

REINVESTIGATION OF THE FINE STRUCTURE OF REINKE'S CRYSTAL IN THE HUMAN TESTICULAR INTERSTITIAL CELL

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ABSTRACT

Testicular biopsy materials of men were examined with the electron microscope equipped with a tilting stage. An optical transformation method was applied for the electron microscope images. An attempt was made to clarify relationships between the contour and the internal pattern of Reinke's crystal in the interstitial cell. The shape of this crystal is a hexagonal prism. Three types of internal patterns are observed in relation to the main planes of the crystal. The crystal consists of 50-Å-thick filaments. It is trigonal, $a = 300 \text{ \AA}$, $c = 450 \text{ \AA}$, being similar to catalase crystals studied by X-ray diffraction and electron microscopy (20), but the dimensions are different. Dislocations of Reinke's crystal are also analyzed. In some interstitial cells without Reinke's crystal, specially arranged 50-Å-thick filaments are observed. They are similar in arrangement to the pattern within Reinke's crystal, but not so closely compacted. Morphological similarities and dissimilarities between this crystal and other crystal and crystalloid structures are discussed.

INTRODUCTION

Many decades ago, crystalloid inclusions in the testicular interstitial cells (Leydig cells) of men were studied by Reinke (23), and since then they have been called Reinke's crystals. He reported that the crystals were found in the interstitial cells of all actively spermatogenic testes of men from 15 to 65 years of age. The crystals were stained with Weigert's stain for fibrin, and also with carmine. He also reported the chemical nature of the crystal in section and after partial isolation, and concluded that the crystal is protein in nature and shows no constant birefringence. In 1912, Winiwarter found smaller inclusions of another kind in human Leydig cells and called them rice-form bodies (27). Since 1956, Reinke's crystal has been investigated by electron microscopy (10-13, 26, 28-30). Fawcett and Burgos published the first

electron micrographs of the crystals and noted that they were composed of globular macromolecules, 150 Å in diameter, uniformly spaced, about 190 Å apart along two axes at right angles to each other (12, 13). Later, Yamada reported a regular hexagonal internal structure which consists of 200-Å tubular hexagons, and he also described other filamentous structures that may relate to the formation of the crystal (28, 29). Sisson and Fahrenbach have reported that Reinke's crystal is composed of 50-Å-thick filaments (26).

This paper deals with further morphological observations on the fine structure of Reinke's crystal and other related structures made by using recently developed techniques for extending the analytical capabilities of the electron microscope. The relationships between the crystal structure

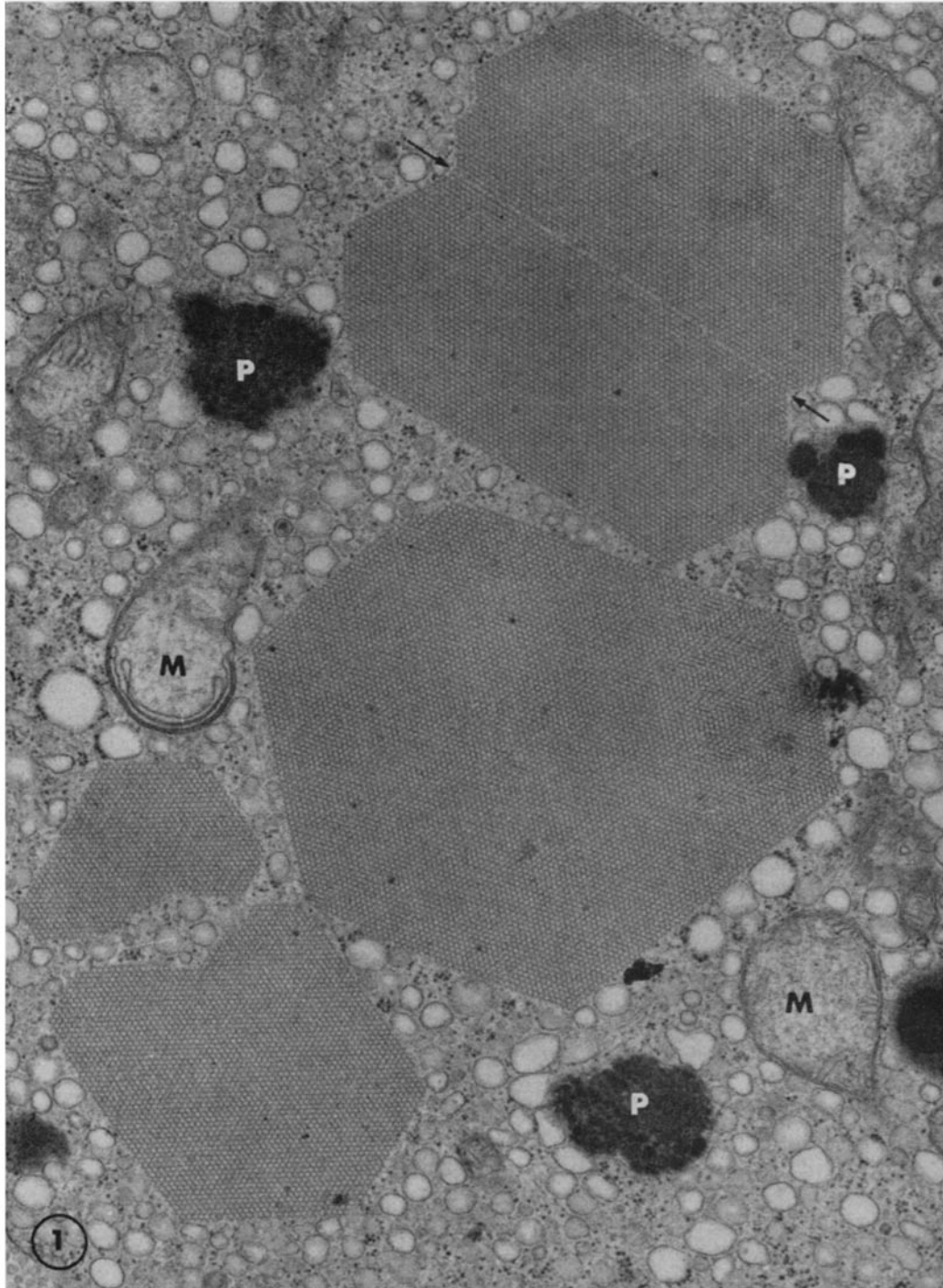


FIGURE 1 A survey electron micrograph showing several crystals of Reinke. They are polygonal in shape, and the edges are sharp. Note that each side is parallel to the corresponding opposite side. The arrows indicate dislocation of the crystal. *M*, mitochondria; *P*, pigment granules. $\times 22,000$.

