Virchows Arch. A Path. Anat. and Histol. 371, 295—303 (1976)

Human Ecchordosis Physaliphora and Chick Embryonic Notochord

A Comparative Electron Microscopic Study

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Received June 25, 1976

Summary. The ecchordosis physaliphora, a small gelatinous mass attached to the midline of the clivus, is characterized ultrastructurally by glycogen-laden intracytoplasmic vacuoles, focally distended endoplasmic reticulum and perinuclear cisterns with cytoplasmic invaginations, large clusters of granular endoplasmic reticulum interdigitating with mitochondria, and an abundant extracellular space.

These morphologic features are also present in the 9-day embryonal chick notochord and the human chordoma and serve to reaffirm the derivation of the ecchordosis and chordoma from notochordal rests.

Key words: Ecchordosis physaliphora — Embryonic chick notochord — Electron Microscopy — Chordoma.

Introduction

Virchow in 1857 provided the first microscopic description of a small gelatinous mass arising in the midline of the clivus which he termed the "ecchondrosis physaliphora". The cartilagenous nature of this mass was questioned by Müller (1858) who proposed a notochordal origin. Ribbert (1894, 1895) confirmed Müller's theory and suggested that the term "chordoma" be applied to all such neoplasms. Stewart and Morin (1926) recognized a marked difference in the biological behavior of clivus chordomas. Some insidiously invaded the skull and cranial cavity while others lay dormant as small incidental masses at autopsy. For this latter group of incidental lesions, they proposed the term "ecchordosis physaliphora".

The ecchordosis has been well described by light microscopists (Stewart and Morin, 1926; Horwitz, 1941). However, Wyatt et al. (1971) have provided the sole electron microscopic report to date. The present study describes the electron microscopic features of an ecchordosis physaliphora and emphasizes the ultrastructural similarities between the ecchordosis and 9-day old embryonal chick notochord.

Materials and Methods

At autopsy, the ecchordosis was identified as a 0.5 cm. in diameter gelatinous, partly cystic mass adherent to the leptomeninges overlying the basilar artery (Fig. 1). A minute stalk extended from this mass through the dura into the midline of the clivus. The ecchordosis was divided into two portions, one for light microscopy and the other for electron microscopic

