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Actin in the Cytoplasm of Adrenocortical Cells (38864)

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Contractile proteins are present in many nonmuscular cells where they may play a role in functions other than unidirectional contraction (1); a correlation has been established between the content of such proteins in a given cell and the morphology of its cytoplasmic filaments (2).

It has been proposed that contractile systems are involved in exocytosis, such as those of insulin (3) or of colloid, linked to the release of thyroxin (4). These processes depend on the intracellular level of cyclic AMP (5) and are influenced by cytochalasin B, an agent which is known to inhibit microfilament function. The ACTH-stimulated acute release of corticosteroids is accompanied by a rise of the intracellular cyclic AMP (5); it is not inconceivable that such release might also depend on a contractile mechanism (6). However, the presence of contractile proteins in adrenocortical cells has not yet been established. In order to clarify this possibility, two techniques were used: (1) immunofluorescent staining of actin using anti-actin autoantibodies (AAA) present in the serum of certain patients with chronic aggressive hepatitis (7); and (2) identification by means of electron microscopy of microfilaments 40–80 Å in diameter (corresponding to the morphologic aspect of actin filaments) (2).

Materials and Methods. Ten male Wistar rats of 100–120 g body wt and five male hamsters, 80–140 g body wt, were used. Cryostat sections from the adrenal glands of five rats and three hamsters were treated with AAA-containing sera from two different patients, followed by the fluorescein-conjugated IgG fraction of sheep anti-human IgG antiserum (7). The titers of the AAA sera, as judged by immunofluorescent staining of rat intestinal smooth muscle, were 1/1280 and 1/640. As a control, serum from normal human donors was used instead of AAA-containing serum. Incubation of AAA-containing sera with purified actin from human blood platelets (thrombosthenin-A) was performed as previously described (7). Small cubes from one of the adrenal glands of five rats and two hamsters were fixed by immersion during 5 hr in 3% glutaraldehyde in cacodylate buffer at pH 7.2–7.4. The blocks were left in buffer at 4° overnight, postfixed in 2% OsO_4 in collidin buffer at pH 7.3–7.4 dehydrated in alcohol and embedded in Epon 812. Sections about 1 μm thick were cut with glass knives on a Reichert ultramicrotome. Thin sections from selected areas of the zona glomerulosa, fasciculata, and reticularis were cut with a diamond knife, mounted on noncoated grids, stained with uranyl acetate and lead citrate, and examined with a Philips 300 electron microscope.

Results. After immunofluorescent staining, AAA binding was noted in the cytoplasm of most adrenocortical cells (Fig. 1b, c). The intensity of the staining was slightly higher in the zona glomerulosa in comparison with the zona fasciculata and reticularis. In single cells the staining was mostly limited to the periphery of the cytoplasm, having a pattern of distribution similar to that reported in hepatocytes with the same antibody (8). The intensity of the staining was higher in adrenals than in liver as controlled in several parallel experiments. Adrenals treated with normal serum or with AAA serum previously incubated with thrombosthenin-A did not show fluorescent staining (Fig. 1, a, d).

On electron microscopic examination, the ultrastructure of the adrenocortical cells was as previously described (9). In addition, most of the cells in the glomerulosa, fasciculata, and reticularis had a peripheral network composed of 40–80 Å-thick microfilaments, in which some denser areas were irregularly scattered (Figs. 2, 3). The network usually occupied a thin layer just beneath the cell

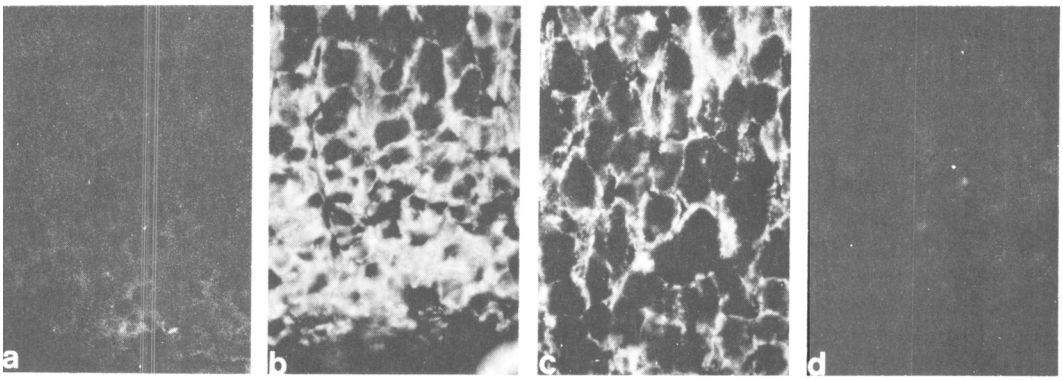

FIG. 1. Specific immunofluorescent staining of adrenocortical cells by AAA. (a) Cryostat section of the rat adrenal (zona glomerulosa) incubated first with normal human serum followed by fluorescent goat anti-human IgG: no staining (×400). (b) An adjacent cryostat section (zona glomerulosa) incubated first with AAA followed by fluorescent goat anti-human IgG: bright fluorescence is seen in the vascular wall of an arteriole and in the peripheral part of the cytoplasm of adrenocortical cells (×400). (c) A cryostat section through the zona fasciculata treated as in (b): a bright fluorescence is seen at the periphery of the cells (×400). (d) Cryostat section through the zona glomerulosa incubated first with AAA absorbed with thrombosthenin-A, followed by goat anti-human IgG: no staining (×400).

