implantation, viable adrenocortical tissue apparently represented no more than 15–20 % of the total graft volume. Grafts were not well demarcated from the spleen parenchyma and showed large areas of necrosis (Fig. 1). Graft cells were irregularly arranged and contained small dark nuclei and atrophic cytoplasm (Fig. 2). Electron microscopy demonstrated that graft tissue, in addition to typical fibroblasts and many macrophages, contained various cell types.

Several small poorly differentiated cells, resembling mesenchymal elements, were observed (Fig. 7). They displayed a rather scanty cytoplasm, containing irregularly shaped mitochondria with laminar cristae, many profiles of rough endoplasmic reticulum and abundant free ribosomes and polysomes, as well as occasional dense bodies.

Among the mesenchyme-like cells there were many degenerating and some viable adrenocortical cells. Degenerating parenchymal cells (Fig. 8) were shrunken and their cytoplasm appeared to be filled with lipid droplets and dense bodies, some of which resembled typical residual bodies. Viable adrenocortical cells (Fig. 9) contained ovoid mitochondria with sparse tubular cristae embedded in a rather light matrix, a poorly developed smooth endoplasmic reticulum and many lipid droplets.

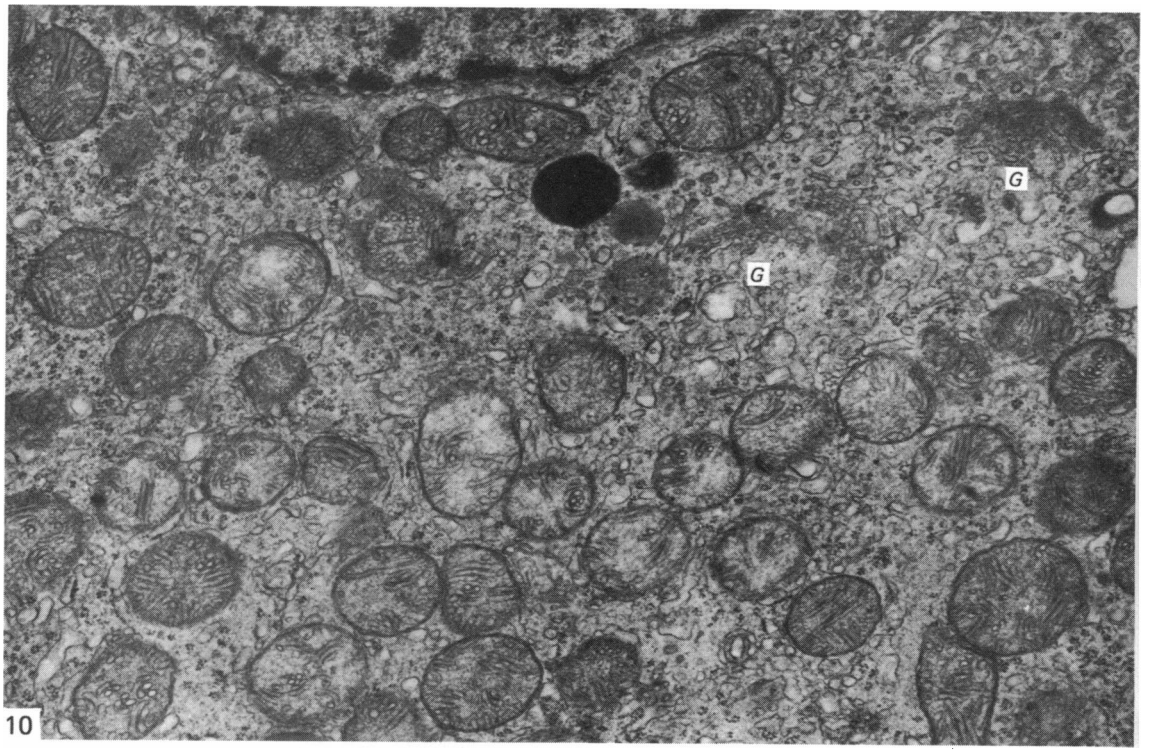
Fifteen days after transplantation, adrenal grafts were very small and not well demarcated from the spleen tissue, but did not display necrotic areas (Fig. 3). Numerous mitoses were observed. Parenchymal cells were noticeably enlarged (Fig. 4) and showed quite homogeneous ultrastructural features (Fig. 10). Mitochondria were round or ovoid and contained a mixture of vesicular and tubular cristae. Some organelles possessed intramatrical areas devoid of cristae. Smooth endoplasmic reticulum was rather abundant and free ribosomes and polysomal rosettes were scattered among its tubules. The juxtanuclear Golgi apparatus and some dense bodies were always present. Lipid droplets appeared to be completely absent.