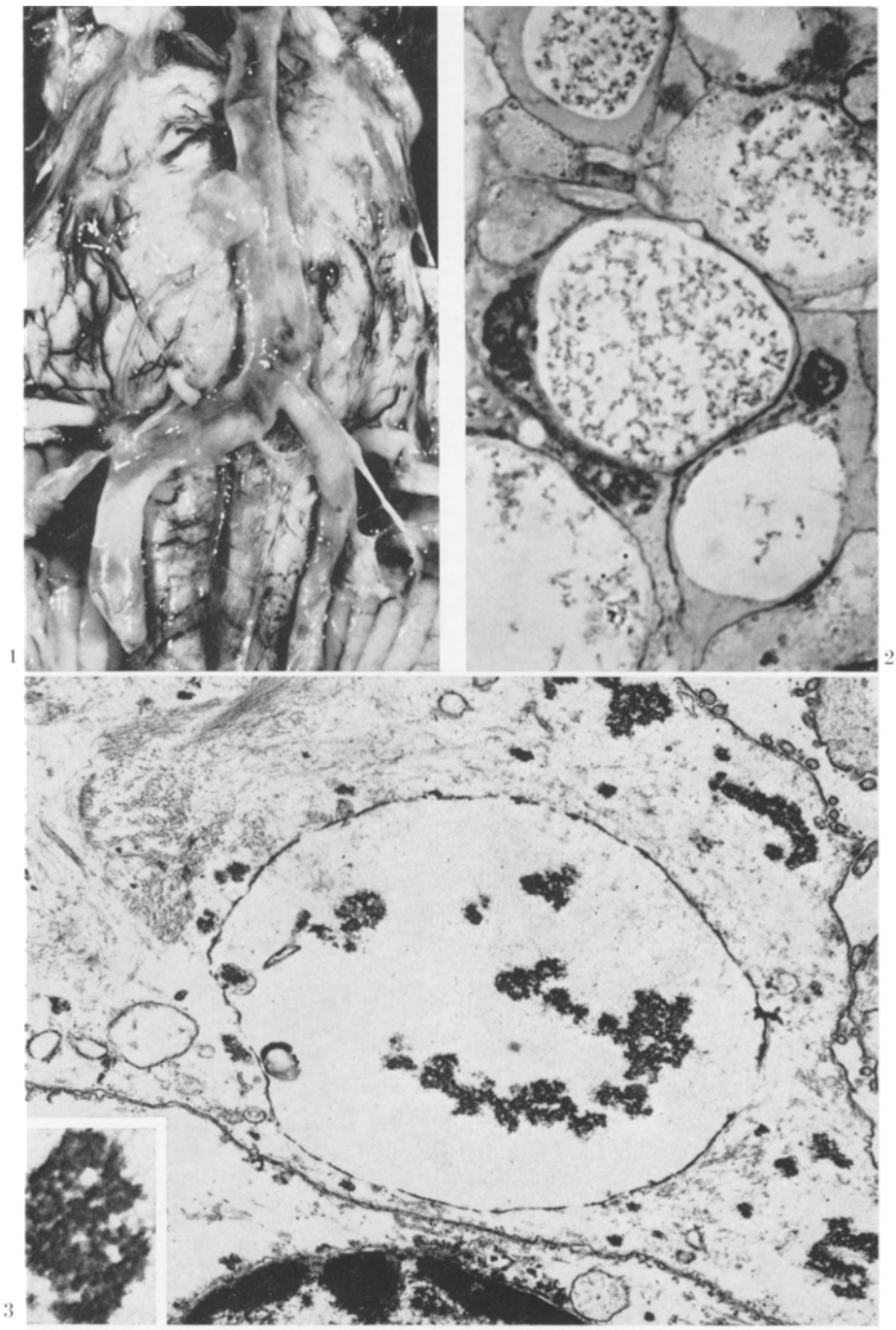

Fig. 1. Ecchordosis physaliphora adherent to leptomeninges overlying the basilar artery and the midline of the pons

examination. The portion for light microscopy was fixed in buffered formalin and stained with hematoxylin and eosin, periodic acid-Schiff (PAS), alcian blue (pH 1.0), and mucicarmine.

The portion for electron microscopy was trimmed immediately into 1 mm^3 fragments in 3.5% glutaraldehyde fixative with 0.06 M cacodylate buffer (pH 7.00; 548 mOsm) and stored in this solution at 4° C. for approximately 2 months. The tissue was then washed in cacodylate buffer with sucrose (pH 7.30; 336 mOsm), osmicated, dehydrated in a graded series of ethanols, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined in a Siemens 1 A electron microscope operating at 80 Kv with a 50 μ copper foil objective aperture.

The notochord was rapidly removed from 9-day old chick embryos and trimmed into 1 mm^3 fragments while immersed in one of the three following fixatives: A. Chilled 2% glutaraldehyde in 0.06 M cacodylate buffer (pH 7.2, 460 mOsm) containing 2% polyvinyl pyrrolidone (PVP, mol. wt. 40,000; Oxford Laboratories, Redwood City, Calif.) for 2 hours, then transferred to chilled 3.5% glutaraldehyde in 0.06 M cacodylate buffer (pH 7.0, 530 mOsm) for 16 hours. The tissue was then washed in cacodylate buffer with sucrose (pH 7.4, 360 mOsm) for 30 minutes before osmication (Abrunhosa, 1972). B. Chilled 3.5% glutaraldehyde with 0.06 M sodium cacocylate buffer (pH 7.0, 530 mOsm) for 18 hours, followed by a wash as described above and osmication. C. Chilled 2% paraformaldehyde, 2.5% glutaraldehyde with 0.1 M cacodylate buffer (pH 7.1, 990 mOsm) for 2 hours (method modified from Glauert, 1974), then washed as described above followed by osmication. Further processing and examination were done with methods used above.

Results

Light Microscopic Observations

The ecchordosis was composed of a delicate lacework of cells interspersed by large and small vacuoles, some of which indented adjacent nuclei and were thus clearly intracellular. The vacuoles were usually devoid of contents but on occasion, a thin margin of granular material remained which stained red with hematoxylin and eosin, pink with mucicarmine, and deep red with the periodic acid-Schiff reaction. The granular material was unstained with alcian blue. One micron sections of glutaraldehyde-fixed, epon-embedded tissue provided greater preservation of cellular detail. Vacuoles were clearly intracellular and contained many discrete granules (Fig. 2).

