A C T A I C H T H Y O L O G I C A E T P I S C A T O R I A

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Fish anatomy

STOMACH AS AN ADDITIONAL RESPIRATORY ORGAN, AS EXEMPLIFIED BY *ANCISTRUS MULTISPINNIS* (COUVIER ET VALENCIENNES, 1937), SILURIFORMES, TELEOSTEI

WYKORZYSTANIE ŻOŁĄDKA JAKO DODATKOWEGO NARZĄDU ODDECHOWEGO NA PRZYKŁADZIE *ANCISTRUS MULTISPINNIS* (COUVIER I VALENCIENNES, 1937), SILURIFORMES, TELEOSTEI

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The micro- and ultrastructure of intestinal wall of 29 individuals of *Ancistrus multispinnis* was examined under the scanning and transmission electron microscopes. The presence of cellular structures, analogous to type I pneumocytes forming the mammalian pulmonary lining, was identified in the stomach corpus. Cells of a morphology similar to that of neuroendocrine cells of the salamander lungs were found as well. The pulmonary-cerebral capillaries were identified in the stomach corpus. The observations demonstrated that the stomach of *A. multispinnis* was able to function as an additional respiratory organ.

INTRODUCTION

Carter and Beadle (1931), who observed the behaviour of *Ancistrus* sp. and analysed the air filling its stomach, put forward a suggestion that the species was using the stomach as an additional respiratory organ.

Preliminary observations of the *A. multispinnis* stomach corpus under the transmission electron microscope (TEM) revealed a single layer of flat epithelial cells the structure of which resembled pneumocytes occurring in mammalian lungs. The stomach part in question contains numerous capillaries situated between corpuses of epithelial cells (Satora 1998). The thinnest barrier separating the capillaries and the gastric lumen was found to measure 0.6 μm. In addition, the epithelial cells were observed to contain oval lamellar bodies (Satora 1998).

The present work was aimed at a detailed examination of the micro- and ultrastructure of *A. multispinnis* stomach in an attempt to resolve whether, and to what extent, Carter and Beadle (1931) were right in venturing an opinion on the role of the species' stomach in breathing. Should this opinion be confirmed, the question of the material (structural) basis of the process ought to be addressed.

MATERIAL AND METHODS

Histological and ultrastructural analyses were made on 29 *A. multispinnis* individuals of varying body weight. Prior to the examination, all the individuals were kept in a 120-dm^3 tank, at about 25°C. Since Carter and Beadle (1931) observed that *Ancistrus* sp. would use its stomach as an additional respiratory organ under hypoxia only, the tank was not aerated. The fish were fed an Ichtio-vit (Tropical) plant food. The individuals observed were classified as belonging to *A. multispinnis*, based on a key worked out by Gosline (1947).

Before fixation, the fish were anesthetised in the aqueous solution of MS-222, weighed, and measured. The intestine was dissected out under an MST 120 stereomicroscope.

Tissues intended for ultrastructural analyses were treated for 2 h in 1% glutaraldehyde with 0.2 M cacodylate buffer. The preparations were soaked for 15 min. in cacodylate buffer. Subsequently, they were transferred to 2% osmium tetraoxide with cacodylate buffer and left soaking for 1.5 h, following which they were dehydrated in an acetone series and embedded in Epon 812. Epon block fragments were cut into ultra-thin sections, collected on a copper netting and contrasted with uranyl acetate and lead citrate. The sections were viewed under a JEOL 100-SX transmission electron microscope. The air-blood barrier thickness was measured with a ruler on TEM microphotographs.

Tissues intended for scanning electron microscope (SEM) examination were rinsed in cacodylate buffer with 2 mN EGTA to remove the mucus. They were then fixed in glutaraldehyde with cacodylate buffer and 1% picrynic acid and dehydrated in acetone series. The mounts were dried at CO_2 critical point, gold-coated, and viewed at 15 kV under a JSM-5410 scanning electron microscope.

RESULTS

The SEM examination of the internal surfaces of the stomach and the duodenum showed the epithelium to be covered by mucus and to be visible only after the mucus was rinsed off. A section through the stomach cardia revealed a single epithelial layer consisting of polygonal, columnar, strictly contiguous cells developing microvilli in their apical part (Fig. 1).

