

## **Intracisternal Crystalline Arrays of Coated Parallel Tubules in Cells of a Human Osteosarcoma**

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**Summary.** Crystalline arrays of coated parallel tubules (CPT) were observed by electron microscopy within dilated cisternae of the rough endoplasmic reticulum of pleomorphic mononuclear cells in a human osteosarcoma. The wall of the peculiar intracisternal tubules consisted of an electron-dense thin membrane-like envelope which appeared to be composed of granular subunits. The electron-lucent tubular core together with the limiting envelope was approximately 15 nm in diameter. A coat of fuzzy material, approximately 10 nm thick, tightly surrounded the membrane-like wall of the tubules. Cross sections of accumulations of CPT showed the tubules to be arranged in a hexagonal crystalline array. The nature and significance of the intracisternal CPT are unknown.

**Key words:** Osteosarcoma – Intracisternal tubules – Crystalline array

### **Introduction**

Intracellular tubuloreticular structures, associated with both rough and smooth endoplasmic reticulum, have been observed in two cases of human osteosarcoma (Jenson et al. 1971; Györkey et al. 1971). Jenson et al. (1971) described two distinct patterns of accumulation of the tubular structures which measured approximately 23 nm in diameter. In one pattern dilated cisternae of the endoplasmic reticulum contained loosely separated branched tubules. In the other, the endoplasmic reticulum formed a tight mantle around compact sieve-like aggregates of tubular profiles. Similar tubuloreticular structures have been found in a great variety of mammalian tissues (Chandra 1968; Uzman et al. 1971; Schaff et al. 1976) and their possible nature and significance have been widely discussed.

In this study another type of intracisternal tubular structure, coated parallel tubules (CPT), is described. The CPT have a highly ordered arrangement and

are associated with the rough endoplasmic reticulum. They were detected during electron microscopic investigation of a case of human osteosarcoma.

### Material and Methods

Biopsy material was excised from a solid tumor present in the upper end of the left tibia of a 24-year-old man. The material was sent to the Institute of General Pathology and Pathological Anatomy of the Technical University in Munich (Director: Prof. Dr. W. Gössner). Histological examination of the tumor tissue showed the presence of an osteosarcoma. The tumor-bearing extremity of the patient was amputated 23 cm above the knee joint. Small pieces of the tumor, which measured about 11 cm in diameter, were obtained for electron microscopic examination. Specimens were fixed in cacodylate-buffered 3% glutaraldehyde, postfixed in chrome-osmium, dehydrated, and embedded in Epon 812. Thin sections were cut with a diamond knife on a Reichert Om U3 ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a TESLA BS 500 electron microscope.

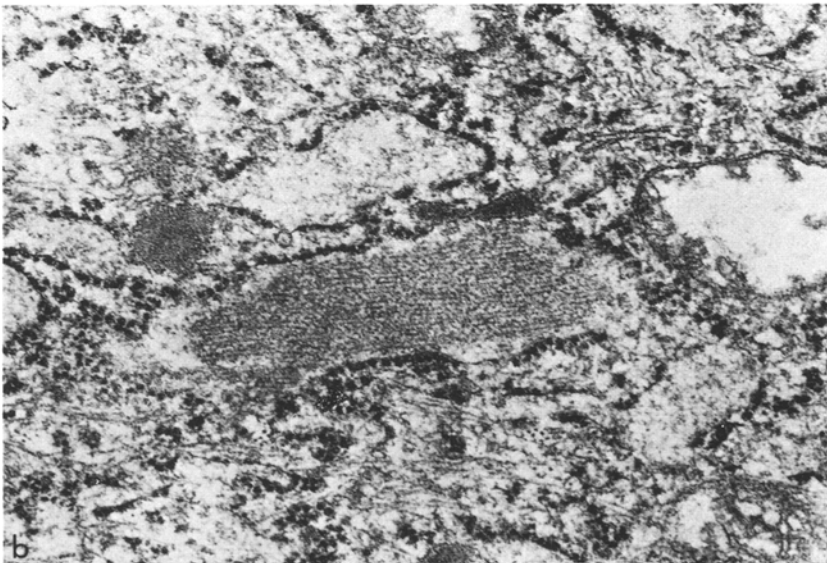
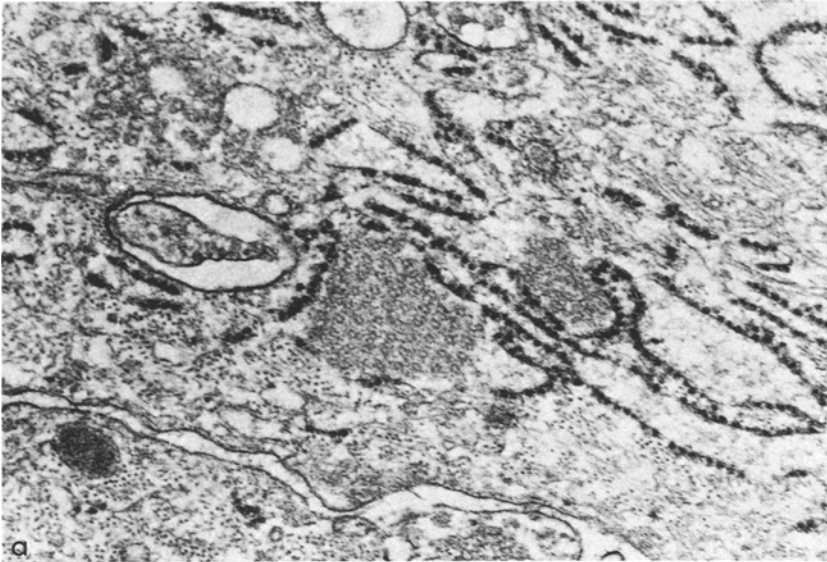
### Results

