A NEW CRYSTAL-CONTAINING CELL IN HUMAN ADRENAL CORTEX

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ABSTRACT

Electron microscope examination of the adrenal cortex from three male human subjects revealed a special type of cell occurring in periendothelial spaces, in all adrenal cortex zones. It is a clear, spindle-shaped cell the principal cytoplasmic features of which are crystalline inclusions with a structure similar to that of the Reinke crystals of human testicular interstitial cells and an abundance of microfilaments. Enzymatic digestions with pronase, pepsin, and ribonuclease were performed, and no digestion of the crystals was obtained. The crystals had no peroxidase or acid phosphatase activities. This cell appears to be exclusive to human males and it may be related to adrenal androgen secretion.

INTRODUCTION

Ultrastructural studies of normal human adrenal cortex have been relatively few because of the difficulty in obtaining properly fixed material. The tissue specimens examined so far have been removed, almost exclusively, from cancer patients during surgical operations (1, 3, 9, 13, 24).

Lately, we have had the opportunity of studying the adrenal cortex from three male patients who underwent unilateral adrenalectomy as medullary therapy of Buerger's disease. Adrenal cortex glands looked entirely normal by light and electron microscope standards, which was to be expected since no adrenal cortex changes have been reported in that condition (14). We have, however, found a special type of perivascular spindle-shaped cell in all zones of the cortex. These not yet described cells contained cytoplasmic crystalline inclusions, similar to the Reinke crystals in human testicular interstitial cells (4, 19, 26).

It is the purpose of the present report to describe the fine morphology of these cells and to discuss their possible functional significance.

MATERIALS AND METHODS

The human adrenal tissue used in this study was obtained from three male patients aged 23, 31, and 48, respectively, whose left adrenal glands were removed for the treatment of Buerger's disease. The patients received thiopentone (Pentothal), succinylcholine (scoline), diallyl-nor-toxiferine (Alloferine), and pethidine as anaesthetic drugs. After removal, the adrenals were quickly cut into small pieces and immersed in the fixative. The samples were fixed, for light microscopy, in 10% neutral Formalin or Bouin's solution, embedded in paraffin, and stained with hematoxylin-eosin. Specimens were fixed, for electron microscopy, in 5% glutaraldehyde (Fisher Scientific Co., Fair Lawn, N. J.) in 0.1 M sodium cacodylate buffer, at pH 7.2, for 2 hr at 4°C, and then were post-fixed with Veronal-acetate-buffered 1% osmium tetroxide, pH 7.3, at 4°C, for 2 hr. After dehydration in a graded series of ethanols, the specimens were embedded in Epon 812 (11). Ultrathin sections were stained with lead citrate (25) for 30 min, or doubly stained with uranyl acetate (saturated aqueous solution) and lead citrate (25). Examination was carried

out in a Siemens Elmiskop IA electron microscope. Semithin sections, 1–2 μ thick, were cut from the same blocks and stained with methylene blue-azur II (23).

Enzymatic Digestions

Ultrathin sections on uncoated copper grids were treated with H_2O_2 for 15 min, washed in distilled water, and immersed in: 0.5% pronase (TAAB Laboratories, Emmer Green, Reading, England) in distilled water, pH 7.4, for 30 and 90 min at 40°C; 0.5% pepsin (Worthington Biochemical Corp., Freehold, N.J.) in 0.1 M HCl, for 60 and 240 min at 38°C; or 0.5% ribonuclease (Koch-Light Laboratories Ltd., Colnbrook, England) in distilled water, pH 6.8, for 60, 120 min, and 5 hr at 38°C (18). Washing in distilled water was followed by staining of sections with uranyl acetate and lead citrate.

Cytochemical Methods

PEROXIDATIC ACTIVITY OF CATALASE: Some adrenal specimens were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, and cut with a Sorvall TC_2 Smith and Farquhar tissue sectioner (Ivan Sorvall, Inc., Norwalk, Conn.) at 30 μ; the sections were incubated for 90 min at 37°C in Novikoff and Goldfischer medium (21) prepared according to Beard and Novikoff (2). The specimens were then postfixed in 1% osmium tetroxide and processed for electron microscopy as above. For controls, some of the sections were incubated in medium without H_2O_2 or DAB (3, 3′-diaminobenzidine), or in complete medium to which 0.02 M aminotriazole (Aldrich Chemical Co., Inc., Milwaukee, Wis.) was added.