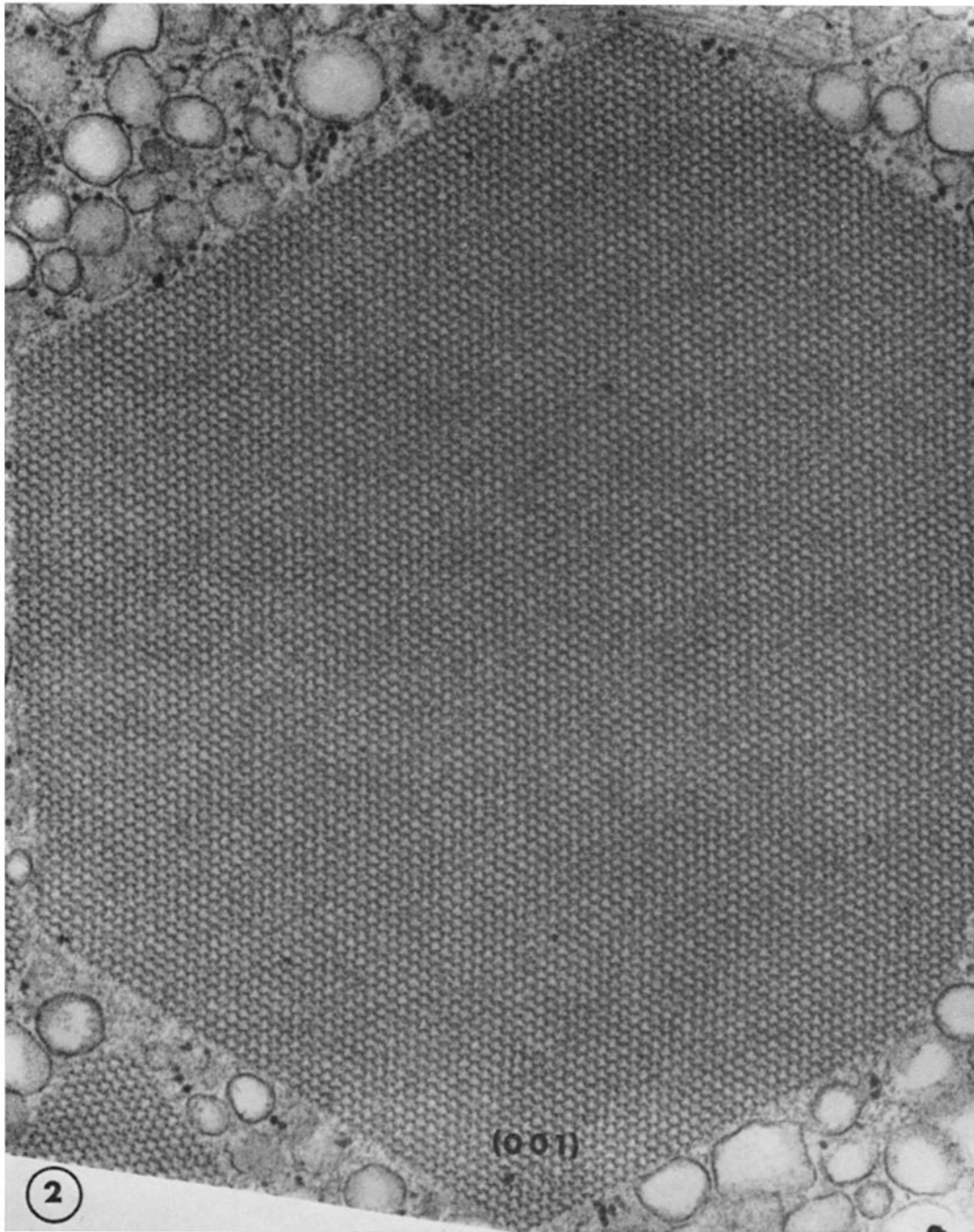


FIGURE 2 A section of the crystal with hexagonal contour. The section is parallel to the (001) plane. A honeycomb structure is seen. $\times 50,000$.