Bundles of tubular structures arranged in parallel, sectioned longitudinally, transversely or at an angle, were occasionally found within dilated cisternae of the prominent rough endoplasmic reticulum of pleomorphic mononuclear osteosarcoma cells (Figs. 1–3). Cells containing such tubules were distributed inhomogeneously in the tumor tissue. They were frequently observed in sections from some tissue blocks but rarely in others. The intracellular distribution pattern of the intracisternal tubules was also very irregular. Sometimes tubular profiles filled either a part or the whole of a single dilated cisterna of the rough endoplasmic reticulum in a cell (Fig. 1a and b) while the rest of the cisternae were dilated but free of tubules. In a few cell sections large areas were occupied by accumulations of tubular structures tightly surrounded by membranes of the rough endoplasmic reticulum (Fig. 2).

The wall of the intracisternal tubules consisted of an electron-dense thin membrane-like envelope. The tubular core was electron-lucent (Figs. 1–3). At high magnification cross and longitudinal sections of the tubules showed that the membrane-like tubular wall was not a unit membrane and appeared to be composed of granular subunits (Fig. 3b). The tubular core together with the limiting envelope was approximately 15 nm in diameter. A coat of fine flocculent material with moderate electron-density tightly surrounded the membrane-like wall of the tubules (Figs. 1–3). The fuzzy coat was approximately 10 nm thick.

The tubular structures were found to be located only within cisternae of the rough endoplasmic reticulum. They were always aligned parallel to one another without undulation or branching, and they were never continuous with the surrounding membranes of the rough endoplasmic reticulum.

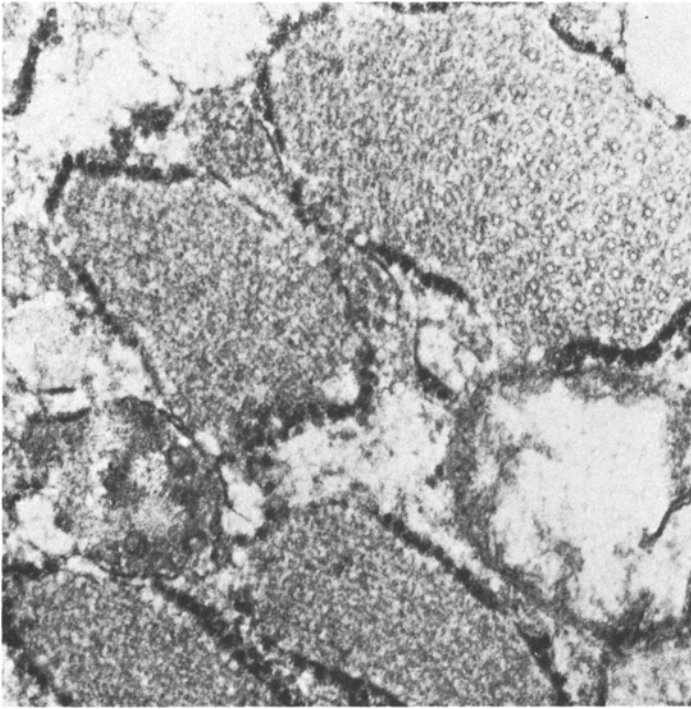
Cross sections of accumulations of CPT showed a highly ordered arrangement of the tubules in a hexagonal crystalline array with a uniform center-to-center spacing of approximately 50–60 nm (Figs. 1a, 2, 3a and b). In longitudinal sections the course of the crystalline arrays was either straight or slightly curved and up to 1.25  $\mu\text{m}$  long (Figs. 1b, 2).