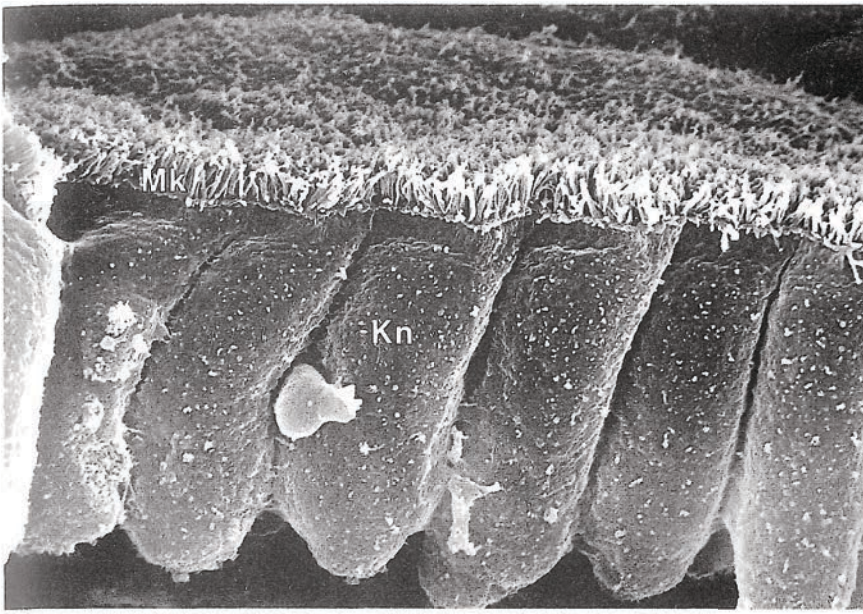

Fig. 1. “Edge” of the stomach cardia epithelium. The photograph shows columnar strictly contiguous epithelial cells. Their apical part is covered with microvilli. EC—columnar epithelial cells; MV—microvilli. (SEM) × 3 400

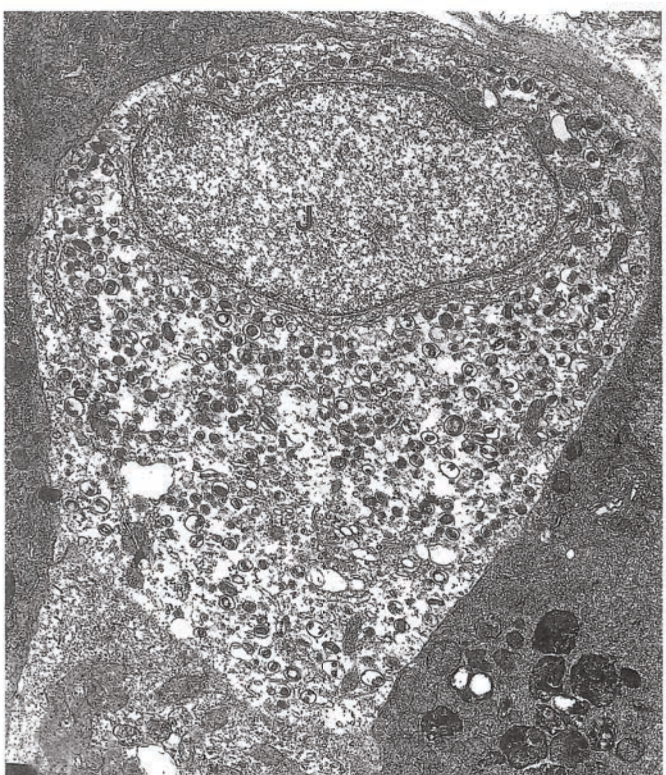

Fig. 2. Ultrastructure of neuroendocrine-like cell of the stomach corpus. N—nucleus. (TEM) × 8 400