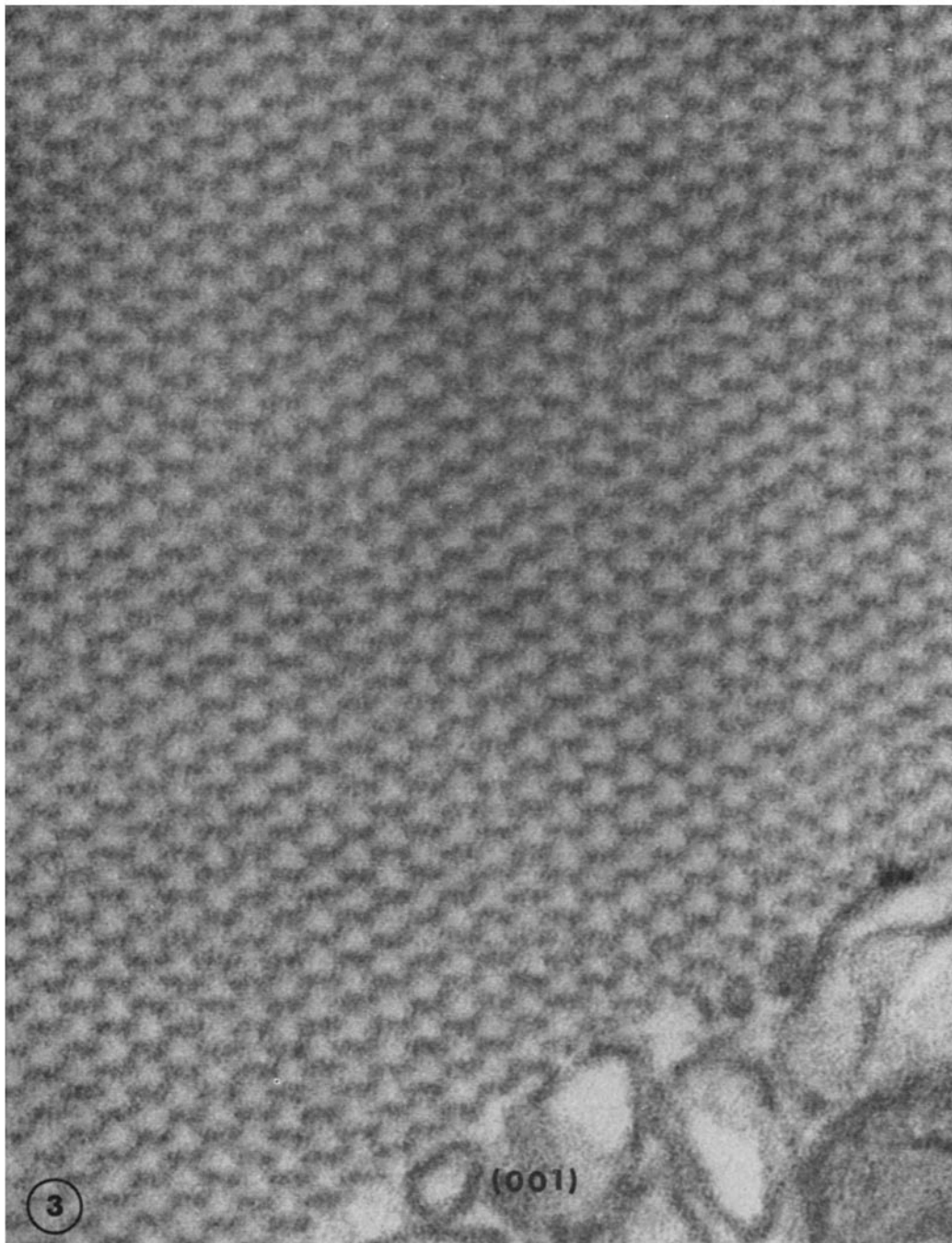


FIGURE 3 Higher magnification view of the crystal sectioned parallel to the (001) plane. The hexagonal pattern consists of 50-Å-thick filaments. The hexagons are 200 Å on each side and are open to the cytoplasm at the edge of the crystal. The filaments show a beaded appearance indicating subunit structure. The diagonal of the hexagons is perpendicular to the side of the crystal. $\times 180,000$.

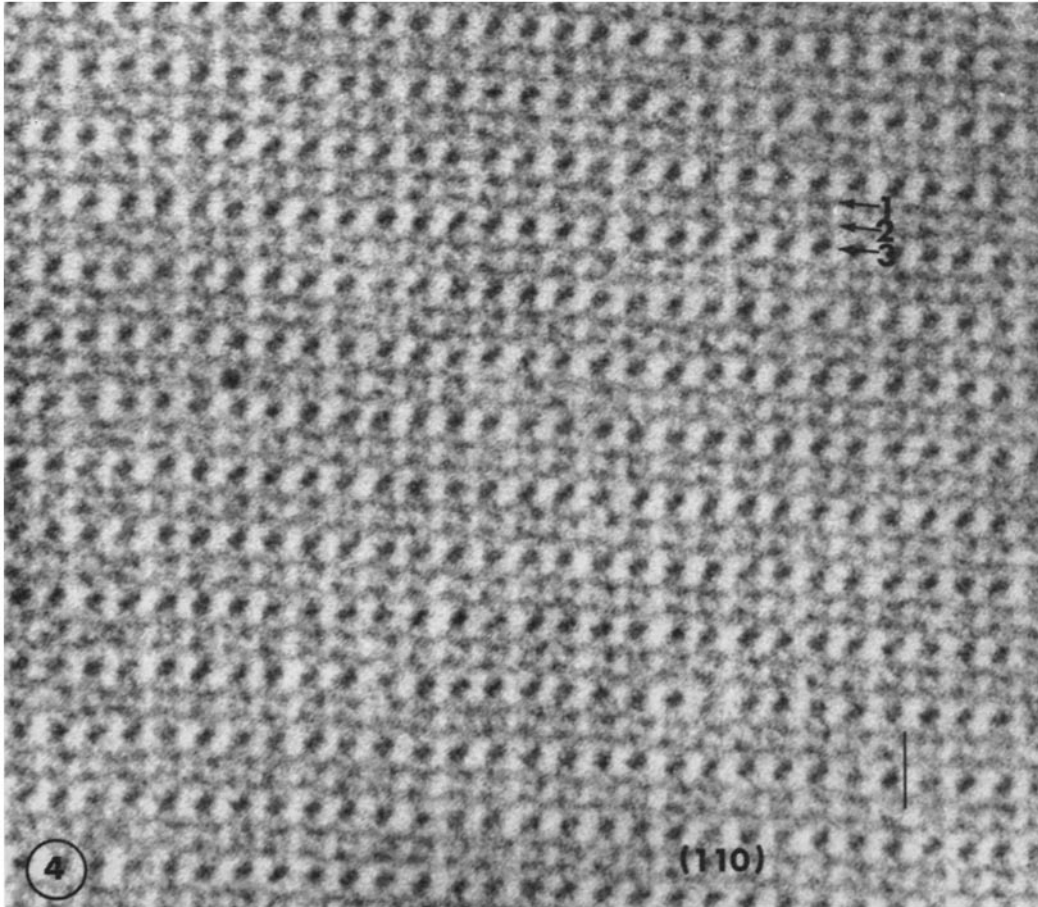


FIGURE 4 Higher magnification showing a section of the crystal parallel to the (110) plane. A set of three parallel lines is observed perpendicular to the long axis (bar). The first (1) and second (2) lines are straight, and the third (3) is seen as a row of dense dots. The lines are arranged at intervals of 150 Å and appear to be connected with each other by thin filaments along the long axis. $\times 180,000$.

and the various internal patterns observed in sectioned crystals are explained.

MATERIALS AND METHODS

Testicular tissue blocks were obtained by biopsy from the Department of Urology, Chiba University Hospital. Most specimens reported here were immersed in 3% glutaraldehyde followed by 2% osmium tetroxide at pH 7.2 with phosphate buffer. Other specimens were fixed with 2% osmium tetroxide in various kinds of buffer solutions. They were dehydrated with ethanol and embedded in Epon 812. Sections were cut with a diamond knife on an MT-1 microtome and stained with both uranyl acetate and lead citrate. They were examined with an HU-11A electron microscope equipped with a tilting goniometer stage of HK-2AM type.

