Cell Tiss. Res. 153, 175—187 (1974)

Electron Microscopical Observations on Kölliker's and Hatschek's Pit and on the Wheel Organ in the Head Region of Amphioxus (*Branchiostoma lanceolatum*)

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Received August 1, 1974

Summary. Kölliker's and Hatschek's pit and the wheel organ in the head region of *Amphioxus* have been studied with the electron microscope. Kölliker's pit is lined by ciliated cells lacking specific ultrastructural characteristics which might suggest an olfactory function. No nerve terminals have been found near this structure. The wheel-organ consists of tall ciliated cells, which occasionally have been found to be innervated and which are clearly marked off the rest of the epithelial lining of the oral cavity. The epithelium of Hatschek's pit again is ciliated and possesses markedly euchromatin rich nuclei. In the area of Hatschek's pit numerous epithelial cells have been observed which possess accumulations of small granules and vesicles in their basal cytoplasm. Such basally granulated cells occur in the immediate neighbourhood of blood spaces of the glomus and other vessels; the connective tissue between blood and epithelium is loosened up or has completely disappeared. The question of homology of the structures investigated with the olfactory groove and hypophysis of vertebrates is briefly discussed.

Key words: Branchiostoma — Kölliker's pit — Hatschek's pit — Wheel organ — Electron microscopy.

Introduction

In the head region of *Amphioxus* two ciliated pits occur which have aroused the interest of comparative anatomists since more than 100 years: Kölliker's pit, an epidermal invagination on the left outer side of the rostral area of the animal, and Hatschek's pit in the roof of the oral cavity.

Kölliker's pit is a remainder of then europorus (for literature see Franz, 1927) and consists of prismatic ciliated cells (Krause, 1921; Franz, 1927). Frequently it is considered to be homologous to the olfactory groove of vertebrates, an idea which was first put forward by Kölliker (1843). Therefore it is also called olfactory pit. Franz (1927) states that it is not innervated, which was confirmed by Wollenhaupt (1934). Thus, an olfactory function appears to be unlikely. Stendell (1914) compares it to the "olfactory-hypophyseal" groove of vertebrates.

Hatschek's pit actually is part of the wheel organ of Johannes Müller, which is either postulated to be of entodermal (Franz, 1927, a.o.) or of ectodermal (Legros, 1898; Drach, 1948) origin. The wheel organ is located in the roof of the oral cavity where it forms strands of prismatic ciliated cells. Hatschek's pit is a

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* With financial support of "Deutsche Forschungsgemeinschaft" (We 380/5).

groove in the frontal area of the wheel organ, which originally has been thought to be a sensory organ (Hatschek, 1884); later the majority of investigators considered it to play a role in the feeding mechanism of *Amphioxus*, producing a water current. This organ is of particular interest since it has been homologized with various structures of tunicates and vertebrates: e.g. with the mouth and adhesive organ of Ganoid fishes (van Wijhe, 1914), with a transformed anterior gut region and in particular with the stomochord of Enteropneusts (Franz, 1927), with the pituitary gland of vertebrates (Legros, 1898; Drach, 1948; a.o.) and with the ciliated groove and the neural gland of Tunicates (Hatschek, 1884; Julin, 1881; Drach, 1948; a.o., further references see Huus, 1937; Seeliger, 1911; Brien, 1948) which also has been postulated to correspond to the vertebrate pituitary gland. Franz (1927) who has studied *Amphioxus* most extensively, rejects this assumed homology between wheel organ, inclusive Hatschek's pit, and the pituitary gland. Drach (1948) mentiones a particular homology between Hatschek's pit and the hypophyseal duct of cyclostomes.

The aim of the present electron-microscopical investigation is to find more substantial evidence for the assumed functions and significances of the above mentioned organs.

Material and Methods

Living specimens of adult *Amphioxus* have been obtained from the Marine Biological Station, Helgoland. 1 mm thick slices of the animals were fixed for two hours in cold 3.5% phosphate-buffered glutaraldehyde (pH 7.4). After repeated rinses in phosphate buffer the tissues were postfixed in 1% osmic acid for 2 hours and after dehydration in ethanol embedded in Araldite. For light microscopical orientation an almost complete series of Richardson-preparations of the head region of 4 animals (cross- and longitudinal sections) has been studied. Sections for electron microscopy have been stained for 5 mins each in uranyl acetate (saturated solution in 70% methanol) and in lead citrate. Electron microscopes: Zeiss EM 9 A Siemens 101.

