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Ultrastructure of the adrenocortical homologue in dexamethasone-treated eels

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INTRODUCTION

As in higher vertebrates, the existence of a pituitary–adrenal axis has been demonstrated in teleosts and other groups of fishes. Thus, hypophysectomy or dexamethasone administration was followed by a reduction in plasma cortisol levels in the trout, *Salmo gairdnerii* (Donaldson & McBride, 1967) and in the European and American eels, *Anguilla anguilla* and *Anguilla rostrata* (Bradshaw & Fontaine-Bertrand, 1968; Butler, Donaldson & Clarke, 1969). Light microscopic studies have also indicated that exogenously administered corticosteroids, such as desoxycorticosterone, cortisone, or cortisol, eventually elicit a cytological atrophy of the adrenocortical homologue (AH) in the cavefish, *Astyanax mexicanus* (Rasquin & Atz, 1952), the mouth-brooder, *Tilapia mossambica* (Basu, Nandi & Bern, 1956), the European eel, *Anguilla anguilla* (Olivereau, 1966) and the catfish, *Heteropneustes fossilis* (Subhedar & Rao, 1974). Some other reports, however, indicated contrary findings. In the mud minnow, *Umbra krameri*, treatment with desoxycorticosterone led first to nuclear atrophy, followed by mitotic activity and hypertrophy (Krauter, 1958). Bovine adrenocortical extract injected into the goldfish, *Carassius auratus*, had no action on the adrenal (Chavin, 1956). Failure of hypophysectomy to induce atrophy of the adrenocortical homologue (AH) in the killifish, *Fundulus heteroclitus*, has also been recorded (Pickford, 1953). It seemed to be of interest to explore the effect of experimental adrenocorticotrophic inhibition on the AH in the eel, which has been an important model for research on corticosteroidogenesis in teleosts (Butler, 1973). The present communication deals with the ultrastructural changes of the AH in North American eels following the injection of dexamethasone, a very potent synthetic corticosteroid known to inhibit corticotropin secretion in mammals (Vanek, 1965).

MATERIALS AND METHODS

Mature female silver eels (*Anguilla rostrata*) (body weight 1000–1800 g) kept in laboratory aquaria at a temperature of $10 \pm 2°$ with constantly flowing dechlorinated tap water were used. Two experimental groups were set up (number of animals given in parentheses): (A) experimental animals (6) treated with dexamethasone (9α-fluoro-16α-methyl prednisolone, E. Merck) at a dose of 20 mg/day for 10 days; (B) sham-injected controls (4) receiving only the vehicle. The injectable steroid was prepared as a suspension in 10% ethyl alcohol in normal saline and each experimental animal received an intraperitoneal injection of the vehicle (2·5 ml) containing the drug. At the conclusion of the experiment the animals were anaesthetized in

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tricaine methanesulphonate solution (0·4 g/l). Fixation was performed by the perfusion through the right postcardinal vein of Tyrode solution followed by 2 % formaldehyde–2·5 % glutaraldehyde in 0·1 M cacodylate buffer (Flickinger, 1967). Details of the perfusion technique have been given elsewhere (Bhattacharyya & Butler, 1979). Following perfusion, regions of the anterior and posterior cardinal veins presumed to contain the AH were excised, and the tissues fixed for an additional period of 2 hours in the fixative at 4 °C. After repeated washings with buffer the specimens were post-fixed in 1 % osmium tetroxide in the same buffer for 2 hours at 4 °C. Tissue blocks were dehydrated in graded ethanol and embedded in Araldite.

Ultrathin sections were cut with glass knives on a Porter–Blum MT-2 microtome and collected on uncoated copper grids. The sections were stained with uranyl acetate and lead citrate and examined in a Philips 201 electron microscope operated at 60 kV. One μm sections were cut for light microscopy and orientation, and stained with 1 % toluidine blue in 1 % borax solution.