Chick notochord was composed of abundantly vacuolated cells, widely dispersed in a loosely textured matrix. This tissue closely resembled the previously described ecchordosis. In most specimens, only small fragments of residual notochordal tissue adhered to the notochordal sheath. Cells immediately adjacent to this sheath contained many small vacuoles while cells further removed from the sheath had few but much larger vacuoles. The centers of well preserved notochordal fragments consisted solely of cells with large, extremely thin-walled vacuoles. The minute granules seen in the vacuoles of the ecchordosis were not present in those of the notochordal cells.

Fig. 2. Ecchordosis physaliphora, composed of cells with large intracytoplasmic vacuoles containing discrete granules. Plastic embedded one-micron section. Mallory's azure II—methylene blue, × 1,000

Fig. 3. Ecchordosis physaliphora. Upper cell contains a large, spherical, intracytoplasmic vacuole, delimited by a single membrane and filled with clusters of dense material. × 14,500. Inset: Detail of dense material resembling aggregates of glycogen. × 68,000

Electron Microscopic Observations

Ultrastructural examination of the ecchordosis revealed elongated cells, many of which contained large spherical intracytoplasmic vacuoles, delimited by a single membrane and filled with varying amounts of electron dense granular material resembling aggregates of glycogen (Fig. 3). Other cells had small vacuoles derived from the focal distension of granular endoplasmic reticulum or perinuclear cisterns. Papillary cytoplasmic infoldings were conspicuous within these structures (Fig. 4). Occasional cells contained deep invaginations of the extracellular space which superficially resembled true intracytoplasmic vacuoles. However the presence of pinocytotic vesicles associated with the limiting membrane of these "vacuoles", confirmed their actual derivation from an infolding of the plasma membrane (Fig. 5). Still other cells, while free of vacuoles, contained dense sheets of cytoplasmic filaments measuring about 100 Å in diameter and large clusters of granular endoplasmic reticulum interdigitating with mitochondria (Fig. 6).

Apart from the remarkable variety of vacuolar structures, the ecchordosis also contained rare microtubules 240–270 Å in diameter, occasional cytoplasmic clusters of glycogen rosettes, and large extracellular spaces with scattered granular-flocculent material and leashes of mature and immature collagen fibrils. Cells were joined by infrequent zonulae adherentes.

The embryonic chick notochord was adequately preserved using any of the three methods described above but method A, employing PVP, provided the best results. Notochordal fine structure was strikingly similar to the ecchordosis and featured spherical, cytoplasmic vacuoles which were often confluent (Fig. 7 and 8), distended cisterns of granular endoplasmic reticulum often containing papillary cytoplasmic infoldings (Fig. 8), distended perinuclear cisterns (Fig. 9), and cytoplasmic invaginations of the extracellular space mimicking the true intracytoplasmic vacuoles (Fig. 10). Other features shared by both notochord and ecchordosis included scattered cytoplasmic filaments (100 Å in diameter), rare clusters of mitochondria interdigitating with granular endoplasmic reticulum, and infrequent zonulae adherentes. The only major point of difference was the lack of granular material within the lumens of the intracytoplasmic notochordal vacuoles.

Discussion

A comparative ultrastructural study of the ecchordosis physaliphora and the embryonic notochord has revealed many morphologic similarities, including large, confluent, intracytoplasmic vacuoles, distended granular endoplasmic reticulum and perinuclear cisterns, cytoplasmic invaginations of the extracellular space, clusters of mitochondria interdigitating with granular endoplasmic reticulum, and occasional broad intercellular spaces. The only major difference between

Fig. 4. Ecchordosis physaliphora. Focal distention of perinuclear cisternae with many papillary cytoplasmic infoldings. ×33,000

Fig. 5. Ecchordosis physaliphora. Cytoplasmic invagination (*) of the extracellular space. Note pinocytotic vesicles on the limiting membrane of the invagination. × 14,500