Observations

A. Kölliker's Pit

Although being in direct continuation with the epidermis, the fine structure of the cells of Kölliker's pit differs profoundly from that of the epidermal cells which has been described by Olsson (1961) and Welsch (1968). The pit cells form a single layered prismatic ciliated epithelium (Fig. 1). The elongated or oval nuclei are rich in euchromatin and contain one or two prominent nucleoli. The pale cytoplasm is characterized by a relative paucity of cell organelles (Fig. 2). Mitochondria are few in number and predominantly located in the apical cytoplasm, they posses a few short cristae and a fine granulated matrix. The small Golgi apparatus is in supranuclear position and is surrounded by small vesicles. Some of its dilated cisterns contain electron dense material. Rough E. R. cisterns again are few in number; the outer nuclear membrane bears a few ribosomes, free ribosomes are scattered throughout the cytoplasm. In the cellular apex light vesicles and bigger granules with heterogeneous contents occur. Near the basal plasma membrane a row of light vesicles is to be found. Distributed throughout the cytoplasm are small bundles of microfilaments. Apically the cells bear a few

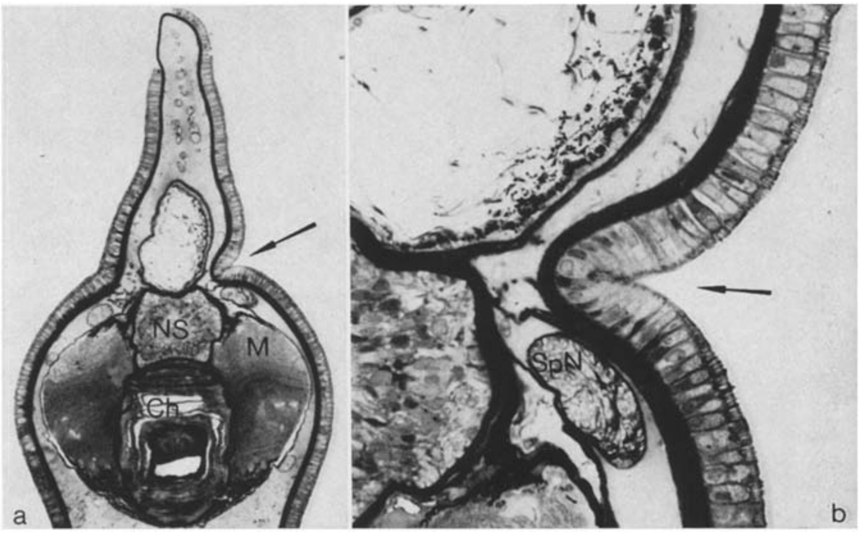

Fig. 1a and b. Kölliker's pit (arrow) transverse section through the head region. *NS* nervous system, *Ch* Chorda dorsalis, *M* muscle, *SpN* spinal nerve. (a) $\times 120$, (b) $\times 360$

microvilli and one cilium each. The latter are characterized by the $9 + 2$ pattern, rootlets and apposed centrioles (Fig. 2). Laterally the cells form short interdigitations.

No typical cell junctions have been observed; apically, however, the plasma membranes of neighbouring cells locally appear to be slightly thickened and the intercellular space is narrowed to some extent.

The epithelium rests upon a thick basal lamina and a voluminous layer of collagen fibres. No nerve cell processes have been detected near the epithelium. In the neighbourhood of the pit a spinal nerve is situated under the collagen layer (cf. Franz, 1927).

B. Oral Cavity, in Particular the Wheel Organ and Hatschek's Pit

a) The Normal Epithelium of the Oral Cavity (Figs. 3, 4) is cuboidal and single layered and rests upon a thick basal lamina and a layer of regularly arranged collagen fibrils. The pale euchromatin-rich nuclei are of variable outlines, frequently possess deep indentations and contain in their periphery a prominent nucleolus. The cyytoplasm contains few organelles and occasionally lipid inclusions. Regularly in the basal cytoplasm rows of vacuoles occur which are surrounded by filament bundles. Frequently the cells contain electron dense pigment granules (Fig. 4). The apex bears microvilli, laterally the cells extend interdigitating processes.

b) Wheel Organ. Two to three cells form a transition from the normal cuboidal epithelium of the oral cavity to the prismatic ciliated epithelium of the wheel organ (Fig. 3). The nuclei of this epithelium form a broad band and are relatively rich in heterochromatin (Figs. 3, 4). Those cells, in which the nucleus is in a more

Fig. 2. Epithelial cell of Kölliker's pit, arrows point at basal vacuoles. ×18000

basal location, generally possess a paler cytoplasm than those with a more apically located nucleus. The cell organelles are predominantly in supranuclear localization, but relatively few in number. Striking are many vesicular inclusions and elements presumably representing residual bodies, since their contents is very heterogeneous and often consists of myelin figures. Occasionally lipid droplets and small fields of glycogen (α-particles) have been observed. Individual cells exhibit apical protrusions containing light vesicles. In the neighbourhood of blood spaces some cells occur which contain basal granular and vesicular inclusions. Corresponding cells are described in more detail under the heading Hatschek's pit etc.