For optical diffraction and filtering methods (14, 17, 18), a helium-neon gas laser (NEC GLG2009) was used as a source. The subject was made of transparent film reversed from the original electron microscopic negative. The subject was placed between the collimating lens and a lens ($f = 120$ cm) which focused the diffracted rays produced by the subject on the diffraction plane, where the diffraction pattern was recorded on a sheet film. After the photographic process, certain diffraction spots on the film were removed so as to make small holes and the film was stained with ink for the opaque mask. Then the mask placed again in the previous position on the diffraction plane allowed the diffracted rays from the subject to pass through. The recombining lens ($f = 50$ cm) for optical filtering produced the image, which was about half the length of the subject.

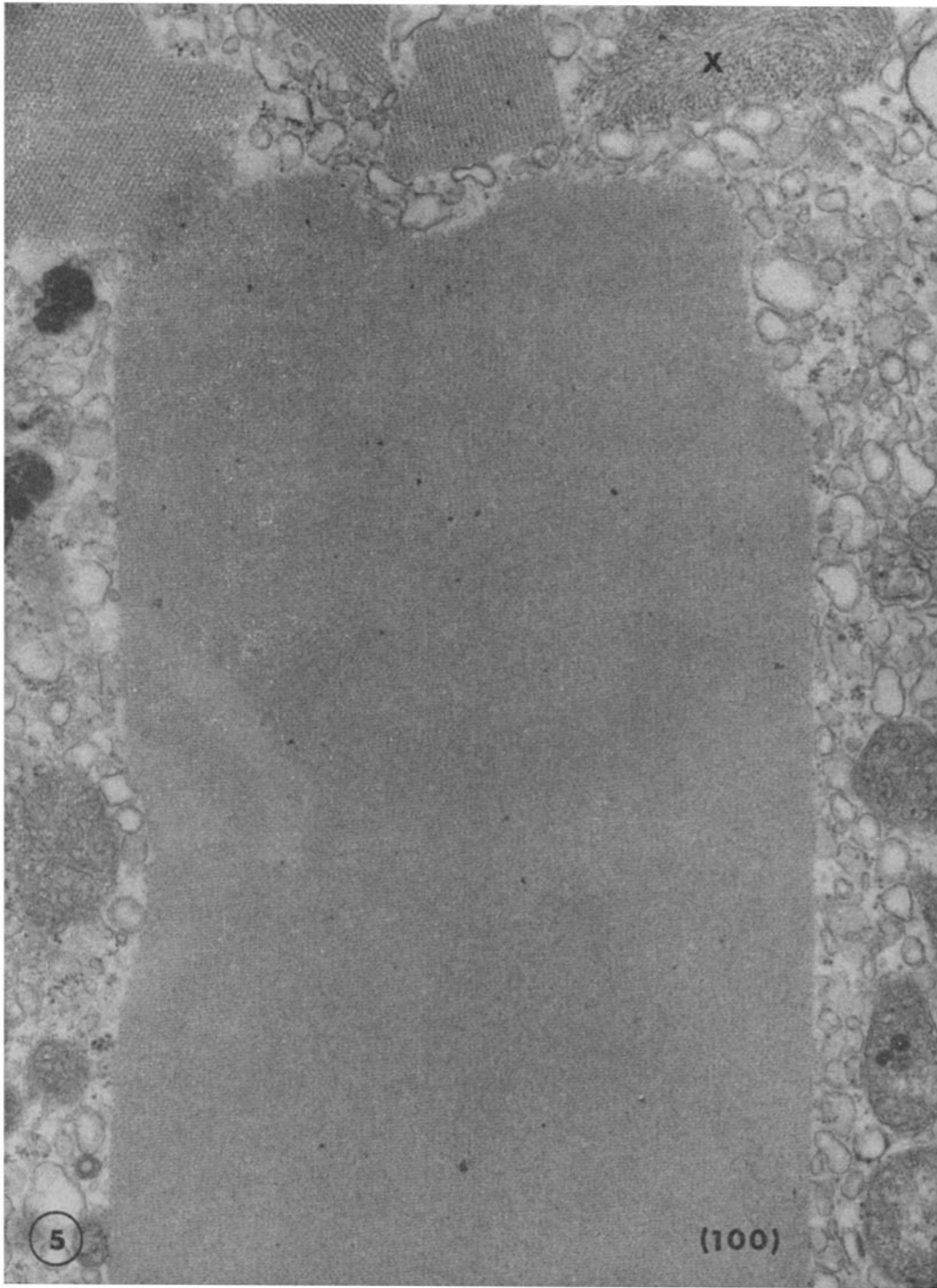


FIGURE 5 An extraordinarily large crystal of rectangular contour is illustrated. Three other crystals are cut at various planes. The large one is sectioned parallel to the (100) plane. A filamentous conglomeration (X) is seen. $\times 21,000$.