Thirty to thirty six days after implantation, a conspicuous regeneration had occurred. Grafts were very large and invariably surrounded by an obvious connective tissue capsule (Fig. 5). Parenchymal cells were voluminous and arranged in irregular cords intermingled with a well developed capillary network (Fig. 6). Occasional mitoses were still visible. No clear signs of histological zonation were found. Electron microscopy showed that all the graft parenchymal cells displayed the typical features of the adult rat zona fasciculata elements (Fig. 11). Mitochondria contained vesicular cristae, smooth endoplasmic reticulum was plentiful, the Golgi apparatus was prominent and some lipid droplets appeared in the cytoplasm.

Fig. 7. At the third day after transplantation, among degenerating adrenocortical cells (*ld*) and typical fibroblasts (*F*), some small poorly differentiated cells resembling mesenchymal elements can be observed in the adrenal graft. These cells contain mitochondria with laminar cristae, profiles of rough endoplasmic reticulum, and many free ribosomes and polysomes. × 20000.

Fig. 8. Degenerating adrenocortical cell in an adrenal autograft 7 days after implantation in the rat spleen. The cell has a shrunken appearance and contains a small dark nucleus, many lipid droplets and numerous dense bodies. × 11500.

Fig. 9. Viable adrenocortical cell from the same specimen as shown in Fig. 8. Mitochondria are ovoid and display scanty tubular cristae embedded in a rather light matrix. The smooth endoplasmic reticulum is poorly developed, while free ribosomes and polysomes are abundant. *ld*, lipid droplets. × 21000.



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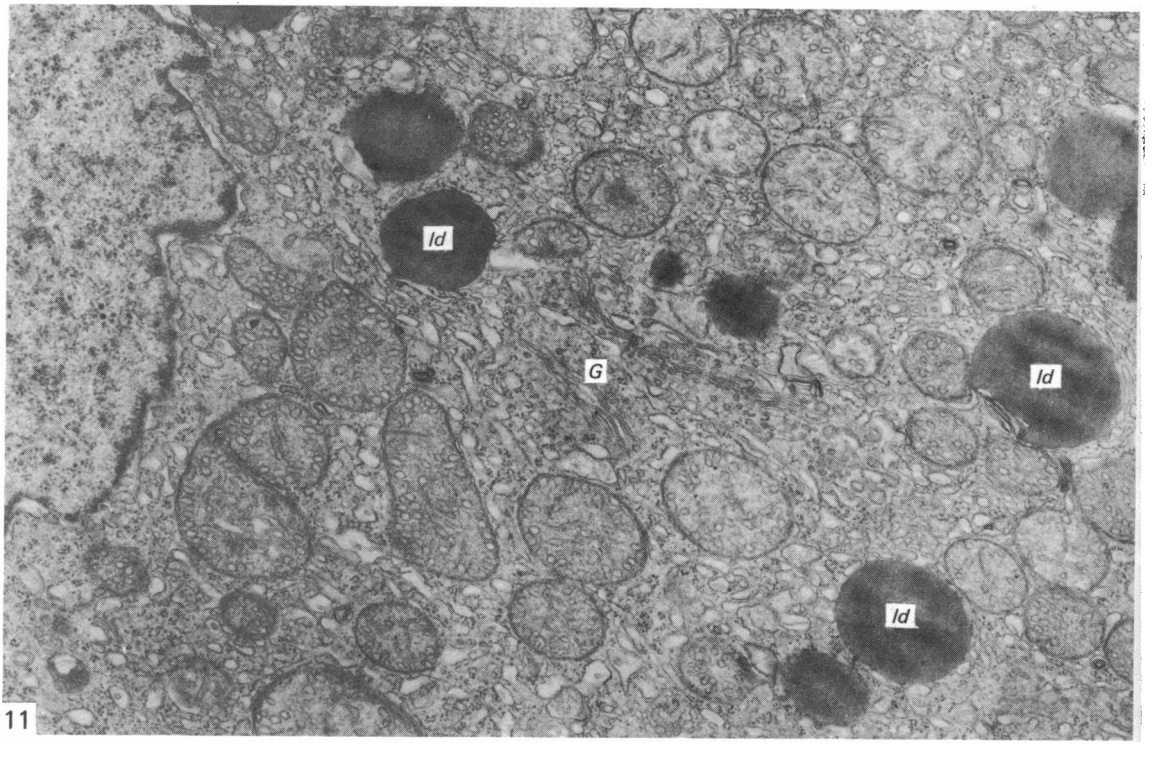