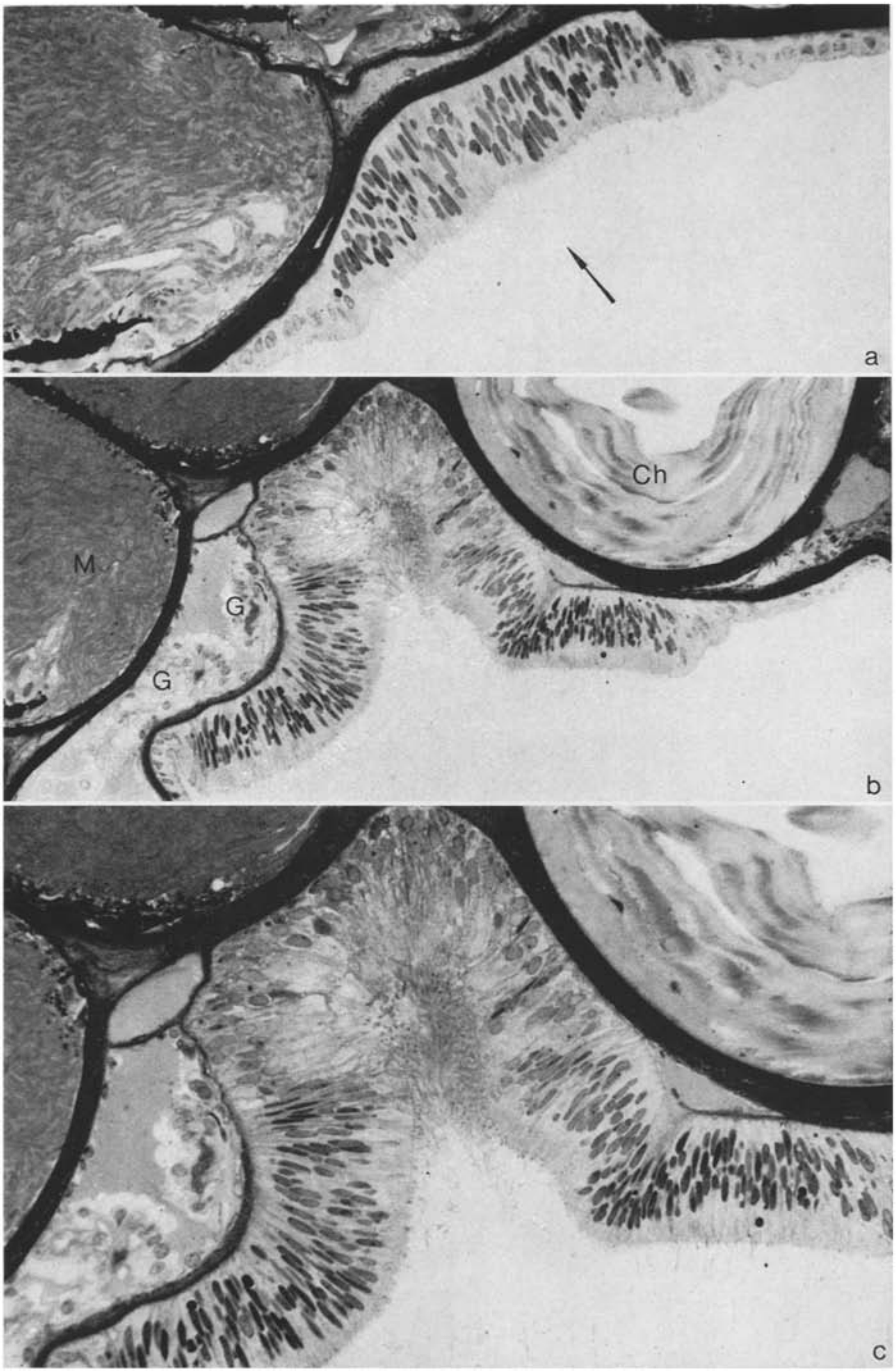

Fig. 3a—c. Wheel organ and Hatschek's pit, transverse sections through the area of the oral cavity. (a) ciliated epithelial ridge of the wheel organ (arrow), ×360; (b, c) Hatschek's pit, note the difference in respect of the nuclear densities of the pit cells (light nuclei) and of the neighbouring cells of the wheel organ (darker nuclei). *M* muscle, *Ch* Chorda dorsalis, *G* Glomus. (b) ×280, (c) ×380