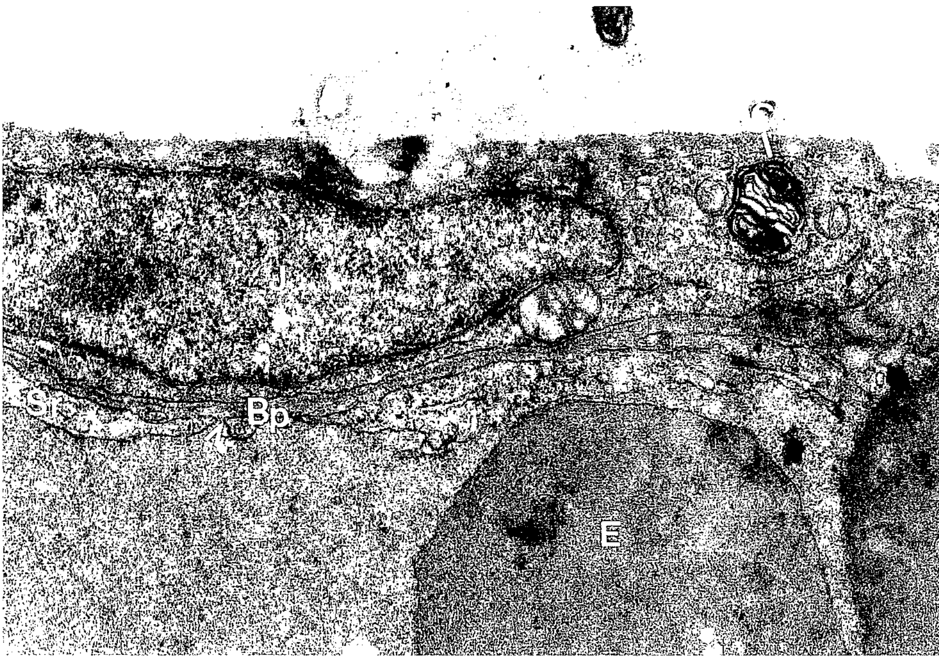

Fig. 3. Section of the stomach corpus epithelium. Arrows indicate single pores. BM—basement membrane; E—erythrocyte; EN—capillary endothelium; GE—gastric epithelium; GL—gastric lumen; LB—lamellar bodies; N—nucleus. (TEM) × 19 740

Numerous pores, which are openings of gastric glands, are seen on the sides of the stomach rugae. The apical parts of some of the rugae show the presence of gastric gland secretion, occurring in the form of plugs and attesting to the secretory activity of this part of the stomach.

The TEM examination of the stomach corpus revealed a single layer of flat epithelial cells, the characteristic structure of which resembled that of type I pneumocytes in the mammalian lungs. Numerous capillaries situated between corpuses of the epithelial cells were visible in this part of the epithelium as well.

In addition, single cells which morphologically resembled the neuroendocrine cells lacking nerve endings were identified immediately underneath the epithelium (Fig. 2). They typically had large nuclei and light-coloured cytoplasm, a well-developed Golgi apparatus, and numerous vesicles with dense content. The vesicles were filled with electron-dense material surrounded by a clearly lighter space.

The capillaries in the stomach corpus were situated between corpuses of the epithelial cells. The strongly flattened epithelial cell microvilli were the only barrier separating the capillaries from the gastric lumen. The capillary walls were lined with strongly flattened endothelium. The only thicker structures visible in the capillary endothelium occurred where nuclei and cell organelles were present.

A characteristic feature of the capillary endothelial cells in the stomach corpus was the presence of solitary pores in the space between the basement membrane and the capillary endothelium (Fig. 3).

DISCUSSION

The cause of the immense species richness of the Amazon area from which *A. multispinnis* originates is the diversity and continuous variability of the tropical rain forest. Its periodic flooding leads to the extraordinary biodiversity as plants and animals have to adapt to ever changing habitats. Flooded forests are short-lived refugia and centres in which evolution-related changes are in progress. In dry seasons (during glaciations), the tropical forest area was divided into a number of isolated sub-areas which served as refugia to numerous organisms. Consequently, original populations were geographically divided into parts the genetic pools of which were unable to mix (Goulding 1993), a situation favouring speciation. During interglacial periods, new species which had emerged in different refugia could disperse and colonise larger and larger areas of the tropical rain forest (Colinvaux 1989).

The theory outlined above is supported by the fact that the Amazon drainage houses the species-richest freshwater ichthyofauna on the Earth, consisting of as many as about

3000 species. The Rio Negro alone harbours more than 130 species feeding, to a higher or lower extent, on detritus. Leaves and lignified plant fragments provide nutrition for numerous fish species, while plant detritus (non-living organic matter) lies at a base of many food chains (Goulding 1993).

Our histological and ultrastructural examination of the stomach corpus epithelium in *A. multispinnis* demonstrated the presence of cells the structure of which is similar to that of type I pneumocytes occurring in lungs of the Dipnoi, *Polypterus*, and mammals (Zacoone et al. 1995). The epithelial cells present in the respiratory part of the stomach are clearly differentiated into a part containing the nuclei (corpus) and a strongly flattened and extended peripheral part (microvilli). The epithelial cells form a single layer, whereby they come in contact with one another along their lateral surfaces only. The blood capillaries are separated from the gastric lumen by a continuous barrier formed by the epithelial cell microvilli. Within the stomach corpus, the capillaries are situated between the epithelial cell corpuses and do not protrude into the gastric lumen, as is the case in amphibian lungs. The present study revealed the presence, in the *A. multispinnis* stomach corpus, of numerous capillaries situated on crests and sides of stomach rugae.

A very interesting problem, particularly in view of the fact that the stomach corpus of *A. multispinnis* contains a thin air-blood barrier of about 0.6 μm minimal thickness (Satora 1998), is how the fish protects its very thin barrier in the stomach (the thin epithelium) from damage by large food particles. Presumably, a preliminary stage of protection of sorts is provided by the strongly developed gill rakers on each of the four gill arches; on the other hand, the presence of a copious amount of mucus in the stomach cardia seems to secure a basic protection from mechanical damage. Similarly, the respiratory intestine of the pond-loach contains mucus cells which most likely play a protective role (Jasiński 1973). Thus there is a functional analogy between mucus-producing cells of the pond-loach and type II pneumocytes occurring in mammalian lungs (Jasiński 1973). Presumably, such is the case in *A. multispinnis* as well.