OBSERVATIONS

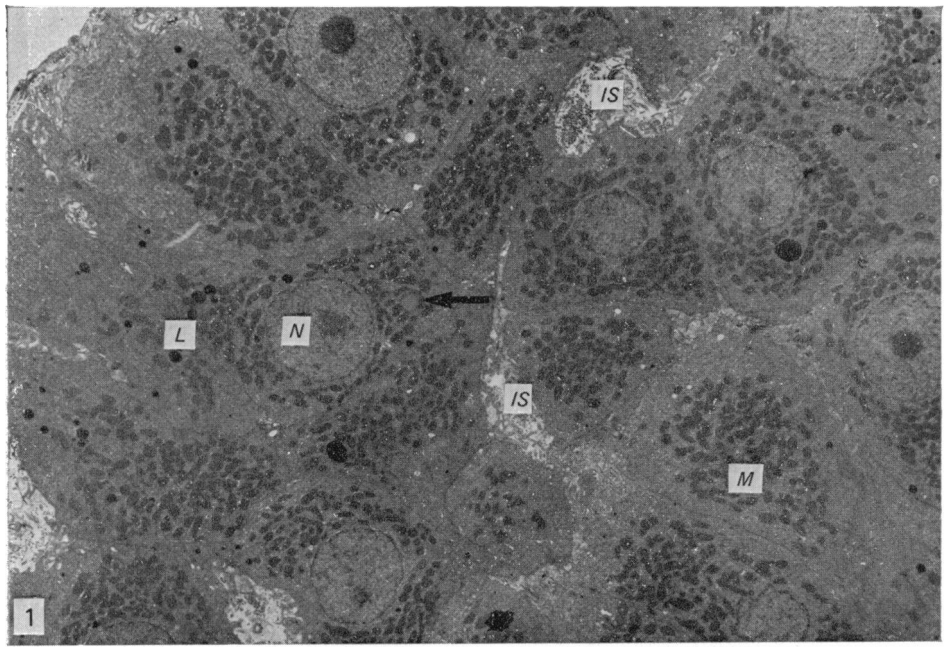
(A) *Control*

The AH, enclosed by a thin capsule of collagenous fibres, are localized in the wall of left and right anterior and posterior cardinal veins close to the heart and are in the form of lobules of cells separated by extensive capillaries. The cells are separated by interstitial spaces containing nerve fibres and chromaffin cells; numerous slender microvilli arising from the free cell surface project towards the interstitial or peri-capillary space and the vein wall (Fig. 1). Each AH cell contains a conspicuous network of tubular smooth endoplasmic reticulum, prominent profiles of Golgi apparatus, and numerous mitochondria with tubular cristae and dense matrix (Fig. 2). In addition, microbody-like structures and two classes of dense bodies, possibly lysosomes, are to be seen (Fig. 2): (*a*) small granular dense bodies; (*b*) filamentous bodies containing whorls of circularly and longitudinally oriented filaments. A few patches of rough-surfaced endoplasmic reticulum, microtubules and filaments are also present.

(B) *Dexamethasone-treated*

Two of the experimental animals showed a moderate response to dexamethasone administration. The parenchymal cells were shrunken, leading to the formation of large intercellular spaces filled with collagen bundles. The nucleoli of the cells were absent and the mitochondria had somewhat lowered matrix density. The dense bodies (*a*) were increased in number to 10–12 per cell. Small autophagic vacuoles with trapped mitochondria were seen. Occasionally a series of desmosomes was formed between adjacent cells (Fig. 5).

More profound modifications were observed in the remaining animals. The nuclei were not remarkably altered except for the absence of nucleoli and the slightly wrinkled outline of the nuclear membrane. Microvilli associated with the free surface of AH cells were reduced in size, number and infoldings. The mitochondria appeared highly electron-lucent due to loss of matrix density (Figs. 3, 4). There was a deposition of numerous small osmiophilic granules over the matrix (Fig. 11) which, by coalescence, had led to the formation of bigger osmiophilic masses. The Golgi apparatus was reduced in size or fragmented, individual lamellae being reduced in number while isolated saccules of the Golgi apparatus were frequently observed