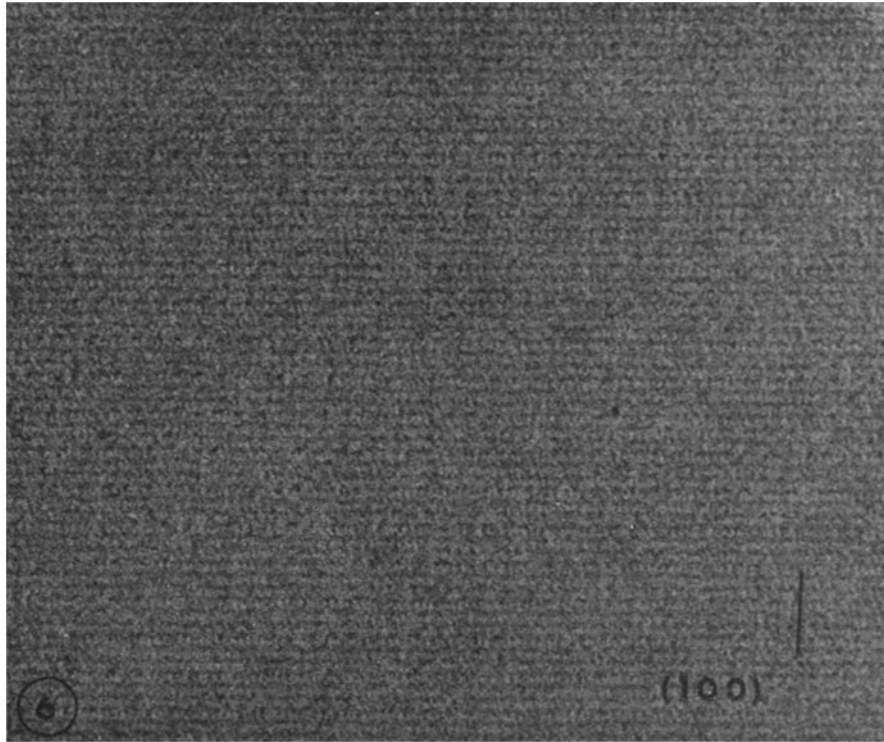


FIGURE 6 Higher magnification of Fig. 5 showing parallel lines at 150-A intervals perpendicular to the long axis (bar). Other parallel lines along the long axis are found when the micrograph is viewed along the long axis. $\times 70,000$.

OBSERVATIONS

The general fine structure of Reinke's crystal has been reported previously (see 13, 29), and further observations will be given in this paper. It is known that the crystals of Reinke are not always present in all interstitial cells, but they appear frequently. The crystals in thin section vary in contour, and their edges are sometimes irregular. However, in favorable preparations, the crystals are polygonal in shape and the edges are sharp at every corner (Fig. 1). Each side of the crystal is almost parallel to the corresponding opposite side. The three-dimensional structure can be reconstructed from outlines of many sections of the crystals. The shape thus obtained is a hexagonal prism or its derivatives. In sections perpendicular to the long axis of the crystal, the contour is hexagonal, while in longitudinal section it is rectangular. In sections of the crystal showing hexagonal surface outline, a hexagonal honeycomb pattern is found in the interior of the crystal

(Figs. 2,3). The plane of section is parallel to the (001) plane. The hexagons are 200 A on each side. The filaments comprising the hexagons are 50 A in diameter and show a beaded appearance indicating the presence of subunits (Fig. 3). The diffraction pattern and filtered image are shown in Figs. 8 and 8 *a*. The diagonal of the hexagons is perpendicular to the side of the crystal (Fig. 3). At the side of the crystal, the hexagons are open to the cytoplasm as was described by Yamada (29). The filaments along the side appear to be folded at an angle of 120° alternately, forming a honeycomb pattern. The holes of the honeycomb are of lower density and are continuous with the cytoplasm.

When the sectioned crystal is rectangular in contour, the internal structure shows two kinds of patterns. One pattern is a periodic repeat of a set of three parallel lines perpendicular to the long axis. The first and the second appear to be straight lines and lower in density, while the third line is a row of dense dots 300 A apart (Fig. 4). This is

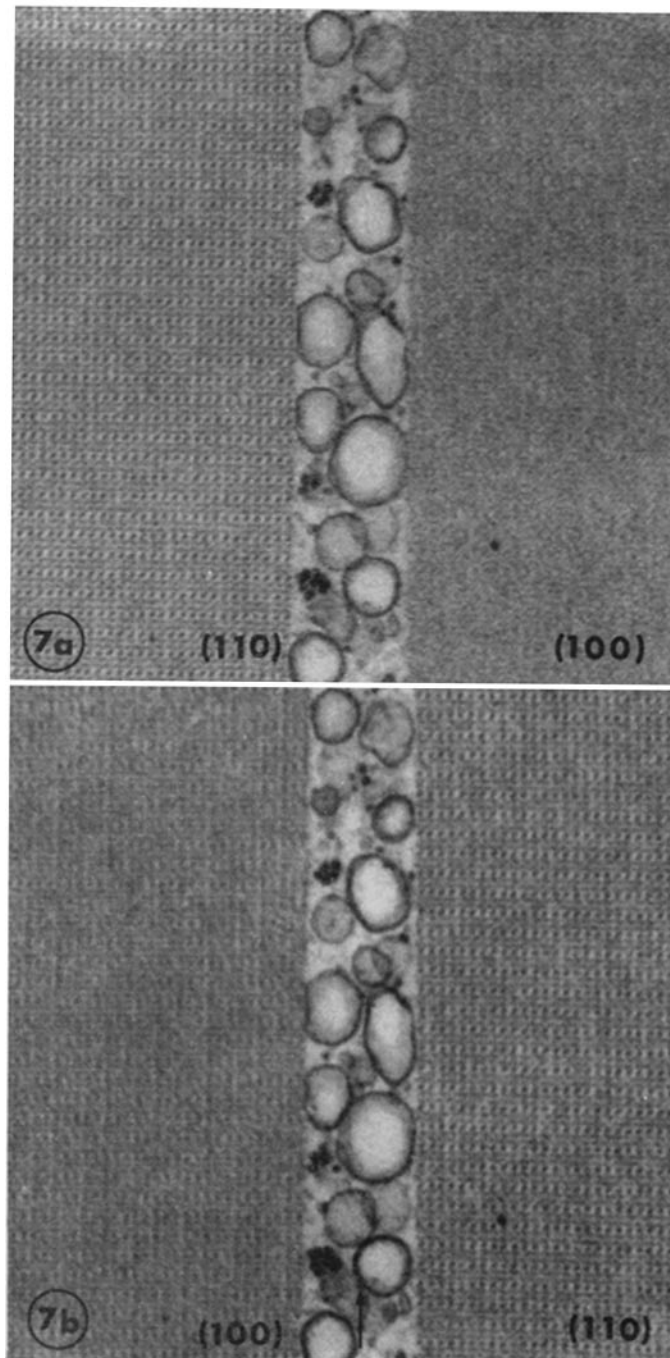
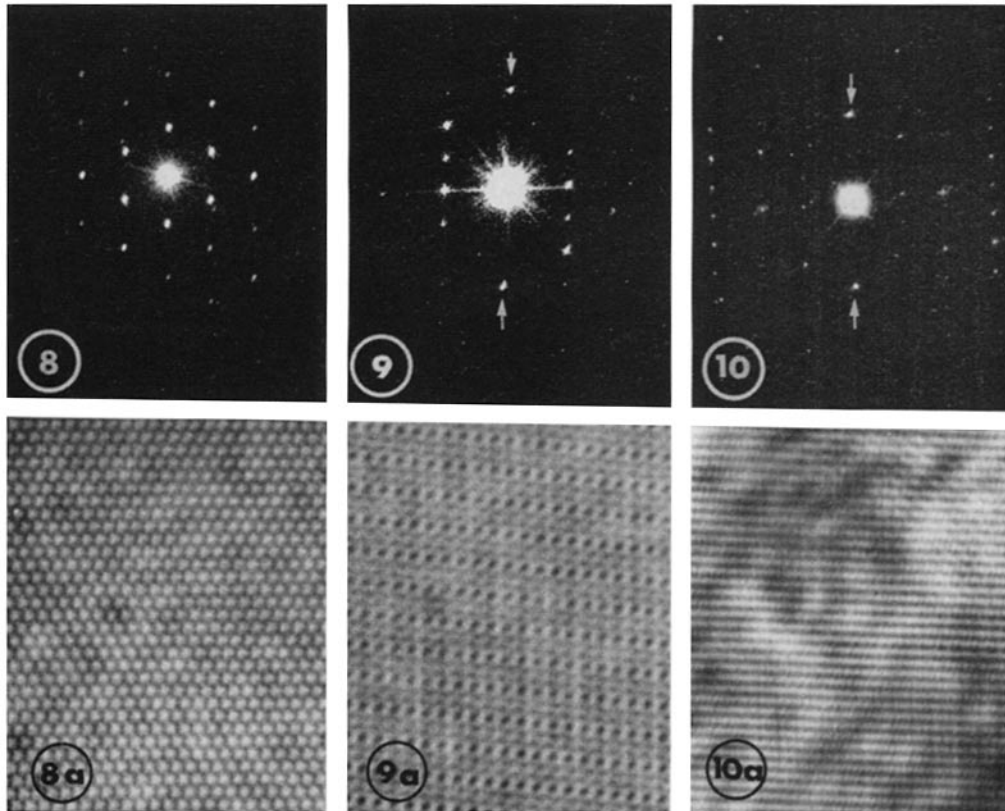