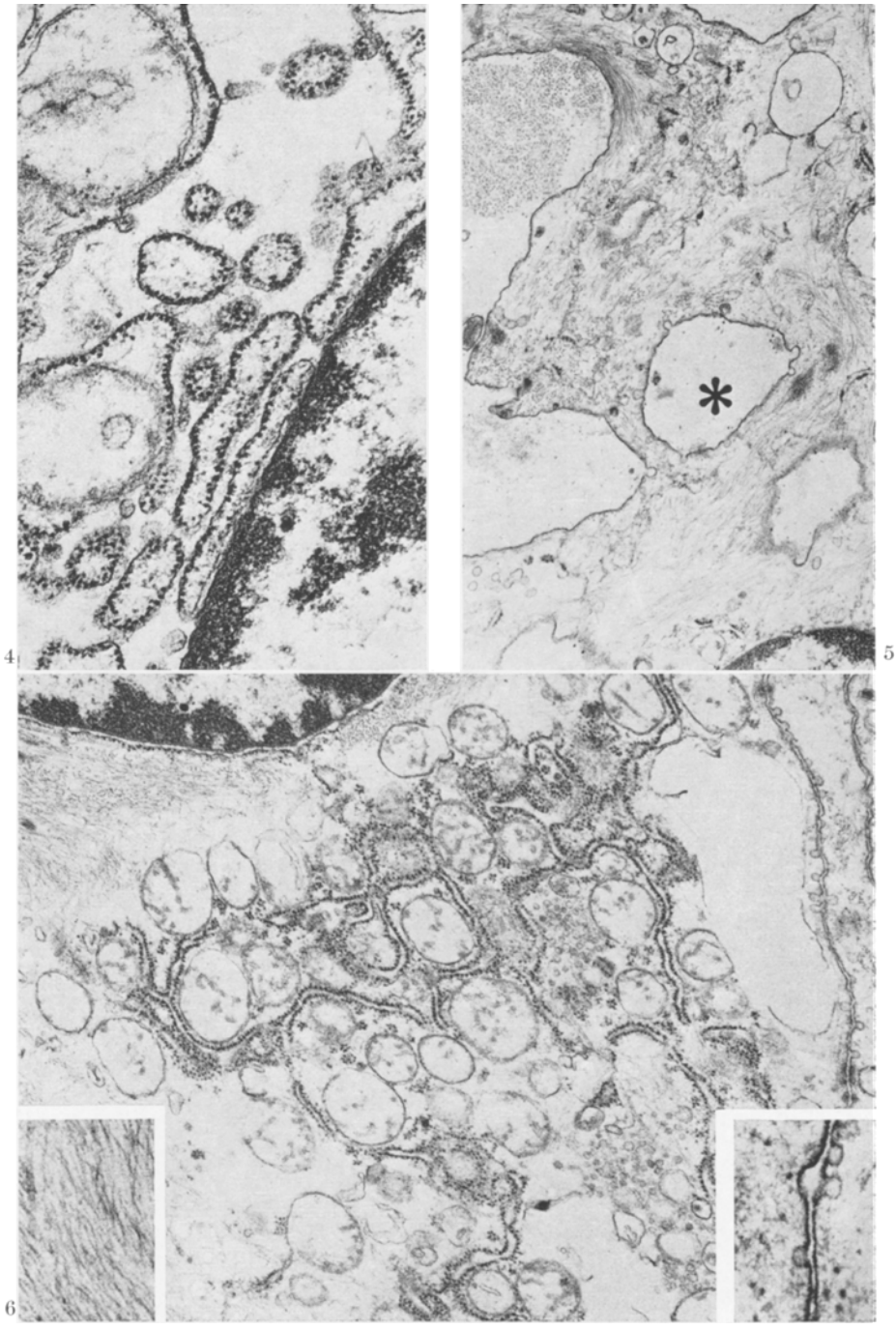

Fig. 6. Ecchordosis physaliphora. Typical cell with sheets of cytoplasmic filaments, clusters of granular endoplasmic reticulum interdigitating with mitochondria, and many pinocytotic vesicles within the plasma membrane. × 16,000. Inset, left: Detail of cytoplasmic filaments. × 38,000. Inset, right: Detail of pinocytotic vesicles. × 50,500