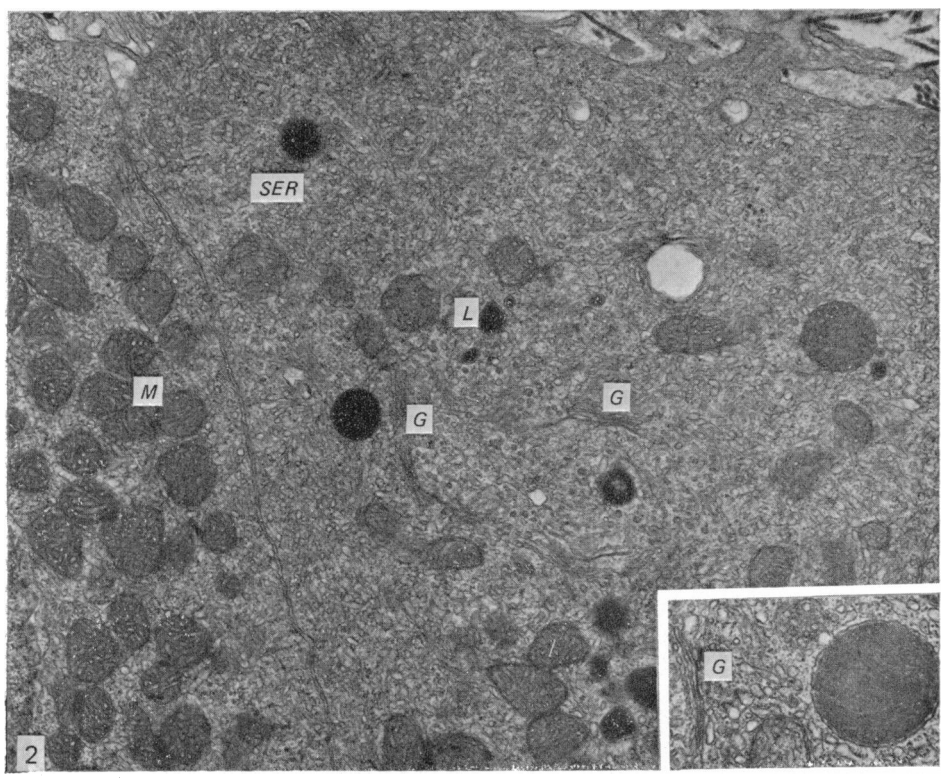
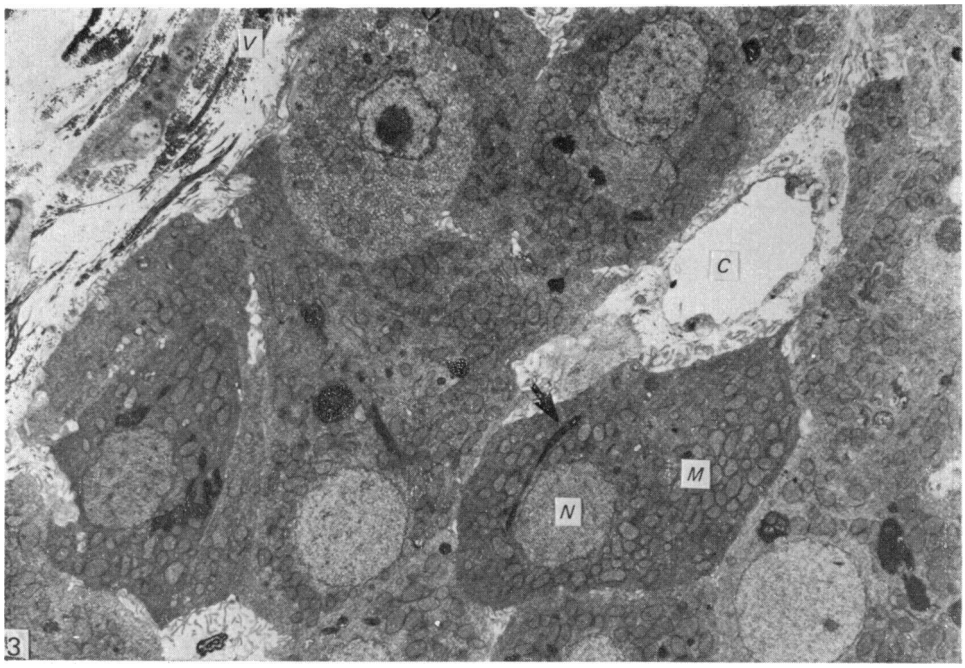