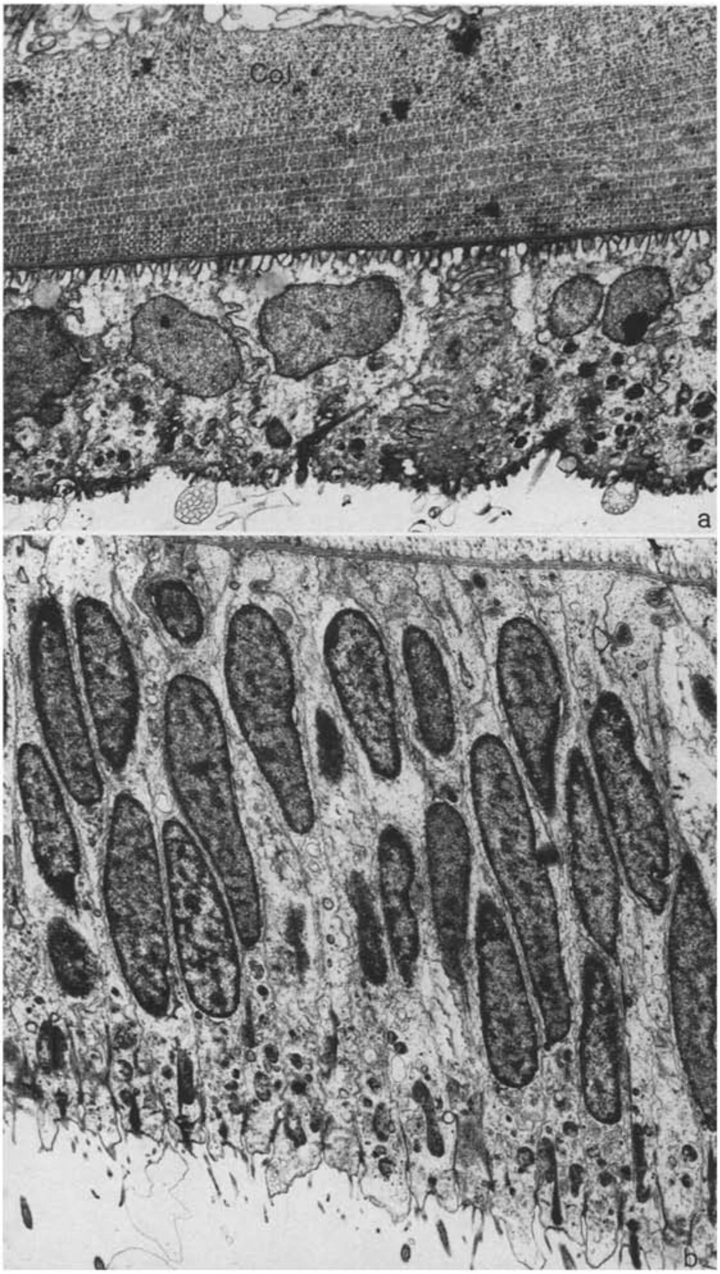

Fig. 4. (a) Normal epithelium of the oral cavity; (b) epithelium of the wheel organ; *Col* dense layer of collagen fibrils. ×18000

In all parts of the wheel organ a small number of nerve cell processes has been found (Fig. 5). These generally occur individually or in groups of 2 to 5 and nor-

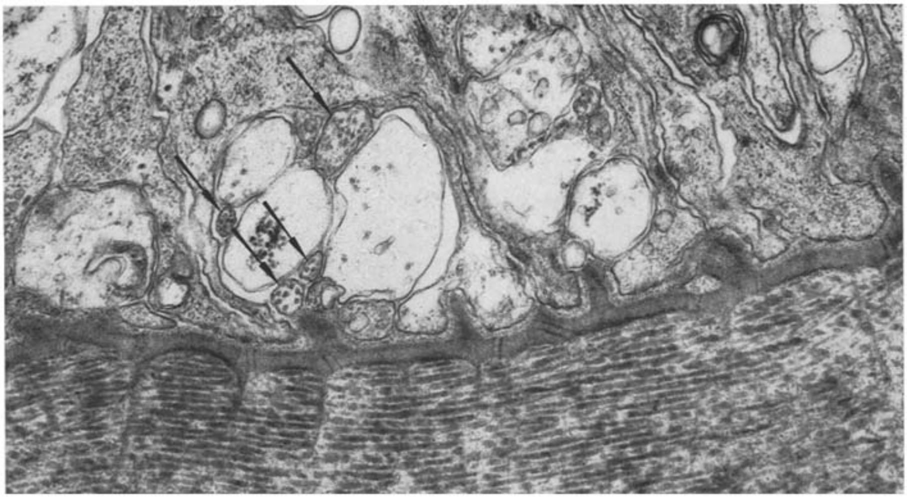

Fig. 5. Nerve cell processes (arrows) containing microtubules. ×18000

mally contain microtubules. Typical nerve terminals have not been seen, however, bigger profiles containing a few light vesicles, glycogen particles (β-particles) and one to two mitochondria possibly correspond to synaptoid structures.