FIGURE 7 *a* and 7 *b* A pair of tilting electron micrographs showing two crystals. The section was tilted at 20° and the tilting axis (arrow) is parallel to the long axis of the crystals. The internal patterns of the two are convertible with respect to each other. The planes of the crystals with respect to the electron beam are indicated. $\times 65,000$.

more evident in the filtered image (Fig. 9 *a*). Each line appears to be interconnected by thin filaments at 300-A intervals parallel to the long axis (Fig. 4). The repeating period of each set of three lines is 450 A. Another pattern shows parallel lines 50 A in diameter perpendicular to the long axis at 150-A intervals (Figs. 5, 6). Other parallel lines along the long axis are found when the micrograph is viewed along the long axis (Fig. 6). The two patterns observed above are interconvertible when the section is tilted along the long axis at 20° (Fig. 7*a-b*). In the diffraction patterns of the (110, 100) planes (Figs. 9, 10), the relative strength of the third-order meridional reflections of the plane indicates the presence of a trigonal (threefold screw) axis along

the long axis. Thus, the structure of the crystal consists of three sets of parallel filaments, 50 A in diameter, which run in the plane perpendicular to the long axis, neighboring sets of filaments rotating by 120° with respect to each other. The dense dots found in the (110) plane result from the 120° folded filaments being parallel to the electron beam in the entire thickness of the section (see Fig. 13).

Dislocation of Reinke's crystal is frequently found, as was described by Fawcett and Burgos (13). As shown in Fig. 1, the region of dislocation is low in density. Dislocation parallel to the long axis of the crystal is found in the plane (110) (Fig. 11). It is obvious in this region that a row of dense dots belonging to each third line appears to be



FIGURES 8-10 Optical diffraction patterns of the electron micrographs of Figs. 2, 4, and 6, respectively.

Fig. 8 shows the hexagonal pattern. In Figs. 9 and 10, the relative strength of the third-order meridional reflections (arrows) indicates the threefold screw axis. Scale: 10 mm corresponds to 180 A in Fig. 8, 200 A in Fig. 9, and 220 A in Fig. 10.

FIGURES 8 *a*-10 *a* Optical filtering of Figs. 2, 4, and 6. \times 60,000, 90,000, and 90,000, respectively.

defective. The first line is connected with the other second line, and the second line is connected with the next first line (Fig. 11). Thus, the space between the first and second lines is wider and the lines are not parallel with respect to each other in

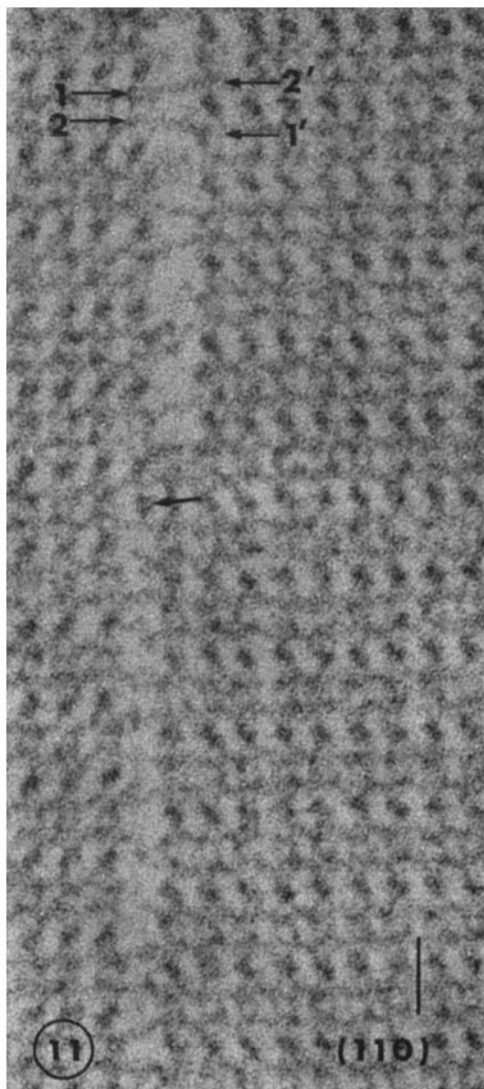


FIGURE 11 Dislocation of the crystal sectioned parallel to the (110) plane. The long axis is indicated by the bar. A row of dense dots parallel to the long axis is lacking in dislocation where the first line (1) is connected to the other second line (2''), and the second (2) is connected to the next first line (1'). At the point (arrow), dislocation is shifted to become perpendicular to the long axis at one phase interval. $\times 190,000$.

dislocation. A model based on the present results is illustrated in Figs. 12-14.