Fig. 1. A survey electron micrograph of a cluster of AH cells from a sham-injected eel. The cells are separated by interstitial spaces (*IS*) that contain collagen fibres and slender microvilli arising from the free cell surface. The cells possess round nuclei (*N*), polymorphic mitochondria (*M*) and a few lysosome-like dense bodies (*L*). An occasionally observed lipid droplet (arrow) is present in a cell. × 2750.

Fig. 2. An enlarged view of AH cells in a control animal. The cytoplasm is permeated by tubules of smooth endoplasmic reticulum (*SER*). Prominent profiles of the Golgi complex (*G*) and mitochondria (*M*) with tubular cristae and dense matrix are also observed. Lysosome-like dense bodies (*L*) are present near the Golgi zone. × 14340. Inset shows a round filamentous dense body near the Golgi complex (*G*). × 19300.



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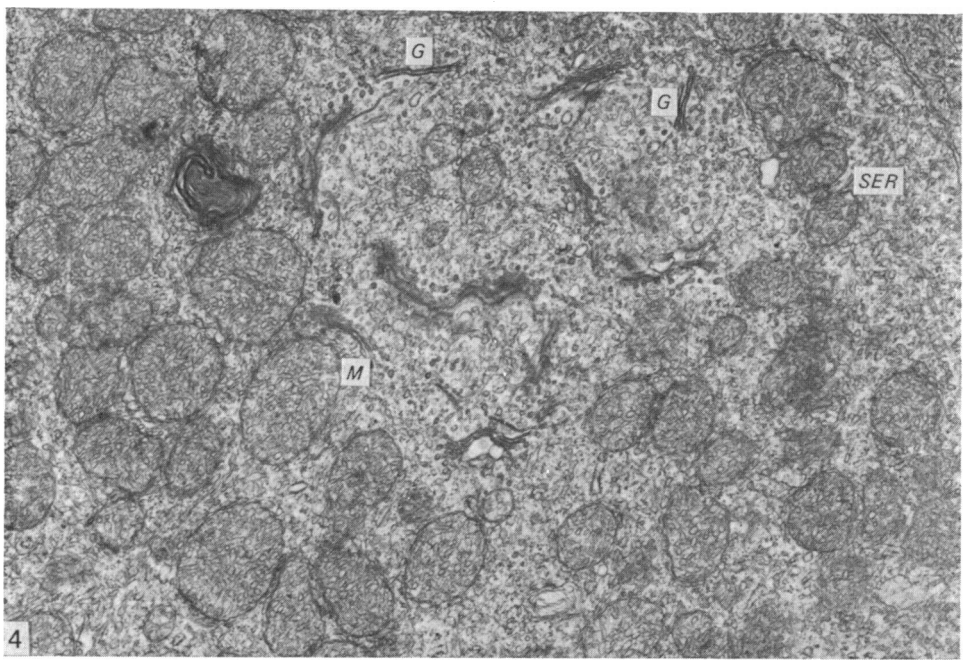

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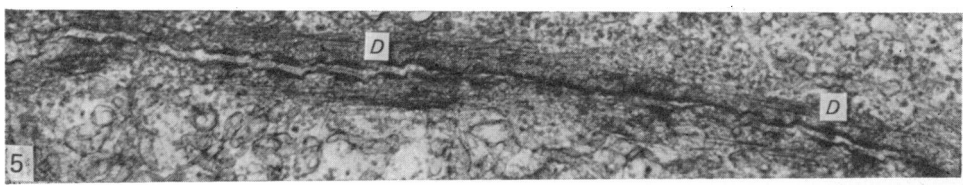

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(Fig. 4). The smooth endoplasmic reticulum appeared as disjoined short tubules or isolated small vesicles. Reduced populations of polyribosomes and coated vesicles were also noted. No significant changes occurred in the filaments, microtubules or the rough-surfaced endoplasmic reticulum.