c) Hatschek's Pit and Those Parts of the Wheel Organ in Its Immediate Neighbourhood. The wall of Hatschek's pit consists of highly prismatic cells which rest upon the usual thick basal lamina and the layer of regularly arranged collagen fibrils (Fig. 3). The nucleus is pale and euchromatin-rich, contains a prominent nucleolus and generally possesses a smooth surface (Figs. 3, 6). The outer nuclear membrane bears ribosomes. The cell organelles are predominantly to be found in apical localization: individual rough E.R. cisterns, roundish mitochondria with a few short cristae and tubules, a small Golgi apparatus and microtubules. Ribosomes occur throughout the cytoplasm but frequently are concentrated in the basal parts of the cells. Around the Golgi apparatus and below the apical plasma membrane numerous vesicular and granular inclusions are to be found: spined vesicles, smooth faced vesicles, small granules with fine floccular or fine particular contents, typical, very irregularly shaped smooth faced vesicular elements (Fig. 6), bigger granules containing homogeneously distributed fine particulate or loosely arranged filamentous material, resembling small mucus droplets. Regularly a few small granules and vesicles also occur near the basal plasmamembrane.

The narrow apex bears a cilium ($9+2$ pattern, long rootlet, associated centriole) and long microvilli containing microfilaments, which in part merge with bundles of microfilaments of the apical cytoplasm. The lateral and basal plasmamembranes take a rather straight course. Typical cell junctions have not been found; they presumably are represented by areas in which the intercellular space is slightly narrowed and a thin layer of electron dense material is opposed to the inner aspects of the plasma membrane.

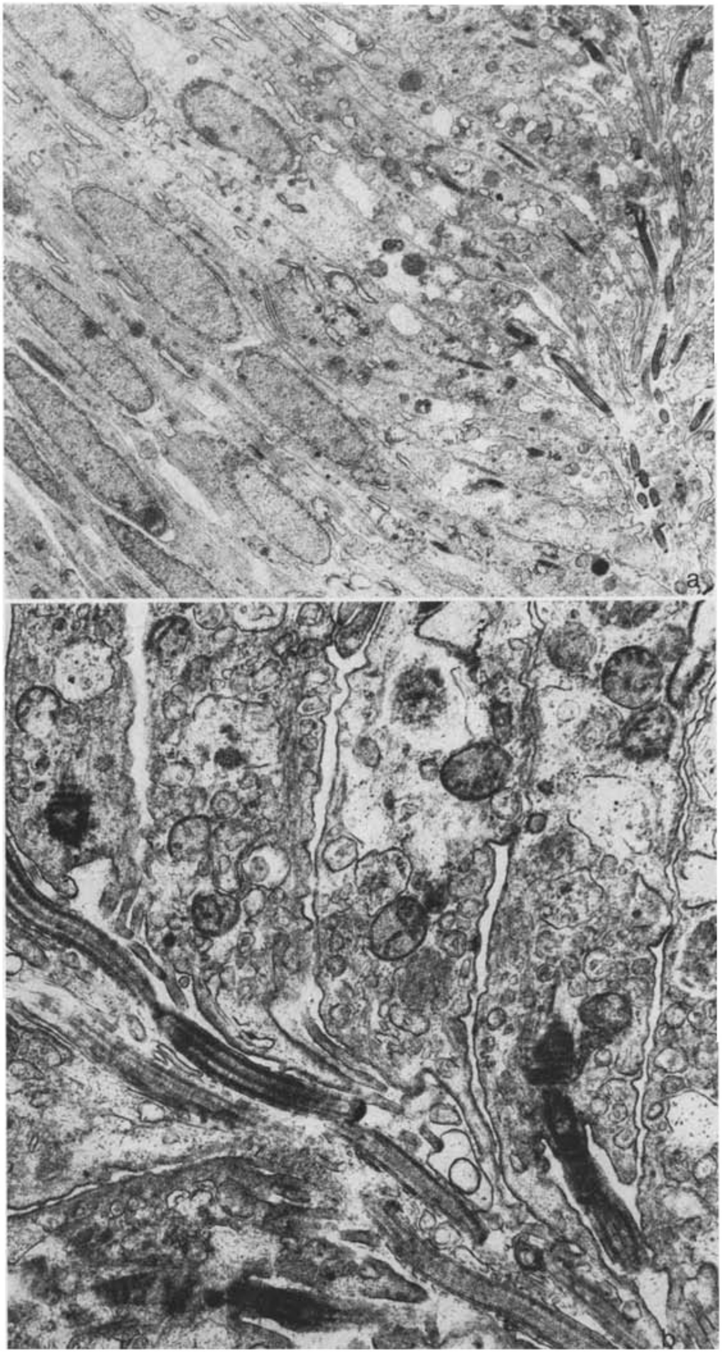

Fig. 6a and b. Epithelium of Hatschek's pit; note the pale nuclei, the cilia indicate the narrow lumen. (b) details of the apical cytoplasm, note the numerous irregularly shaped vesicular elements. (a) ×6000, (b) ×18000