ACID PHOSPHATASE: Some specimens fixed in glutaraldehyde as for fine morphology were cut with the tissue sectioner at 100 μ, and the sections were incubated for 45 min at 37°C in Gomori medium for acid phosphatase, prepared according to Miller and Palade (16). The samples were postfixed in 1% OsO_4 and embedded in Epon 812. For controls, specimens were incubated in media without substrate.

RESULTS

Morphology

By light microscopy, the adrenal cortex looked entirely normal and no pathological changes could be detected in any of the three subjects studied. In the electron microscope, the appearance of the tissue was found to be similar to that described in normal human adrenals by other authors (3, 9, 12, 13, 24). However, an elongated, fusiform cell with light cytoplasm occurred in the perivascular space (Figs. 1 and 4), in all zones of the cortex. This cell could not be discerned in paraffin or semithin Epon sections. The cell surface was regular, with few indentations and practically no microvilli, and it was not surrounded by a basal lamina. The nucleus was centrally located, usually ovoid, and occasionally folded. It contained one nucleolus and fine granular dispersed chromatin usually condensed at the nuclear periphery (Fig. 1); a sharply defined dark band of uniform thickness, similar to the fibrous lamina described in other cells, was noted edging the nuclear membrane.

The cytoplasm was light and usually contained one or several crystalline bodies in each sectioned cell. The location of the crystals within the cell was variable, as was their shape which appeared rectangular (Fig. 3), irregularly hexagonal (Fig. 4), or rhomboidal. Highly regular patterns were seen at high magnification. A pattern of dense parallel lines, ~66 A thick, separated by light spaces, ~76 A thick, was usually apparent when the sectioned crystal was rectangular in contour (Fig. 3). In other planes of section the crystal displayed a honeycomb appearance (Fig. 6) similar to that described in the Reinke crystals (19). Very occasionally, the plane of section disclosed successive sets of three parallel lines, two of them dense, straight, and thick, and the third one a row of dense dots, 138 A in diameter, separated from one another by a 120 A space (Fig. 5). Furthermore, "dislocations" or potential planes of cleavage were at times observed in the crystals (Fig. 4). The appearance of the crystal and its lattice was the same when lead citrate, or double uranyl acetate-lead citrate, stainings were used.

Another feature of these cells was a large quantity of filamentous components which varied in amount from cell to cell (Figs. 2, 3, and 6). These microfilaments were straight, of indefinite length, and measured 60–80 A in diameter; very frequently, they occurred in arrays round the nucleus (Fig. 2), or appeared aggregated in conspicuous bundles near the cell membrane (Fig. 3). Microfilaments showed a tendency to be intimately associated with the crystals (Figs. 2 and 6). The cell had a number of elongated, rough endoplasmic reticulum profiles, which were often dilated and exhibited moderately dense content (Figs. 1–3). Free polyribosomes were found singly or clustered irregularly throughout the cytoplasm (Fig. 3). No elements of smooth endoplasmic reticulum were observed. A well-developed Golgi complex