The most noteworthy feature in steroid-treated eels, however, was the presence of a variety of cytoplasmic inclusions in the vicinity of the Golgi complex.

(1) Some were membrane-limited, round, rectangular or elongated with a finely granular matrix (Fig. 6). Parts of such inclusions were more electron-opaque and possessed concentric lamellae. The central region of the inclusion consisted of a rectangular electron-lucid area with an elongated osmiophilic mass surrounded by membrane fragments. The diameter of similar round inclusions was about 0·5–1 μm.

(2) Other inclusions were membranous whorls which enclosed portions of the ground cytoplasm and contained vesicles of smooth endoplasmic reticulum, ribosomes and mitochondria (Figs. 7–9).

(3) Others again were complex bodies composed predominantly of membranous whorls (Figs. 10, 11): these varied immensely in size, the smallest consisting of about four membranous vesicles associated with osmiophilic granular deposits either inside them or in the surrounding matrix. Profiles of cytoplasm in components, particularly mitochondria, were sequestered within such bodies, and also globular and filamentous structures bearing a resemblance to the filamentous dense bodies of untreated controls (Fig. 11).

(4) Other still more complex bodies of varying shape were constituted solely of osmiophilic parallel, straight or concentric membranous whorls (Fig. 12), and attained a gigantic size (5–6 μm in length). These frequently showed bizarre profiles with myelin-like membranes of varying density, profiles of mitochondria, filamentous bodies and highly electron-dense matrix. A limiting membrane was not always distinguishable around these complex inclusions.

DISCUSSION

Dexamethasone inhibits the release of ACTH from the adenohypophysis, thereby decreasing the rate of corticosteroidogenesis (Martini, Motta & Müller, 1964; Rose & Conklin, 1978). In teleosts, corticosteroids inhibit ACTH secretion with ultimate decrease in cortisol secretion (Sage & Purrott, 1969; Fryer & Peter, 1977). In the European eel, comparable doses of cortisol have produced cytological inactivation of pituitary ACTH cells and the AH (Olivereau, 1966; Hanke, Bergerhoff & Chan, 1967). Subcellular alterations in response to dexamethasone indicate repression in steroidogenesis and functional atrophy of the cell.

A striking feature of the eel adrenocortical cell is the extensive development of the smooth endoplasmic reticulum which suggests intense steroidogenic secretory

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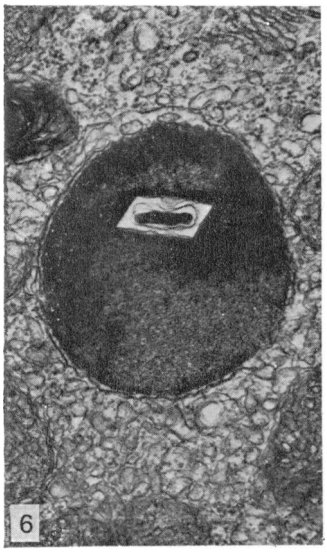
Fig. 3. A survey electron micrograph of AH cells in a dexamethasone-treated eel. Some degree of cellular shrinkage with retraction of surface microvilli is observed. The mitochondria (*M*) appear pale, and gigantic cytoplasmic inclusions (arrow) have appeared in the cytoplasm. *N*, nucleus; *C*, capillary; *V*, vein wall. × 10300.

Fig. 4. A high power view of an AH cell in a dexamethasone-treated animal. The smooth endoplasmic reticulum (*SER*) tubules are disorganized and reduced in quantity. The Golgi complexes (*G*) are atrophied and fragmented. The mitochondria (*M*) have lost matrix density. × 13 760.