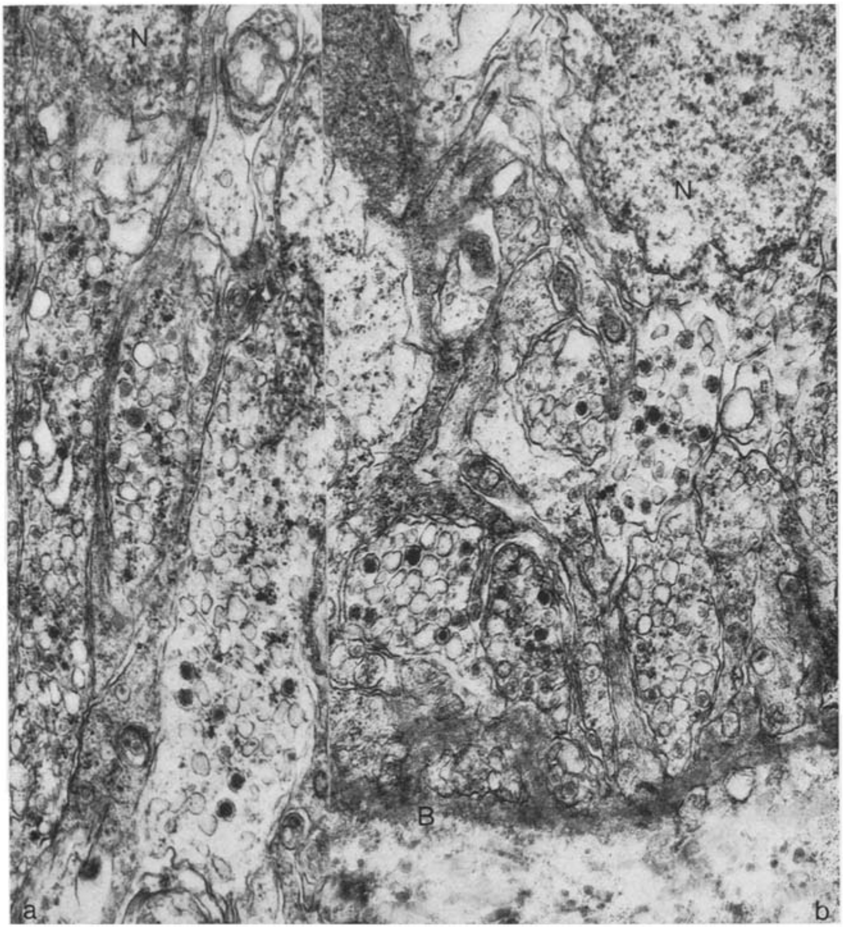

Fig. 7 a and b. Basal parts of epithelial cells in Hatschek's pit containing granules and vesicles. *N* nucleus (euchromatin-rich), *B* basement lamina. ×18000

The epithelium of the pit proper, as described above, merges at the surface of the pit with that of the wheel organ which is easily recognized by its darker nuclei (Fig. 3). In the transitory zone of the two types of epithelial cells, cellular elements occur which contain relatively numerous rough E.R. cisterns and abundant small vesicles and granules in their basal cytoplasm (Fig. 7). It is difficult to decide whether all of these cells reach the surface of the epithelium and whether they belong to the pit cells or to the typical cells of the wheel organ. Their nuclei often are relatively pale as those of the pit and often exhibit deep indentations. Frequently the cells contain both basally located vesicles and granules (Figs. 7), sometimes vesicles predominate, sometimes the granules form the majority. Some of the cells containing accumulations of basal granules definitely seem to belong to the wheel organ, since their nuclei are rich in heterochromation and since they contain myelin figures in residual bodies. In these cells the cytoplasm