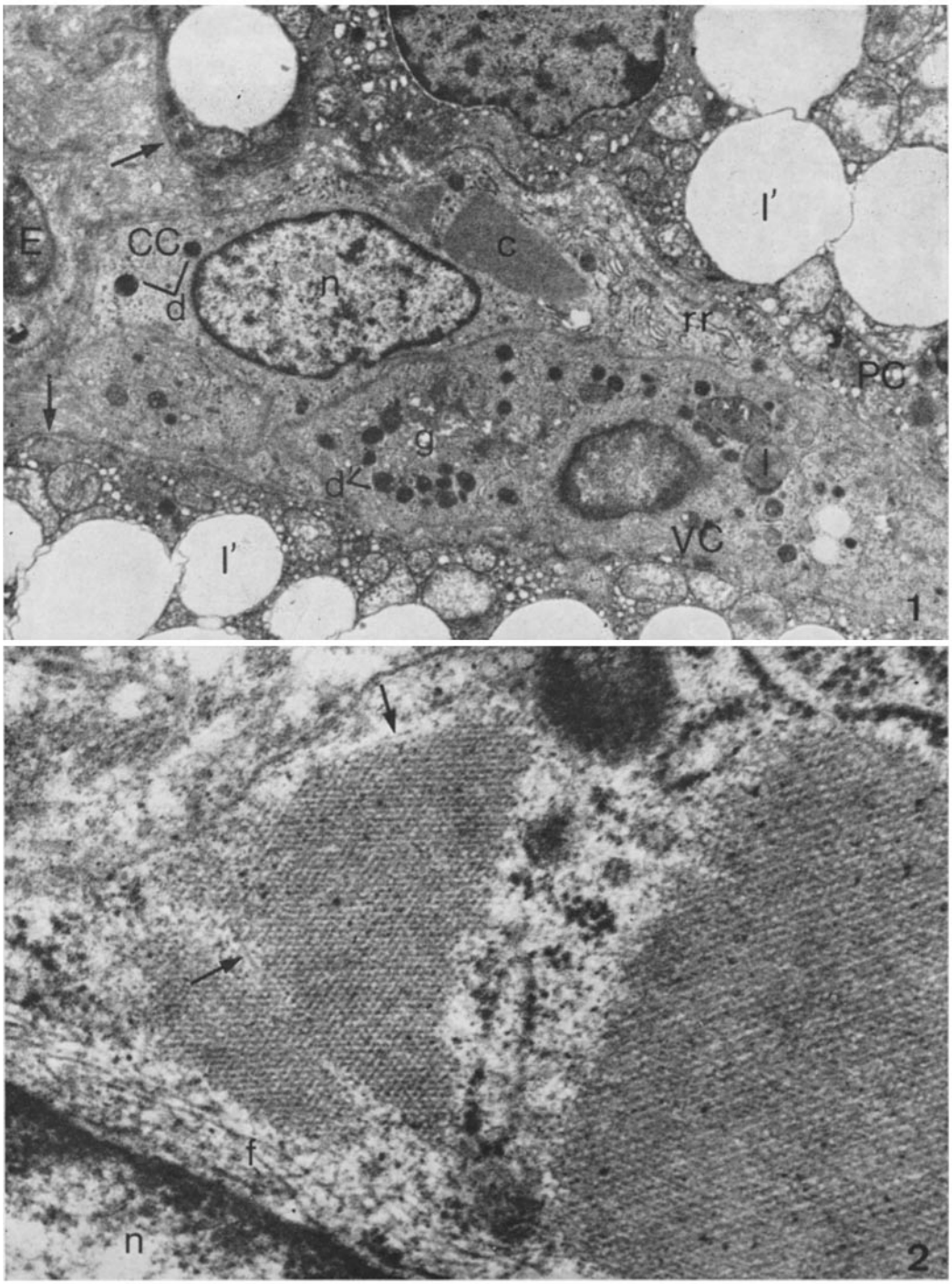

FIGURE 1 Zona glomerulosa of the human adrenal cortex. The low power electron micrograph shows a crystal-containing cell (*CC*) and another perivascular cell (*VC*), as well as two parenchymal cells (*PC*). The *CC* has an ovoid nucleus (*n*) and light cytoplasm containing two crystalline bodies (*c*) cut at different planes, a number of elongated profiles of rough endoplasmic reticulum (*rr*), and a few dense bodies (*d*). In the *VC* the cytoplasm is darker and there is an abundance of lipid droplets (*l*) and dense bodies (*d'*). A well-developed Golgi complex (*g*) is visible. In *PC*, there are numerous lipid droplets (*l'*). *E*, endothelial cell; arrows, basement membrane. Lead citrate. × 8800.

FIGURE 2 Higher magnification of the crystal-containing cell of Fig. 1 showing the regular pattern of the crystals and the microfilaments (*f*) between them and the nucleus (*n*). Microfilaments are intimately associated with the crystal (arrows). Lead citrate. × 60,000.