Fig. 5. Formation of a series of desmosomes (*D*) between the plasma membranes of adjoining cells in steroid-treated eel. × 40000.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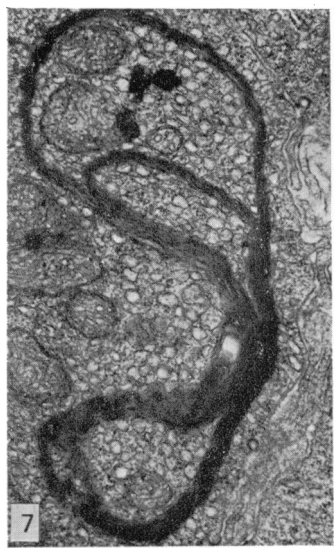
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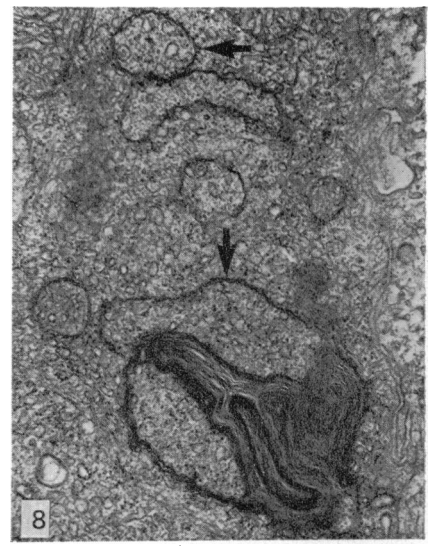
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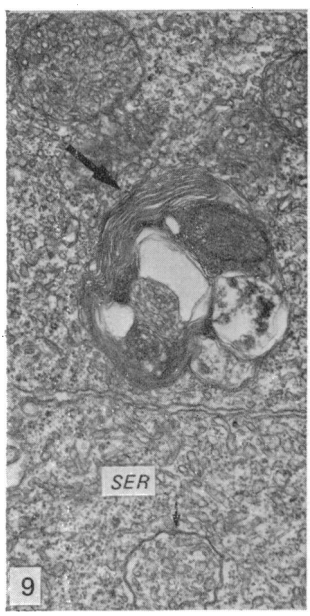

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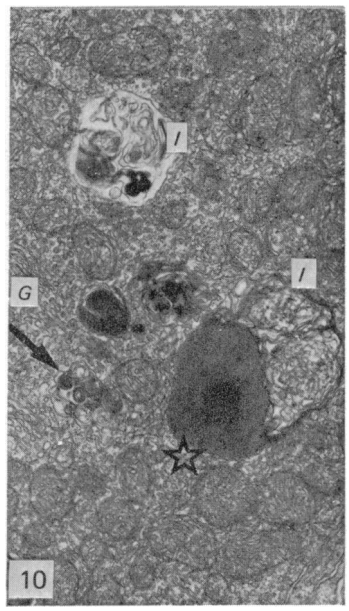

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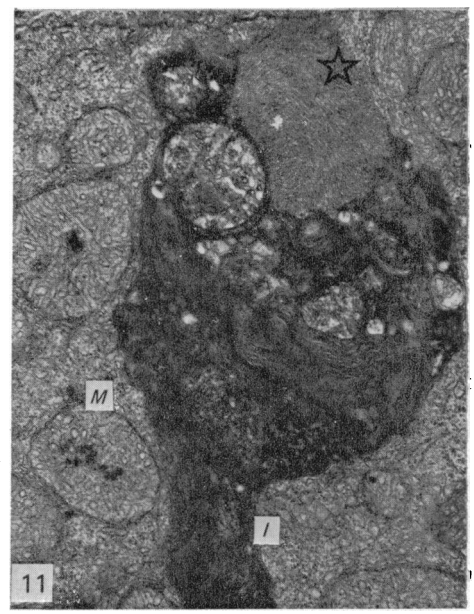