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DISCUSSION

Our results are in agreement with the previous ones indicating the great regeneration capacity of adrenal autotransplants (Wyman & tum Suden, 1932; Ingle & Higgins, 1938; Geiringer, 1954). According to Penney *et al.* (1963) noticeable adrenocortical cell degeneration occurs only during the first week after transplantation. Degenerating cells closely resemble the apoptotic elements described by Wyllie, Kerr, Macaskill & Currie (1973) and by Wyllie, Kerr & Currie (1973) and this finding confirms the view that apoptosis may be an important mechanism of cell death and deletion in several tissues (Wyllie, Kerr & Currie, 1980). This impressive parenchymal cell death may conceivably be due to the altered blood supply in the adrenal explants and may easily explain the noticeable reduction in the volume of the grafts two weeks after transplantation.

Among the degenerating parenchymal cells, viable, but poorly differentiated adrenocortical cells and numerous mesenchyme-like elements are also observed during the early phases of regeneration. Mesenchyme-like cells, resembling those found in adult rat adrenal gland cultured *in vitro* in the absence of ACTH (Armato & Nussdorfer, 1972), seem to be primarily engaged in the synthesis of their structural proteins, Whether or not these cells should be considered de-differentiated adrenocortical cells, or subcapsular stem elements involved in adrenal tissue regeneration, remains to be elucidated. Dividing mesenchyme-like cells were never seen, but we stress that demonstration of mitosis is not a simple task at the ultrastructural level. To gain further insight into this problem, we plan to investigate (by light and electron microscopic autoradiography) the regeneration of adrenal fragments, previously *in vivo*, labelled with 3H-thymidine.

Regeneration of the adrenal autotransplants seems to be closely linked to the ultrastructural differentiation of parenchymal cells. Our data indicate that the most conspicuous structural changes involve mitochondria and smooth endoplasmic reticulum. Mitochondrial cristae, which at the seventh day after transplantation are scarce and of the tubular type, increase in number and eventually transform in a homogeneous population of vesicles. Smooth endoplasmic reticulum displays an evident proliferation. These morphological changes are similar to those described during the enucleation-induced adrenal regeneration (Seki, Sekiyama, Miyahara & Ichii, 1969; Yago *et al.* 1972). Current evidence indicates that enzymes of steroid synthesis are located in the smooth endoplasmic reticulum and the mitochondrial cristae (see Tamaoki, 1973, for review). Hence, it is conceivable that these ultrastructural changes are the morphological counterpart of the resumption by adrenocortical cells of their steroidogenic capacity (see Nussdorfer, Mazzocchi & Meneghelli, 1978, for review). The presence of a well developed Golgi apparatus in the parenchymal cells 15–30 days after transplantation is also in keeping with

Fig. 10. Adrenocortical cell from an autograft 15 days after implantation in the rat spleen. Round or ovoid-shaped mitochondria prevalently contain tubular cristae. Smooth endoplasmic reticulum is quite well represented and several polysomal rosettes are scattered in the cytoplasm. G. Golgi apparatus. ×25000.

