Fine Structural and Enzymehistochemical Observations on the Notochord of *Ichthyophis glutinosus* and *Ichthyophis kohtaoensis* (Gymnophiona, Amphibia)*

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**Summary.** The notochord of *Ichthyophis glutinosus* and *I. kohtaoensis* consists of peripheral flattened cells characterized by a well-developed system of rough endoplasmic reticulum, bundles of tonofilaments, and abundant glycogen particles. These cells contain furthermore fairly high activities of α-naphtyl-acetate esterase and 4-chloro-5-bromoindoxyl acetate esterase as well as acid phosphatase which was found in lysosomal localization. The huge intracellular vacuoles of the centrally situated cells possibly originate from electron translucent spaces within the glycogen fields of the peripheral cells.

The notochord sheath consists of variously differentiated layers of collagen fibers and of an elastica externa. The diameters of the collagen fibers increase from the inner towards the outer region of the sheath. A peculiar feature of the *Ichthyophis* notochord sheath is a ring of mineralized collagen. The notochord of the caecilians investigated is compared with that of anurans, urodeles, and several groups of fish.

**Key-Words:** Notochord — Caecilians — Histochemistry — Ultrastructure.

**Introduction**

The electron microscope has revealed completely different types of notochord organization in the non-vertebrate chordates and hemichordates: In *Branchiostoma* the notochord consists of specialized muscle-plates, in ascidians of solid yolk- and glycogen-containing epithelial cells, in appendicularians of flat epithelial cells surrounding an extracellular viscous material, in enteropneusts of vacuolated cells, in part bearing surface specializations like microvilli and cilia (Cloney, 1969; Flood, 1968; Olsson, 1965; Welsch, 1968; Welsch and Storch, 1970a,b).

In contrast to this heterogeneous assembly the notochord of the few vertebrate chordates so far examined on the ultrastructural level appears to be built up in a rather uniform way: In different species of lampreys (Schwarz, 1961; Welsch and Storch, 1970), in the hagfish (Flood, 1969), in teleosts (Welsch and Storch, 1970) and bullfrog tadpoles (Bruns and Gross, 1970) it consists as in the hemichordates of vacuolated fibre containing cells, the epithelial character of which has been particularly stressed by Schwarz (1961).

The aberrant structure of the notochord tissue of avian and mammalian embryos (Duncan, 1957; Jurand, 1962; Leeson and Leeson, 1958) appears to be due

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to the negligible supportive function of the notochord in these animals. In con-
sequence of our own work and the recent paper by Bruns and Gross (1970), in the
present study the fine structure of the notochord of the tropical caecilians has
been investigated. This group is of particular comparative anatomical interest
because of its numerous primitive characters and its isolated position within the
amphibians. Since according to light microscopical studies (Remane, 1936) there
is a great deal of variation in the construction of the vertebrate notochord sheath,
special emphasis has been laid on this structure.

Material and Methods

4 cm long embryos of *Ichthyophis kohtaoensis* collected in Chiang Mai (Northern Thailand)
and 10 cm long young specimens of *Ichthyophis glutinosus* collected near Medan (Northern
Sumatra/Indonesia) have been used for the present study. For electron microscopy tissues
containing parts of the notochord were fixed in cold phosphate buffered 3.5% glutaraldehyde
for two hours, rinsed in phosphate buffer (pH 7.5), postfixed in 4% osmic acid, dehydrated in
ethanol, and embedded in araldite. Thin sections were stained for five minutes each with uranyl
acetate (saturated solution in 70% methanol) and lead citrate, and examined in a Zeiss EM 9 A
and Siemens E 101 microscope.

For enzyme histochemistry the animals were fixed for 6 h in cold neutral 10% formaline,
stored for one week in a cold gum-sucrose solution. Positive results were obtained for acid
phosphatase (Barka and Anderson, 1963), α-naphthyl-acetate esterase (Barka and Anderson,
1963) and 4-chloro-5-bromoindoxyl acetate esterase (Holt, 1958).

Observations

At the periphery of the notochord the cells are of flattened shape, whereas
those in the center are polygonal containing a huge intracellular vacuole. All noto-
chord cells are interconnected by relatively infrequent desmosomes. The plasma
membranes of neighbouring peripheral cells are extensively interdigitated. The
most prominent cytoplasmic structures of these peripheral cells are 1) well devel-
oped cisterns of rough endoplasmic reticulum, which often are slightly dilated and
contain a fine-granular material, 2) bundles of tonofilaments occurring predomi-
nantly in the cellular periphery, 3) frequent microtubules, 4) fields of glyco-
gen particles (α- and β-particles). These glycogen fields often contain vacuole-like
spaces which can assume considerable size (Fig. 1). Less frequent are membrane-
bound granules of various densities which appear to be concentrated at the basis
of the cells. This region is further characterized by rare pinocytotic vesicles
(spined and smooth-surfaced) and halfdesmosomes attached to the basement
lamina surrounding the whole notochord. Mitochondria with a rather dense
matrix and sparse cristae are rather infrequent. The nucleus is of elongated shape
and contains two typical nucleoli.

The centrally situated cells are dominated by a single huge intracellular
vacuole being not surrounded by any recognisable membrane. It contains in
loosely distributed fashion glycoegen particles. The cytoplasmic particles are
greatly reduced in number, except for the tonofilaments. The nucleus appears
to be rather inactive being filled largely with dense chromatin.

The notochord cells are surrounded by a fibrous sheath which is closely atta-
cched to the notochord basement lamina. It consists 1) of a relatively thin layer of