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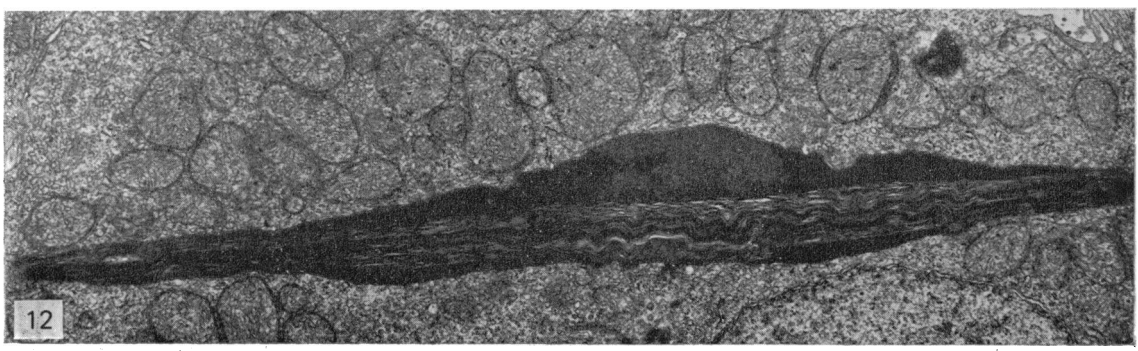
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activity (Pudney & Fawcett, 1977). This is supported by the fact that the microsomal fraction of the teleost adrenal, which is almost all smooth endoplasmic reticulum, has been shown to be the site of synthesis of enzymes involved in steroidogenesis (Sandor, Fazekas & Robinson, 1976). The fragmentation of the smooth endoplasmic reticulum in dexamethasone-treated eels therefore suggests retarded cortisol biosynthesis.

Eel AH mitochondria also contain steroidogenic enzymes, for example C_{20}-22 desmolase which is responsible for transforming cholesterol to pregnenolone, and 11β-hydroxylase which converts 17α-hydroxyprogesterone to cortisol (Sandor, 1969; Sandor *et al.* 1976). The reduction in mitochondrial matrix density in the experimental eels is possibly indicative also of lowered synthesis of steroidogenic enzymes. The electron-opaque matrix granules which appeared in steroid-inhibited animals might represent sites of accumulation of calcium ions in the absence of stimulatory action of ACTH. Such mitochondrial granules in adrenocortical cells have been verified as being due to the deposition of calcium ions (Suyama, Long & Ramachandran, 1977). The regression of the Golgi apparatus, along with morphological inhibition of other steroidogenic organelles, points to its integral role in steroid synthesis or secretion by the eel AH. In hypophysectomized goldfish, however, no clear Golgi-related changes were noted (Ogawa, 1967). The Golgi complex of adrenocortical cells in mammals has been accepted as a focal point of corticotrophic stimulation and inhibition, although its precise function in relation to steroid metabolism is not very clear (Fawcett, Long & Jones, 1969).

The non-accumulation of lipid droplets in regressed AH cells is noteworthy in view of the reported proliferation and accumulation of lipid droplets in adrenocortical cells of higher vertebrates chronically treated with cortisone, dexamethasone or subjected to hypophysectomy, this being interpreted as a phenomenon resulting

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Fig. 6. A membrane-limited inclusion body in a dexamethasone-treated animal. The matrix of the body is granular and also contains electron-opaque lamellae. The rectangular electron-lucent zone gives the impression of a dissolved crystal. ×26000.

Fig. 7. A whorled membranous body that appeared after dexamethasone administration. This inclusion seems to engulf a part of the cytoplasm containing mitochondria and vesicles of smooth endoplasmic reticulum. ×15350.

Fig. 8. Membrane-limited areas of cytoplasm, observed after dexamethasone treatment, which have isolated fragments of smooth endoplasmic reticulum. The boundary membranes belonging to these structures are shown by arrows. The lower inclusion also shows the formation of membrane whorls. ×17000.

Fig. 9. A multimembrane-limited inclusion (large arrow) which is more heterogeneous in organization, with images of trapped mitochondria, electron-lucent zones and vacuoles containing granular deposits. The smooth endoplasmic reticulum (*SER*) is fragmented. An early formation of such a structure (small arrow) is observed in a neighbouring cell. ×17000.

Fig. 10. Abundance of inclusion bodies (*I*) near the Golgi complex (*G*), one of which is an aggregate of membranous vesicles (arrow). A filamentous body (*) seems to have coalesced with a large inclusion body. ×12800.

Fig. 11. An inclusion body (I) that appears as a heterogeneous complex aggregate comprised of whorls and membranes, dense osmiophilic granular deposits, and profiles of sequestered mitochondria. A filamentous body (*) has formed at the upper region of the aggregate. The neighbouring mitochondria (*M*) show osmiophilic matrix granules. ×16200.