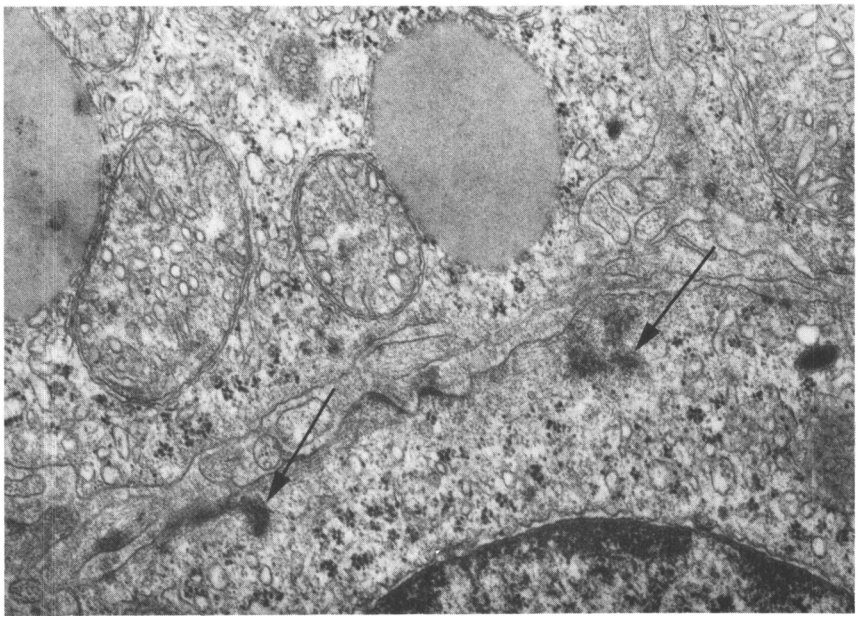

FIG. 2. Low-magnification electron micrograph showing the cytoplasm of an adrenocortical cell of the rat's fasciculata. Note a thin filamentous network at the periphery of the cytoplasm of one cell. Electron-dense zones (arrows) are scattered within the network (×19,000).

membrane but occasionally it penetrated more deeply in the cytoplasm and was in contact with liposomes and mitochondria. To our knowledge such a network has not yet been described in adrenocortical cells.

Discussion. The correlation of immunofluorescent and electron microscopic findings indicates that the peripheral microfilamentous network in adrenocortical cells consists, at least in part, of actin. The presence of such an apparatus supports the assumption that contractile proteins are, directly or indirectly,

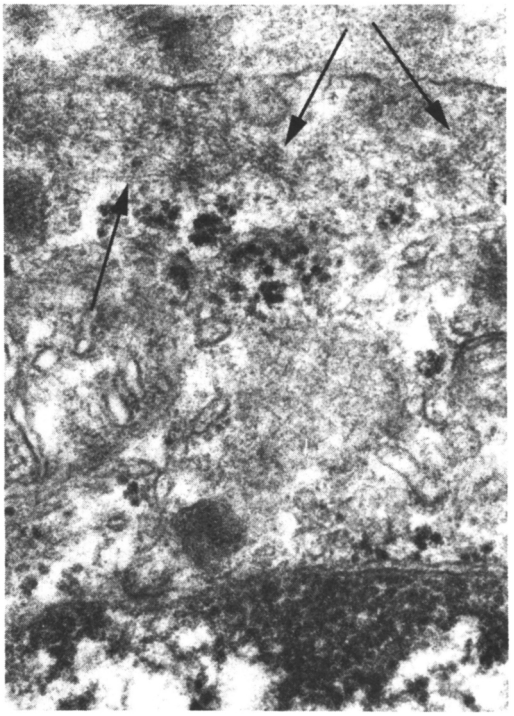

FIG. 3. Higher magnification of the peripheral network in another adrenocortical cell shows that this is composed of 40–80 Å filaments (arrows) (×80,000).

involved in the excretion of adrenocortical hormones.

It has been known for a few years that the serum of patients with chronic aggressive hepatitis contains antibodies which selectively bind to smooth muscle cells (8). The recent observation (7) that incubation of several of these sera with thrombosthenin-A abolishes the ability of such sera to bind to smooth muscle, platelets, and the I-bands of striated muscle, strongly suggests that these antibodies are directed against the actin moiety of actomyosin. Although a full comparison of the amino acid sequences of the actins from platelets and from striated muscle can not yet be made, it is nevertheless well established that thrombosthenin-A and actin from skeletal muscle are very similar (10). Hence, it can be concluded that the serum of patients with chronic aggressive hepatitis contains crossreacting anti-actin autoantibodies.

The combination of immunofluorescence and electron microscopy has been useful in demonstrating the presence of a contractile apparatus in several nonmuscular cells such as fibroblasts of granulation tissue (myofibroblasts) (11), interstitial cells of the lungs (12), regenerating epidermal cells (13), normal (7) or regenerating hepatocytes (13), and β cells of Langerhans islets (14). The presence of contractile proteins in the cytoplasmic periphery of adrenocortical cells could be conceivably correlated with their secretory activity suggesting a common mechanism for exocytosis of secretory products in different glands such as adrenal cortex, Langerhans islets, and liver.

Summary. Rat or hamster adrenocortical cells contain a network of filaments mostly 40–80 Å in diameter, which is particularly evident at the cell periphery. In frozen sections, adrenocortical cells fix (particularly at their periphery) anti-actin autoantibodies present in sera of patients with chronic aggressive hepatitis. It is concluded that the peripheral network of adrenocortical cells contains contractile proteins (at least actin) and the implication of such proteins in steroid secretion is discussed.

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