In addition to the crystal, aggregation of filaments in random directions is occasionally observed (Fig. 5). The filaments are slightly thicker than those of the crystal. In some interstitial cells without Reinke's crystal, groups of filamentous structures are found (Fig. 15). They are irregular in shape and consist of filaments running in different directions. In cross-sections of these structures as shown in Fig. 16, filaments making up groups of hexagons are found. Seven hexagons are closely applied to each other, resembling the hexagonal pattern of Reinke's crystal. The size of the hexagons is almost the same as in the crystal. The groups appear to be connected less closely with the filaments.

DISCUSSION

To determine the parameters of crystal structure, one of the best methods to use is X-ray diffraction

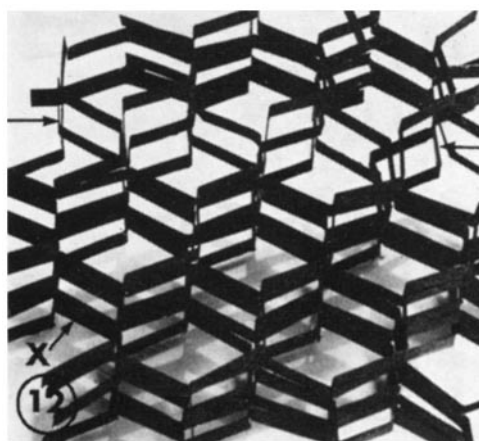


FIGURE 12 A model of the crystal is illustrated. The model was actually made in such a way that each chain folded 120° alternately was related to its neighbor by a twofold axis running parallel to and halfway between the chains in the plane perpendicular to the long axis, and that in the next plane on the previous chains, a similar set of the chains was related to the next by a rotation of 120° . This process was repeated to make the model. The space between the parallel chains perpendicular to the long axis is reduced, so that the model is compressed along the long axis. When the model is viewed horizontally along the X, the image is that shown in Fig. 13. The arrows indicate dislocation of the crystal lacking a row of the filaments. When the model is viewed along the arrows, the image is that seen in Fig. 14.

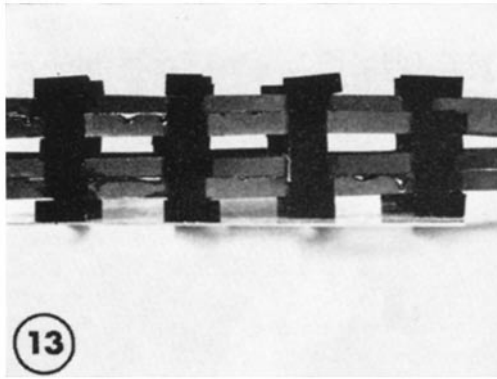


FIGURE 13 The model parallel to the (110) plane shows dense dots, lines, and clear zones similar to Fig. 4, but there is no space along the long axis in the model.

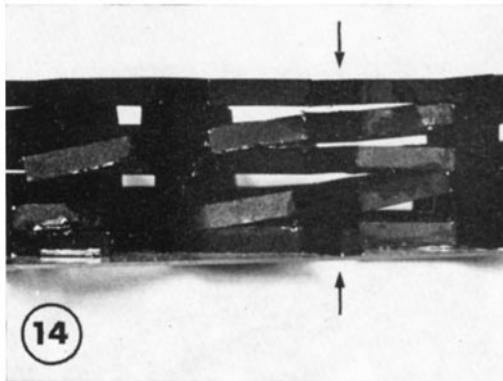


FIGURE 14 The model illustrating dislocation (arrows) as shown in Fig. 11.

of isolated crystals. The intracellular crystal structure of the amphibian yolk, for instance, has been studied (16) by this method. Although the crystal of Reinke is well known in the human testicular interstitial cells, it is doubtful whether the crystals can be isolated for X-ray diffraction analysis except by means of the primitive method used by Reinke himself (23). One of the difficulties of isolation would be the fact that Reinke's crystal is found only in man. The fine structure of testicular interstitial cells has been studied in mammals (4-7, 9, 19, 21, 22, 24, 25), reptiles (8), and amphibia (3). No crystal has been found in the interstitial cells of animals so far studied. The common cytoplasmic elements in the interstitial cells, including those of man, are a well developed agranular reticulum, lipid droplets, mitochondria with tubular cristae, and Golgi complex. In an earlier

paper on Reinke's crystal, Fawcett and Burgos reported that the crystal consisted of particles 150 A in diameter, uniformly spaced 190 A apart, and that the textile-like pattern appeared to represent the arrangement of macromolecules in the lattice of a protein crystal (13). Yamada's illustrations show the hexagonal tubules, 200 A on each side, open to the cytoplasm at the edge of the crystal (29). In the textbook by Bloom and Fawcett, the crystal is said to be made up not of globular macromolecules but of closely compacted microtubules (2). However, as shown here, the crystal is not a simple bundle of microtubules, but is much more complicated. Thus, it is rather difficult to analyze the crystals by thin-section electron microscopy alone. However, in the case of crystal inclusions of the salamander hepatocyte, the face-centered cubic crystal structure has been demonstrated successfully by the thin-section method (15).