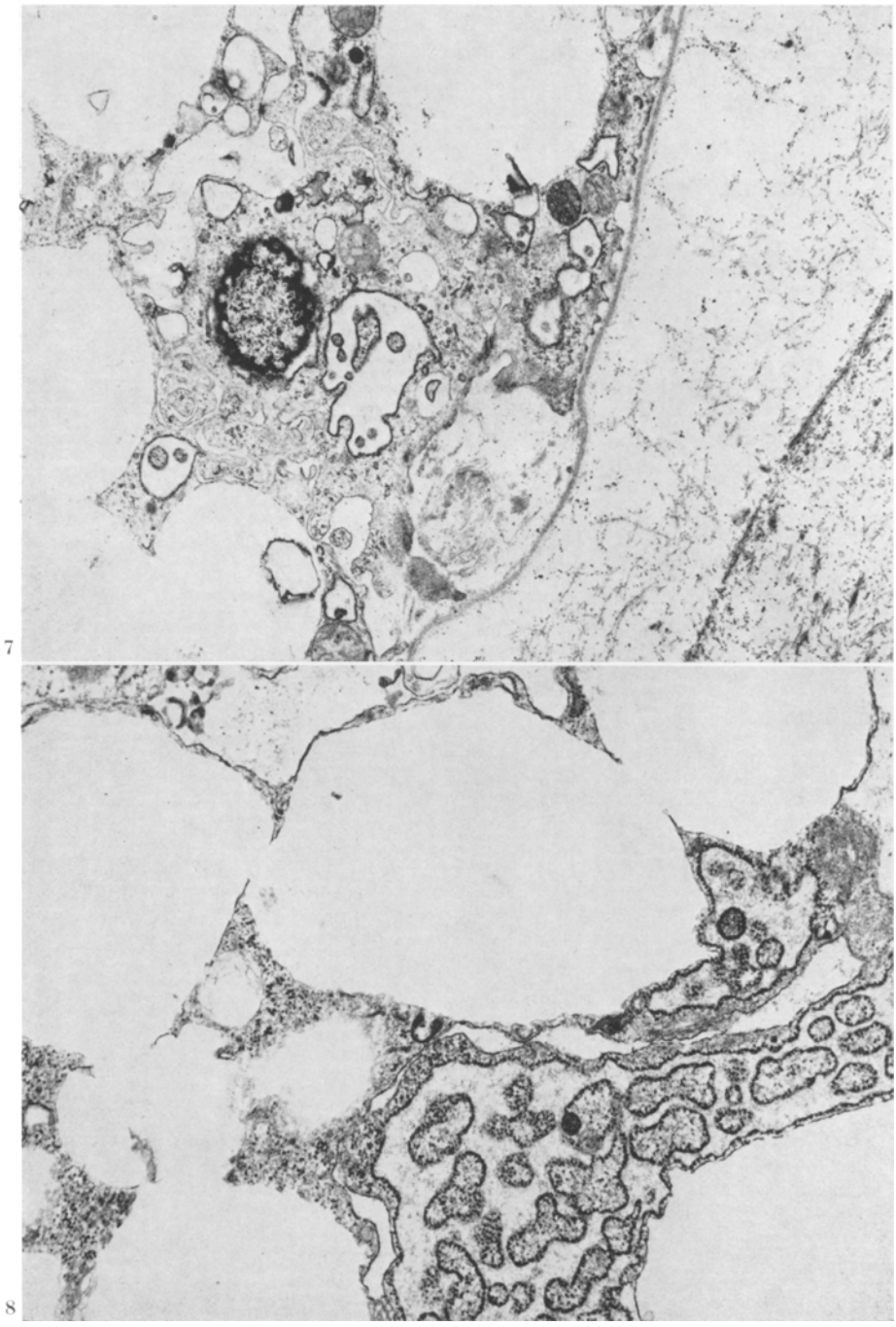

Fig. 7. Embryonic chick notochord. Confluent cytoplasmic vacuoles and distended cisternae of granular endoplasmic reticulum. Note the extracellular notochordal sheath (right lateral field). × 11,000