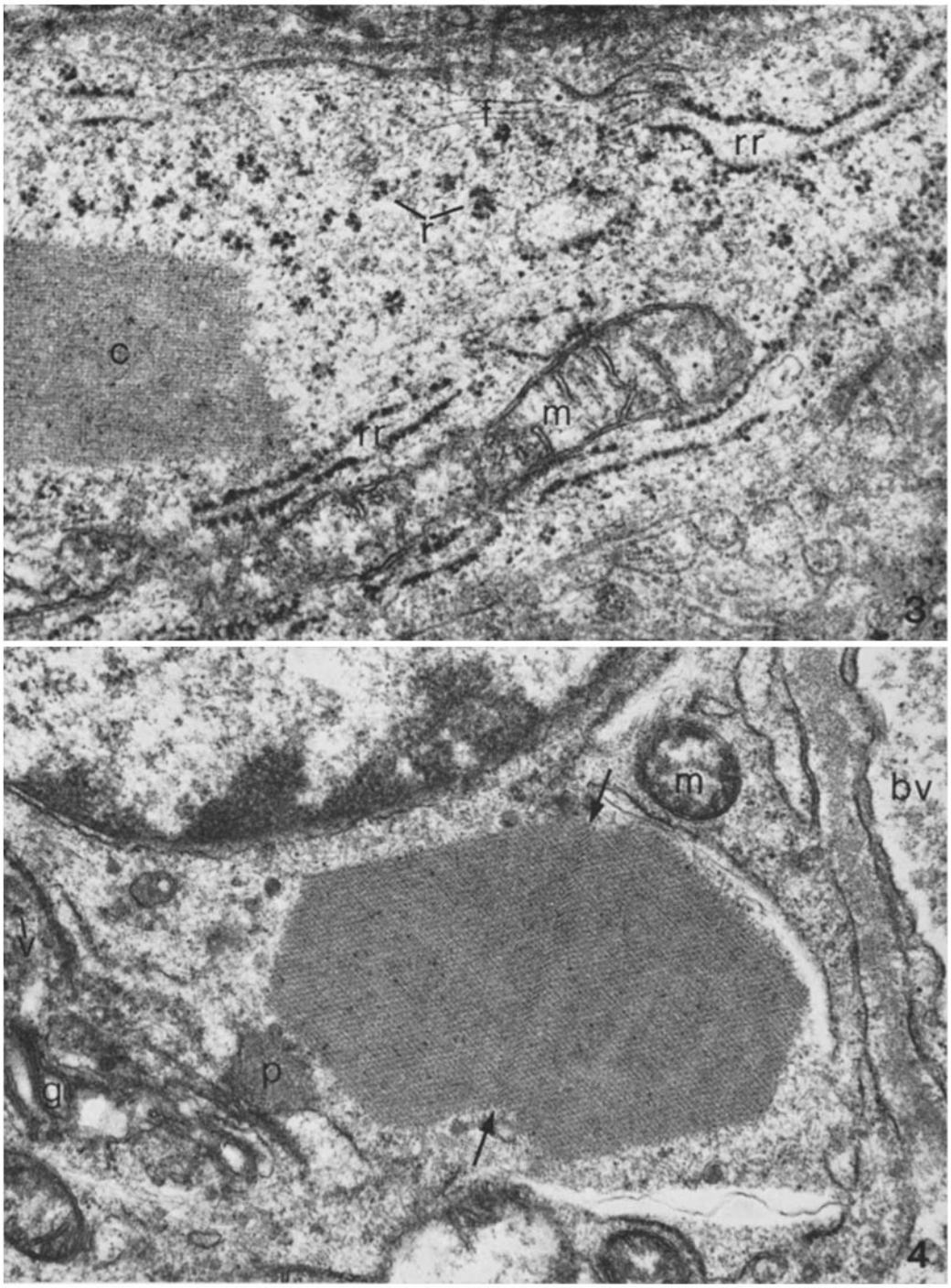

FIGURE 3 A section of a cell with a crystalline inclusion of rectangular contour (*c*). Some profiles of rough endoplasmic reticulum (*rr*) are seen. Conspicuous bundles of filaments (*f*) are observed near the cell membrane. Free or clustered ribosomes (*r*) are scattered in the cytoplasm. Elongated mitochondria (*m*) with laminar cristae are seen. Lead citrate. × 40,000.

FIGURE 4 Detail of a crystal-containing cell from the zona reticularis, after incubation in medium containing DAB. The cell is situated near a blood vessel (*bv*). The crystal is of polygonal, irregularly hexagonal shape, with sharp edges, and no reaction product is observed in the matrix. There is dislocation of the crystal (arrows). Near the Golgi zone (*g*) are some coated vesicles (*v*) and one very dense patch (*p*). *m*, mitochondria. Lead citrate. × 28,000.