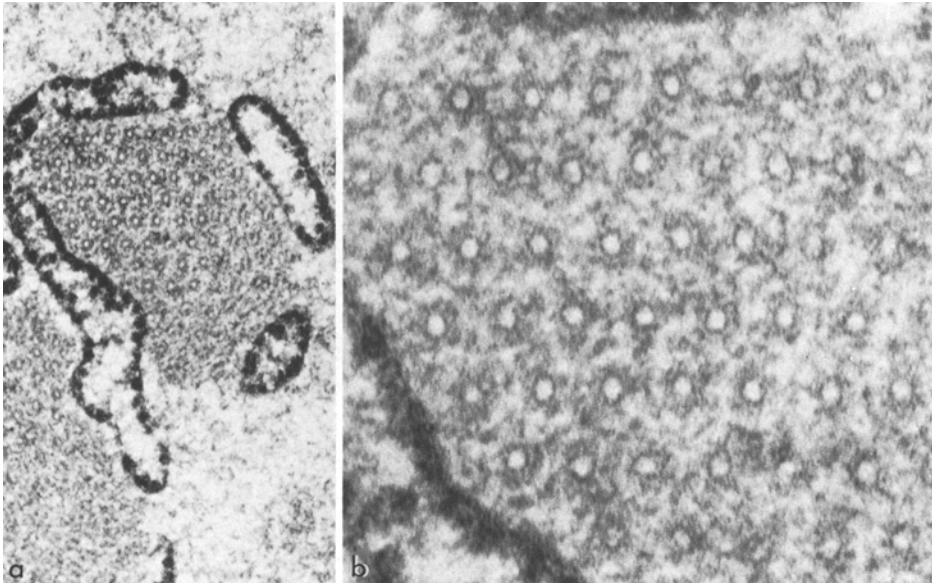
**Fig. 1a, b.** Parallel tubular structures within single dilated cisternae of the rough endoplasmic reticulum of human osteosarcoma cells. **a** In cross section.  $\times 64,000$ . **b** In longitudinal section.  $\times 48,000$

### Discussion

The intracisternal CPT observed in cells of a human osteosarcoma differ in their morphology from the intracisternal tubuloreticular structures previously



**Fig. 2.** Large accumulations of parallel tubules tightly surrounded by membranes of the rough endoplasmic reticulum. The tubules filling the cisterna in the upper right part of the picture are cut at an angle to the left and transversely to the right. The tubules within the other cisternae are sectioned longitudinally. Hexagonal arrangement of cross-sectioned tubules can be seen.  $\times 64,000$



**Fig. 3a, b.** Hexagonal crystalline array of transversely sectioned intracisternal CPT. **a** Overall view.  $\times 57,600$ . **b** Part of **a** at high magnification. The moderately electron-dense coat tightly surrounding each tubule is clearly visible.  $\times 180,000$

reported in two cases of human osteosarcoma (Jenson et al. 1971; Györkey et al. 1971). The CPT have a smaller diameter, are coated with fuzzy material, are arranged in parallel without undulation or branching, are never continuous with the surrounding membranes of the rough endoplasmic reticulum, and form crystalline bodies.

Only two communications could be found in the literature in which tubular structures possibly similar to the CPT reported here are described. Sengel et al. (1971) observed intracisternal "tubulogranular inclusions" in chondrocytes of a case of Morquio's disease (mucopolysaccharidosis type IV). The tubules were arranged in parallel and measured 20 nm in diameter. The electron-lucent tubular core had a diameter of 12 nm and was limited by dense granules. The tubules were encircled by a fine fibrillar and faintly osmiophilic coat. Crystalline arrays of the tubulogranular structures were not reported by the authors. Mackay and Ayala (1980) found aggregates of parallel microtubules within cisternae of the rough endoplasmic reticulum in cells of six human metastatic melanomas. Each tubule had a wall that resembled a unit membrane, surrounded by a fuzzy indistinct outer layer. The internal diameter of a tubule was 30 nm, and the centers of two adjacent tubules were separated by a distance of 60 nm. The authors did not describe hexagonal crystalline arrays of the tubules.

The nature and significance of the intracisternal CPT in cells of a human osteosarcoma are unknown. The tubular structures could be interpreted either as the accumulated abnormal products of disturbed protein synthesis of the rough endoplasmic reticulum of the tumor cells or as subunits of an unknown virus. The latter speculation is supported by reports in which either intracytoplasmic membrane-bound or intracisternal accumulations of parallel tubules are described in virus-infected cells and represent viral core or coat material (Murphy et al. 1971; Gerber et al. 1974).

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