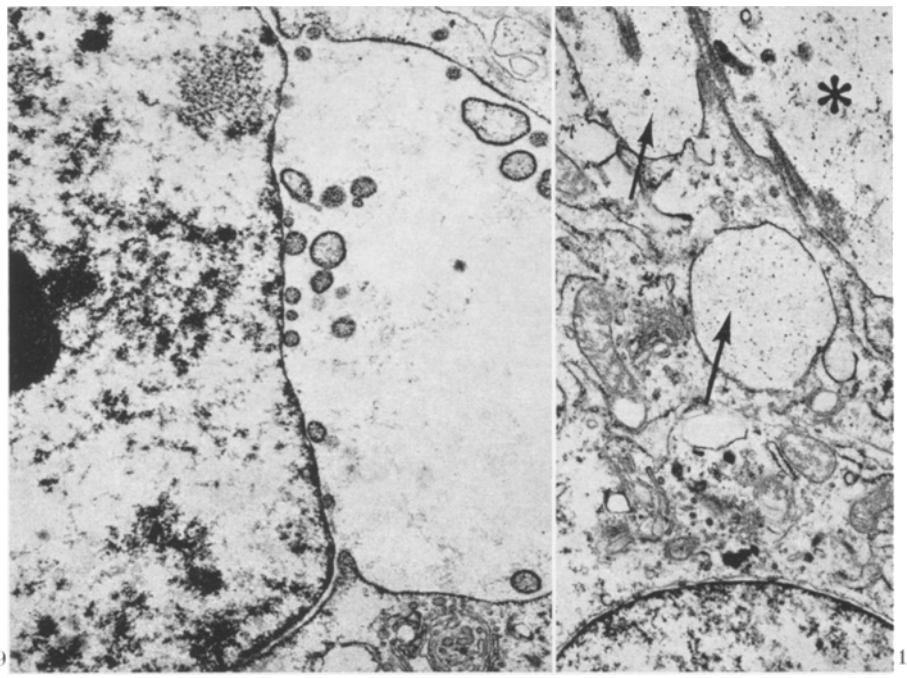

Fig. 9. Embryonic chick notochord. Distended perinuclear cistern containing scattered small papillary cytoplasmic infoldings. Compare with Figure 8. × 15,500

Fig. 10. Embryonic chick notochord. Extracellular invaginations (arrows) mimicking true intracytoplasmic vacuoles. Note the similarity of the vacuolar contents to the extracellular material (*). × 13,000

the ecchordosis and the notochord concerned the contents of the intracytoplasmic vacuoles. In the ecchordosis the vacuoles contained granules resembling glycogen; the notochordal vacuoles however were empty. In a related electron microscopic study of the chordoma *in vivo* and *in vitro* (Horten and Montague, 1976), granular and filamentous deposits were observed in cytoplasmic vacuoles, suggesting the presence of mucopolysaccharides.

By light microscopy, both the ecchordosis and notochord were largely composed of vacuoles which have been variously regarded as intra- or extracellular. With electron microscopy, it is evident that both intracellular vacuoles and large intercellular spaces are present. Apart from the obvious difference in limiting membranes (e.g., the intercellular spaces are associated with pinocytotic vesicles) the vacuoles and intercellular space may also be distinguished by the ultrastructural features of their contents. Vacuoles as previously stated are filled

Fig. 8. Embryonic chick notochord. Confluent cytoplasmic vacuoles and distended cisternae of granular endoplasmic reticulum containing many papillary cytoplasmic infoldings. The endoplasmic reticulum is bordered on both sides by clear cytoplasmic vacuoles. × 21,000

either with dense granular deposits resembling glycogen (ecchordosis) or are empty (notochord), while the intercellular spaces contain clusters of collagen and its precursors.

The origin of the intracellular vacuoles in notochordal tissue and its presumed derivatives remains controversial. In the only prior electron microscopic study of the ecchordosis (Wyatt et al., 1971), no definite source for the vacuoles was noted. Similarly in the present report, the intracellular vacuoles in the ecchordosis were never observed to merge with other cytoplasmic structures. There are many prior ultrastructural investigations of the notochord in laboratory animals including the rat (Cancilla et al., 1964; Peña et al. (1970), the guinea pig (Spjut and Luse, 1964), the rabbit (Leeson and Leeson, 1958), the urodele (Waddington and Perry, 1962), the mouse (Jurand, 1974), and the chick (Jurand, 1962; Ruggeri, 1972) as well as a report of the nucleus pulposus in the juvenile human (Meachim and Cornah, 1970). These studies are remarkably consistent in their observation of focally dilated granular endoplasmic reticulum as the source of notochordal vacuoles. However, in the present report, although the endoplasmic reticulum was focally expanded, there was no evidence of an actual merging of reticulum with the cytoplasmic notochordal vacuoles. Only in the chordoma maintained in organ culture was there an occasional protrusion of a focally distended vesicle of endoplasmic reticulum into a cytoplasmic vacuole (Horten and Montague, 1976).

It is customary to regard the ecchordosis and chordoma as derivatives of notochord (Horwitz, 1941). This light microscopic and clinical impression may now be supplemented by the remarkably similar ultrastructural features shared by all three entities. The ecchordosis is virtually identical with embryonic notochord, as would be anticipated if the ecchordosis is indeed a notochordal remnant. In contrast the chordoma, although retaining all the intra- and extracellular features of notochord, has an extensive Golgi apparatus and many clusters of mitochondria and endoplasmic reticulum appropriate to an actively expanding tumor.

The authors wish to thank Dr. Mary M. Herman for her help and guidance in this work. Supported by Graduate Neuropathology Training Grant 5T01 NS-5500-10 from the National Institute of Neurological and Communicative Disorders and Stroke, U.S. Public Health Service.

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