The presence of lamellar bodies in the *A. multispinnis* stomach corpus seems interesting. It is quite likely that the material they synthesise and accumulate serves a function similar to that played by the mammalian lung surfactant, and/or is a protection from toxic effects of oxygen and from desiccation. The function involving reduction of surface tension is not plausible because the *A. multispinnis* stomach is not vesicular in its structure as opposed to the mammalian lungs.

Our study showed the structure of the capillaries present in the respiratory part of the *A. multispinnis* stomach to be similar to that displayed by the respiratory intestine of the pond-loach (Jasiński 1973) and by the pulmonary-cerebral capillaries occurring in the mammalian lungs. In those cases, the capillary endothelium is strongly distended and separated from outside by a solid layer of the basement membrane. The most distended and flattened part of the endothelium lacks cellular organelles and is filled with an electron-dense substance instead. The capillaries present in additional respiratory organs of fishes show, too, a strongly flattened endothelium facilitating gas exchange (Munshi and Hughes 1992; Olson 1994; Graham 1997).

The structural similarities between the pulmonary-cerebral capillaries and those present in the respiratory part of the *A. multispinnis* stomach may reflect a secondary adaptation of the latter to respiratory function (facilitation of gas diffusion). There are, however, some structural differences between them and the pulmonary-cerebral capillaries. While solitary, diaphragm-covered pores are few in the capillaries present in the *A. multispinnis* stomach corpus, they are absent in the pulmonary-cerebral capillary type (Karrer 1956).

One can contend that, initially, all the capillaries present in the stomach of *A. multispinnis* were of the visceral type. Adaptation of the stomach corpus to assist in breathing led most probably to changes in the capillary structure. Retention of few pores in the endothelium lining of the respiratory stomach capillaries may be interpreted as a trace of the original visceral type of capillaries occurring in that part of the stomach. On the other hand, the presence of so few pores may be also related to the digestive activity going on in the part of the stomach under study.

The *A. multispinnis* stomach corpus shows the presence of solitary, nerveless, neuroendocrine-like cells lacking any contact with the gastric lumen. Such cells were described in salamander and were regarded as belonging to the first stage of the endocrine evolution system proposed by Goniakowska-Witalińska (1997). Immunohistochemical examination of the content of the neuroendocrine-like cells present in the *A. multispinnis* stomach would be necessary to elucidate their role.

CONCLUDING REMARKS

Histological and ultrastructural examination of the *Ancistrus multispinnis* stomach epithelium, the results obtained, and their confrontation with what others had done when studying the various structures which provide a peripheral component for the material dimension of the fish respiration and differ in their topography, origin, and macro- and microstructure, allow to conclude that:

First, the doubtless presence of cells, analogous to type I pneumocytes known from respiratory surfaces of other animals, including higher vertebrates, is an irrefutable proof that the gastric epithelium of the species studied serves respiratory function.

Second, histological peculiarities of structures typical of respiratory surfaces, i.e., the basement membranes and a dense (denser than in other parts of the stomach) network of capillaries, provide additional substantiation to the first conclusion.

Third, the study described shows that, among a rich variety of adaptations in the form of additional breathing organs in fish, the stomach, too, can serve such a function.

Fourth, the use of the anterior part of the alimentary tract as an additional breathing organ, as is the case in *A. multispinnis*, has resulted in the presence of a large stomach, the structure which is basically useless and very seldom present in plant-feeding fish.

Fifth, the presence of the neuroendocrine-like cells is a separate problem; perhaps they serve as chemoreceptors and are responsible for the formation of type I pneumocytes in the *A. multispinnis* stomach.

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STRESZCZENIE

Badania mikro- i ultrastruktury żołądka przeprowadzono na 29 osobnikach *Ancistrus multispinnis*. Analizę przeprowadzono w mikroskopie elektronowym skaningowym oraz elektronowym transmisyjnym. W obrębie trzonu żołądka stwierdzono obecność struktur komórkowych analogicznych do pneumocytów I typu stanowiących wyściółkę płuc ssaków. Pod nabłonkiem w żołądku stwierdzono obecność komórek morfologicznie podobnych do komórek neuroendokrynowych typu zamkniętego występujących w płucach salamandry. Ponadto w trzonie żołądka zaobserwowano włosowate naczynia krwionośne typu płucno-mózgowego. Przeprowadzone badania i uzyskane wyniki skłaniają do wysnucia konkluzji, że żołądek *A. multispinnis* może spełniać funkcję dodatkowego narządu oddechowego.

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