Fig. 11. Adrenocortical cell from an autograft 30 days after implantation in the rat spleen. Mitochondria show vesicular cristae, the smooth endoplasmic reticulum is well developed and some lipid droplets (*ld*) are present. G, Golgi apparatus. ×25000.

this interpretation, because a large number of data suggest that this organelle is 'integral to steroid genesis' (Christensen & Gillim, 1969).

On these grounds, the behaviour of the lipid droplets during graft regeneration may also easily be explained. The noticeable lipid depletion on the fifteenth day after transplantation may be interpreted as the expression of the utilization in steroid synthesis of the cholesterol stored in the lipid droplets (Moses, Davis, Rosenthal & Garren, 1969; Sand, Frühling, Penasse & Claude, 1972). Conversely, the presence of a moderate number of lipid droplets after one month of regeneration may be ascribed to the further proliferation and maturation of smooth endoplasmic reticulum, inasmuch as it is involved in cholesterol synthesis from acetate and glucose (see Christensen, 1965; Malamed, 1975; Nussdorfer *et al.* 1978, for review).

The modalities of adrenal autograft regeneration deserve some further comments, since, at variance with Penney *et al.* (1963), signs of histological and fine structural zonation were not found five weeks after transplantation. This may be due to the different techniques of graft preparation employed. In fact, Penney *et al.* used adrenal halves, whereas we implanted outer cortex fragments of about 1 mm in diameter.

The relative rapidity of regeneration of our autotransplants, which was similar to that of enucleated adrenal gland *in situ* (Ingle & Higgins, 1938), is not surprising if the site of implantation is considered. It appears that the splenic parenchyma, with its good blood flow, may represent the most favourable environment for adrenal regeneration. Moreover, adrenal regeneration is under the control of ACTH (Fortier & De Groot, 1959; Nakayama, Nickerson & Skelton, 1969) and we suggest that in our experimental model the negative feed-back mechanism (which initially enhances ACTH release in response to the bilateral adrenalectomy) may be operative also in the late regeneration phases, since venous blood from the spleen via the portal vein drains into the liver, where newly synthesized adrenocortical hormones are rapidly metabolized. This hypothesis is currently under investigation by employing rats with portocaval anastomosis and by assaying the plasma steroid concentration.

In conclusion, we believe that this experimental model, which is easy to set up and has a high degree of success, will be very useful in the investigation of adrenal cytophysiology.

SUMMARY

The regeneration of adrenocortical autotransplants in the rat spleen has been investigated by light and electron microscopy. Up to the seventh day after implantation, adrenal grafts showed large areas of necrosis and contained many degenerating (apoptotic) adrenocortical cells, some mesenchyme-like poorly differentiated elements, and occasional viable parenchymal cells. These last cells possessed mitochondria with scanty tubular cristae and few profiles of smooth endoplasmic reticulum. After 15 days of regeneration, adrenal grafts were reduced in volume, but contained only viable adrenocortical cells; after 30–36 days, autotransplants were noticeably enlarged and surrounded by an evident connective tissue capsule. Regeneration was closely associated with the morphological differentiation of adrenocortical cells, which one month after transplantation were found to assume all the typical features of adult rat zona fasciculata elements (i.e. mitochondria with vesicular cristae, abundant smooth endoplasmic reticulum, some lipid droplets and a well developed Golgi apparatus).

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