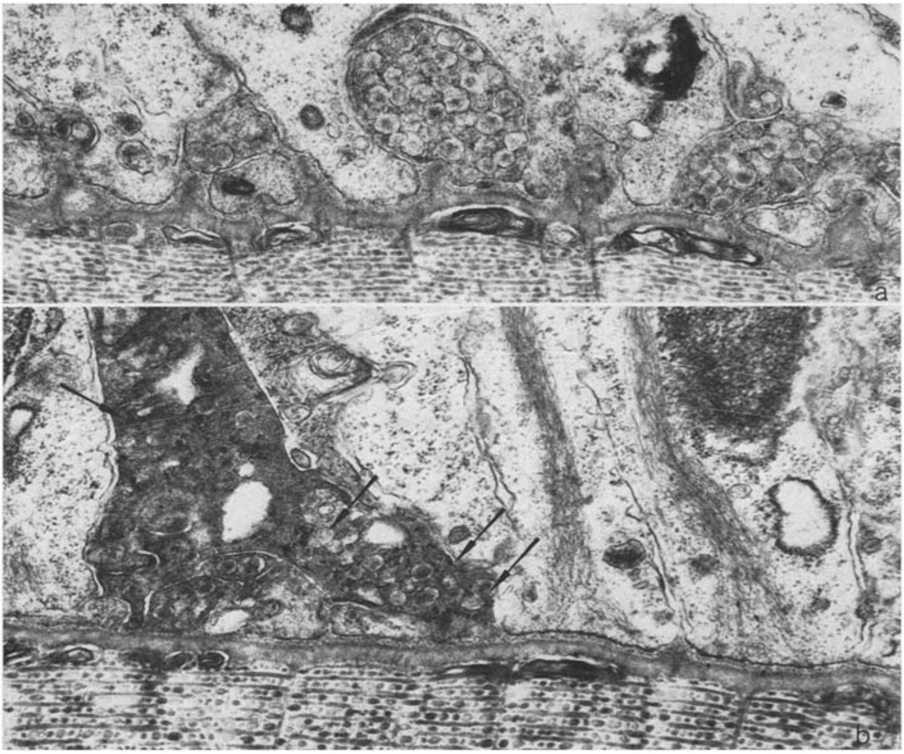

Fig. 8a and b. Basal parts of epithelial cells in the area of Hatschek's pit possessing a relatively dense cytoplasm and basal granules. (a) section through basal processes (arrows) containing small granules. (b) basal foot-like process, arrows point at granules. ×18000

often is relatively dense (Fig. 8), an unexplained phenomenon which has also been observed in a number of nerve cells of *Amphioxus* by Meves (1973). Similar cells have been found in other parts of the wheel organ. The basal parts of these cells can be transformed into stout processes resembling profiles of neurosecretory neurons (Meves, 1973). Such extensions can be found individually between the bases of the other epithelial cells (Fig. 8), but have never been observed to break through the basal lamina.

Below these basally granulated cells blood spaces of the glomus, a pulsating part of the carotid artery (Johannes Müller, 1844; Franz, 1927), and of other vessels occur. The intervening parts of the collagen layer are markedly loosened up or even completely disappear (Fig. 9), leaving only the basal lamina between blood space and epithelium.

Discussion

The electron microscopical investigation of Kölliker's and Hatschek's pit and of the wheel organ of *Amphioxus* allows some comments on the possible function of these structures. We do not intend to give a complete survey over the extensive literature on them.