On the other hand, catalase crystals which were purified from bovine liver cells have been studied with X-ray diffraction and electron microscopy (20). Surprisingly, Longley's electron micrographs showing all faces of the catalase crystal correspond with those of Reinke's crystal presented here, though they are different in dimensions. In his study of catalase crystals, he reported that the crystal is trigonal, $a = 173$ A, $c = 237$ A, with six molecules in the unit cell. Optical diffraction patterns of the electron micrographs of the present material also appear to be quite similar to those of the catalase crystal. Although the exact subunit structure of the 50-A-diameter filaments composing Reinke's crystal has not been detected clearly, one can speculate that the model of Reinke's crystal would have the same crystalline arrangement as catalase. Further, in diffraction patterns of Reinke's crystal, the trigonal (three-fold screw) axis is observed, and no other axes of symmetry can be found. This result would indicate that the crystal would be isotropic, in agreement with Reinke's report (23). It is not clear how less-than-50-A filaments are connected between the parallel 50-A-thick filaments in the direction perpendicular to the long axis. The dense dots found in the (110) plane are explained by the fact that the twofold 50-A-thick filaments are almost parallel to the electron beam in the entire thickness of the section. By the tilting specimen technique, a complete image conversion between the (110) plane and the (100) plane should require

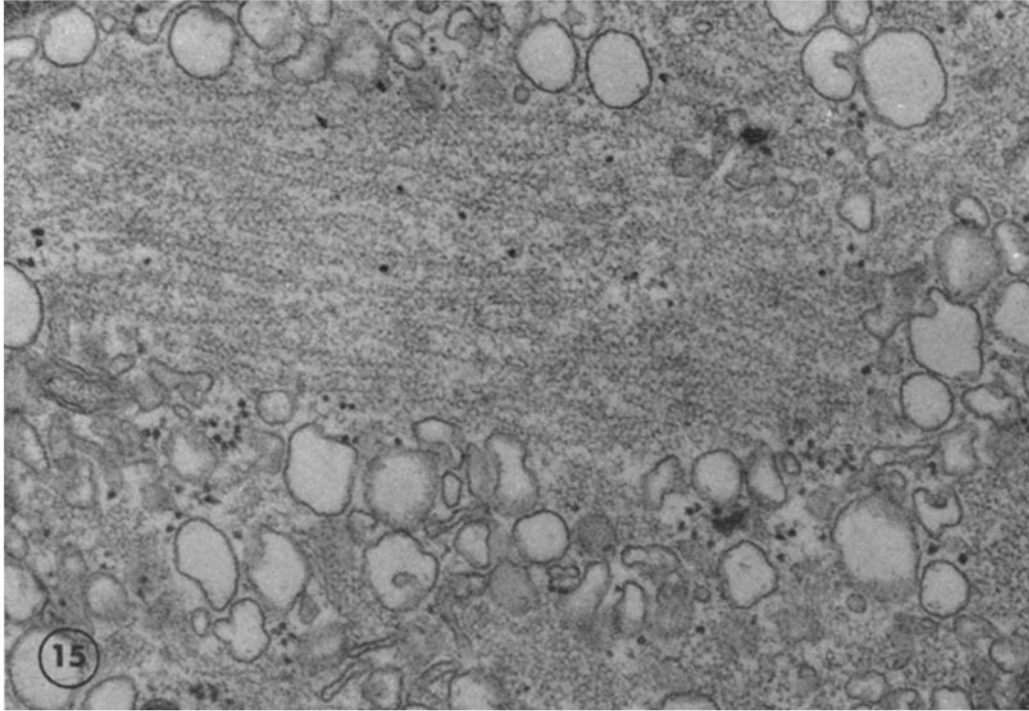


FIGURE 15 Cytoplasm of the interstitial cell without Reinke's crystals. The filamentous structure can be seen. Some of the filaments appear to be cut longitudinally and others obliquely. $\times 55,000$.

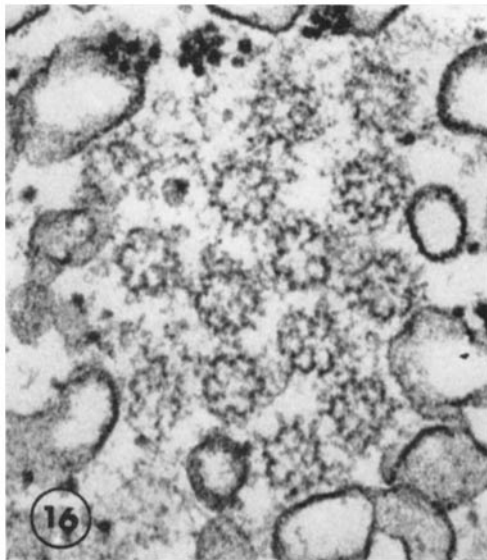


FIGURE 16 A higher magnification of cross-section of the filamentous structure. Each group appears to consist of seven hexagons about 200 Å on each side, and to be connected with others by filaments. $\times 75,000$.

tilting 30° along the long axis. The actual result is an acceptable agreement, as shown in Fig. 7a-b.

Since no morphological relationships between the crystal and agranular reticulum have been observed, Reinke's crystal is different from the cytoplasmic crystalloids found in certain other steroid-producing cells, such as the interstitial cells of the antibrachial organ (26) and the adrenocortical cells (31). It is also different from the microtubular crystals found in mammalian cells *in vitro* after treatment with some alkaloids (1). Sisson and Fahrenbach have also noted the difference between Reinke's crystal and the crystalloid of the agranular reticulum (26).

It is explained that dislocation of the crystal of Reinke is due to absence of a row of the filaments in the plane parallel to the long axis, so that one phase is dislocated as in Fig. 11. Whether the plane of dislocation is also a potential plane of cleavage is not clear.

The filamentous structure found in some interstitial cells devoid of Reinke's crystal may corre-

spond to the rice-form bodies described by Winiwarter (27). Similar structures have been reported in both the cytoplasm and nucleus (11, 29, 30). Because of similarity of structure, the rice-form bodies may be a developmental stage of Reinke's crystal. It seems conceivable that the filaments are condensed and oriented to form a crystal by some unknown mechanism.

The functional significance of the crystal of Reinke is not known. It is hoped that the crystal can be isolated in sufficient quantity to be investigated chemically and by X-ray diffraction.

This paper is dedicated to Dr. H. Stanley Bennett in celebration of his 60th birthday. A part of this work was presented at the IXth International Congress of Anatomists in Leningrad. The abstract appeared in *Morphological Aspects of Andrology*. 1970. A. F. Holstein and E. Horstmann, editors. Grosse Verlag, Berlin.

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After submission of the manuscript, a review article entitled "Fine structure of the testis and its functional significance" by Burgos, M. H., R. Vital-Calpe, and A. Aoki. 1970. In *The Testis*. A. D. Johnson, W. R. Gomes, and N. L. Vandemark, editors, Academic Press Inc., New York. 1:551, was published. Fig. 91 in their paper is quite similar to Fig. 4 in the present paper, but their interpretation is rather different from ours.

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