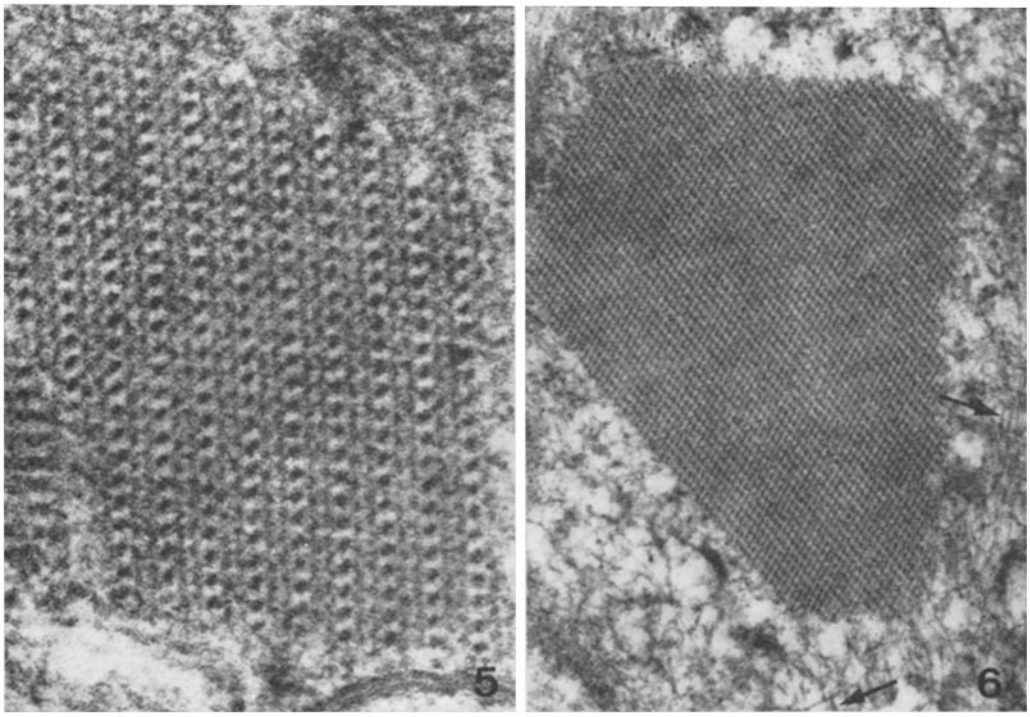

FIGURE 5 A section of the crystal showing sets of three parallel lines, two of them straight and the third one a row of dense dots. Uranyl acetate and lead citrate. × 114,000.

FIGURE 6 Additional detail of a crystal-containing cell of the zona fasciculata of the human adrenal cortex, incubated in medium for acid phosphatase. In this micrograph the crystal displays a honeycomb structure, and no reaction product occurs in the matrix. Note the abundance of filamentous components (arrows) which intimately surround the crystal. Lead citrate. × 50,000.

with coated vesicles was usually situated near the nucleus; small patches of high density with a filamentous structure, not limited by a membrane, appeared in the vicinity of the Golgi complex (Fig. 4). Some round or elongated mitochondria, which did not have the inner membrane structure usual in adrenal glandular cells, were present in the cell (Figs. 3 and 4). As a rule, lipid droplets and dense bodies were scarce, some dense bodies appearing as heterogeneous osmiophilic bodies (Figs. 1 and 2).

In the youngest of the three subjects studied, some cells were observed that were similar to the crystal-containing ones but contained no crystals. These cells had clusters of twisted filaments in the cytoplasm. Other spindle-shaped cells also occurred in the perivascular spaces in the three subjects. These cells, however, had a darker cytoplasm, were rich in lipid droplets and dense bodies, and were devoid of crystalline inclusions (Fig. 1).

Enzymatic Digestions

PRONASE: After treatment of the specimens for 30 and 90 min, the crystalline bodies remained intact whereas the collagen fibrils mostly disappeared from the sections.

PEPSIN: The material was very damaged by the treatment. However, it was possible to see that the crystals were unaffected; no digestion of cytoplasmic filaments was noted.

RIBONUCLEASE: The crystals were unchanged, irrespective of the treatment and the time used for digestion.

Cytochemistry

With regard to the peroxidatic activity of catalase (Fig. 4) and acid phosphatase activity (Fig. 6), no reaction product was observed in the crystalline bodies of these cells. Dense bodies were acid phosphatase positive. Controls were unreactive.