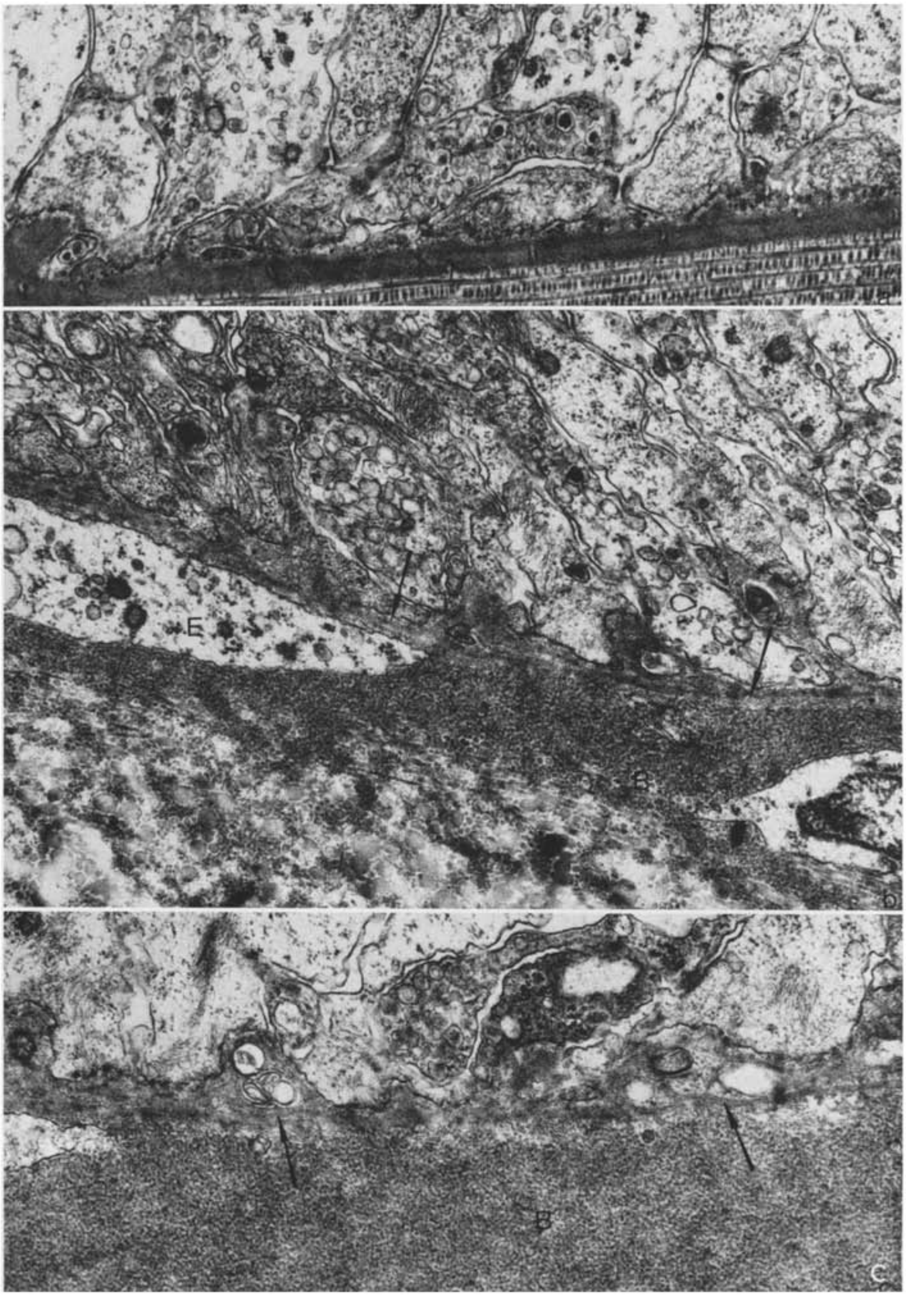

Fig. 9a—c. Basal granule-containing parts of various epithelial cells in the area of Hatschek's pit, (a) over a typical basement lamina and a thick layer of collagen fibrils, (b, c) in areas where blood spaces come into close contact with the epithelium. The blood space (*B*) is separated from the epithelium only by a relatively thin basement lamina (arrows); *E*: possibly endothelial cell. ×18000

Our findings do not support the assumption that Kölliker's pit represents an olfactory organ, since no indications of an innervation have been found—confirming Franz (1927) and Wollenhaupt (1934)—and since the epithelial cells do not exhibit any structural specializations which e.g. can be found in the innervated receptor cells of the cirri of *Amphioxus* (Tjoa, in preparation). Franz (1927) feels that the ciliary beat of the epithelium of Kölliker's pit brings fresh water into the neighbourhood of the brain vesicle.

The main finding in respect of the wheel organ and Hatschek's pit appears to be that they contain epithelial cells with basal accumulations of vesicles and granules and that nerve fibres have been detected in the wheel organ. Since such basally granulated cells occur near underlying blood spaces, which at least in part are said to be pulsating (Müller, 1844), we assume that these cells are endocrine secretory elements, which extrude their basal vesicles and granules into the blood space. This idea is supported by the fact that the normally rather tight layer of collagen under the epithelium of the oral cavity is loosened up or even has completely disappeared in areas where the blood spaces approach the cells containing basal secretory granules. Possibly two types of these cells occur, one resembling the cells of Hatschek's pit and one those of the neighbouring wheel organ. Vesicles and granules in one cell suggest that two types of secretory products may be released by one cell. Especially those cells resembling the typical epithelial cells of Hatschek's pit exhibit further specializations: an increased amount of rough E.R. and an irregularly shaped indented nucleus.

The significance of the innervation of the wheel organ remains unclear, since no typical terminals have been found. The exact relations between nervous system and wheel organ and Hatschek's pit need further studies.

There are no specific agreements in the fine structure of Hatschek's pit and the wheel organ of *Amphioxus* on the one side and of the neural gland of tunicates (Georges, 1967; Lane, 1971).

The above mentioned findings suggest that the wheel organ and Hatschek's pit contain cells which have a function which might correspond to that of the vertebrate adenohypophysis. This supports the idea of a homology of wheel organ inclusive Hatschek's pit and adenohypophysis put forward by several earlier authors because of an agreement of location (Legros, 1898 and many others). The answer to the question whether wheel organ and Hatschek's pit are derived from the ecto- or from the entoderm is not of a particular importance for a solution of the question of homology (Remane, 1951).

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