Fig. 12. A bizarre shaped cytoplasmic inclusion composed of undulating myelin-like membranes of varying density. Some peripheral regions of this structure are highly osmiophilic and also possess a granular matrix. Note the disappearance of the smooth endoplasmic reticulum and the pale appearance of the mitochondria in the field. ×11250.

from non-utilization of steroid precursors (Zelander, 1959; Sasano, Miyazawa, Shimizu & Koizumi, 1966; Kjaerheim, 1968; Fujita, 1972; Bhattacharyya, Calas & Assenmacher, 1975). Lipid droplets in adrenal cells are reservoirs of cholesterol and its esters, triglycerides and fatty acids (Deane, 1962; Moses, Davis, Rosenthal & Garren, 1969) and an exchange of steroid precursors or substrates is thought to occur between lipid droplets, mitochondria and the smooth endoplasmic reticulum during steroidogenesis (Rhodin, 1971; Frühling & Pecheux, 1976). In normal eel AH cells, too, lipid droplets are occasionally noted and their non-appearance in the inactive organ evidently signifies their minor role in steroidogenesis in the eel. It is to be noted, however, that considerable amounts of unesterified cholesterol have been located in the tubules of the smooth endoplasmic reticulum in AH cells (Bhattacharyya & Butler, 1979).

The appearance of different classes of cytoplasmic inclusions is another significant indicator of steroid-induced atrophy of the AH (cf. Shelton & Jones, 1971). The Type I inclusions in our material are somewhat comparable to the crystal-shaped bodies observed in dexamethasone-treated rats (Rhodin, 1971) which are believed to be cholesterol crystals. The variety and abundance of other categories of inclusions (b and c) conceivably represent various stages of internal remodelling in these structures. It would seem that simple membranous whorls enclose bits of cytoplasm and organelles, and these represent the earliest stages in the formation of these inclusions. Such structures are apparently similar to autophagic vacuoles (Novikoff & Shin, 1978) and may be involved in the sequestration of degraded enzyme proteins. At a later stage these entities become more heterogeneous, with the persistence of undigested membranous components and residues of degenerating cellular organelles. The consistent presence of filamentous bodies inside these complex aggregates suggests a lysosomal function. The opaque gigantic inclusions of more complex nature may be end-products of lysosomal degradation. A comparable mechanism of autophagy and subsequent lysosomal association has been described in metamorphosing insect tissue (Locke & Sykes, 1975).

It is interesting to note that following chronic corticoid treatment, in both mammals or birds, adrenocortical cells show pigment bodies (Zelander, 1959), lysosomes (Nickerson, 1972), and/or lysosomal degradation of lipid droplets (Kjaerheim, 1968), but cellular autophagy has not been clearly illustrated. In the eel, endogenous autophagy appears to be the major mechanism involved in cellular regression in the AH deprived of ACTH stimulation (through negative feedback effect of exogenous steroid). In recent years autophagy has been shown to play a prominent role also in the regression of the corpus luteum in a number of mammals (Quatacker, 1971; Gemmell, Stacy & Thorburn, 1976; Paavola, 1977).

SUMMARY

The ultrastructural modifications of the adrenocortical homologue (AH) in the North American eel (*Anguilla rostrata*) were studied following a 10 day treatment with dexamethasone (20 mg/day). The principal changes were: disorganization of smooth endoplasmic reticulum, regression and fragmentation of the Golgi apparatus, and a lowering of matrix density in the mitochondria. Steroid treatment also induced the appearance of numerous cytoplasmic inclusions: (*a*) lamellated bodies with electron-lucent cores; (*b*) membranous whorls isolating cytoplasmic regions containing smooth endoplasmic reticulum and mitochondria and (*c*) complex aggregates

showing whorls of membranes, residues of cytoplasmic organelles, and dense matrix. The non-accumulation of lipid droplets in repressed AH cells was noteworthy.

These subcellular changes indicate endogenous cellular autophagy in the AH as a result of steroid-induced suppression of ACTH production by the pituitary.

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