DISCUSSION

The ultrastructure of the normal human adrenal cortex has been described by several authors (1, 3, 9, 12, 13, 24). Glandular cells exhibited a widespread agranular endoplasmic reticulum, numerous mitochondria with tubulovesicular cristae dispersed among the reticulum, a prominent Golgi complex, and numerous lipid droplets. Scarce mention has been made of connective tissue cells in human glands in those descriptions, with the exception of some perivascular fibroblast-like cells rich in rough endoplasmic reticulum which were reported by Luse in the subendothelial spaces of the human fetus and adult subjects (12). In the latter, these cells were described as having numerous lipid droplets and showing signs of endocytic activity similar to those displayed by analogous cells in the adult mouse (12).

The crystal-containing cells which are here described have not been reported before to the best of our knowledge. From their perivascular location, fusiform shape, and richness in rough endoplasmic reticulum, these cells appear to be perivascular fibroblastic elements. They are, however, quite different from the lipid-containing perivascular cells of Luse which were also observed in our material (Fig. 1) but which are much darker and have no crystalline inclusions or microfilaments in the cytoplasm. On the other hand, our cells did not exhibit any features suggestive of endocytic functions, such as pseudopodia, pinocytic vesicles, or abundance of lysosomes, nor did they contain pigment bodies as described in the perivascular cells of the mouse by Zelander (27).

The crystalline bodies and the microfilaments are very similar to those present in testicular interstitial cells (4, 19, 26). Indeed, the shape, contour, and edges of the crystal, as well as the occasional honeycomb appearance of the internal structure, strongly resemble the patterns described by Fawcett and Burgos (4), Yamada (26), and Nagano and Ohtsuki (19) in the Reinke crystal in the human testis. Leydig's cells are, however, much larger than the crystal-containing cells of the adrenal cortex and have a well-developed agranular reticulum, which is absent from adrenal cells. Curiously, in the testis of human males there are also certain fusiform cells which contain scarce smooth vesicles, a few lipid droplets, and fine filamentous components, but no crystals. These cells are believed to be precursors of mature interstitial cells (4). It is possible that the cells with twisted filamentous structures seen in the youngest patient may similarly be precursors of the clear crystal-containing cells.

In order to tentatively elucidate the nature of the crystalline bodies, enzymatic studies were carried out and it was seen that the inclusion was not digested by pronase, pepsin, or ribonuclease. Although the crystalline structure of the inclusions strongly suggests a protein nature these results do not permit such confirmation or point out a RNA viral nature. However, it must be remarked that negative enzymatic digestions on Epon sections are not conclusive since incomplete enzyme penetration of the embedding medium is always possible with this procedure (18).

Although the crystal lattice was similar to that of catalase (10), the catalase DAB reaction was negative suggesting that the crystalline inclusion had a different nature. In fact, catalase crystals are known to yield a positive DAB reaction (6). A lysosomal nature, as suggested for certain bodies containing crystalloid inclusions (15), cannot be taken into account since these inclusions neither had a peripheral membrane nor showed acid phosphatase activity. It seems that only further isolation of the crystals and subsequent study with chemical and X-ray diffraction techniques may elucidate their composition.

The fact that we found these cells in normal glands from human males whereas previous studies of normal human adrenal ultrastructure were carried out almost exclusively on glands from female subjects is noteworthy. The adrenal cortex is well known to secrete androgens, namely testosterone, as well as their precursors, dehydroepiandrosterone and androsterone (5). On the other hand, in female rats treated with androgens, parenchymal adrenal cells have been seen to develop filamentous structures (20), while arrhenoblastoma tumor cells (a masculinizing tumor of the ovary) have features evoking the interstitial cells of the testis including a Reinke-like crystal (7).

It is therefore plausible that this cell might be exclusive to human males and concerned with androgen secretion. In some ultrastructural descriptions of hyperfunctional adrenals recently published (8, 13, 17, 22), these cells were not mentioned although part of the material was obtained from males. It is difficult to know whether these cells were indeed absent in such pathological

conditions or whether they were overlooked in those descriptions.

Finally, we must stress that this cell does not seem to have any relation to Buerger's disease. No adrenal cortex changes have been seen to occur in this disease (14); this was also the case in our material. However, confirmation of the presence of these cells is required in patients not suffering from Buerger's disease.

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