ESTUDO ULTRA-ESTRUTURAL DA BEXIGA NATATÓRIA DE PIRARUCU (*Arapaima gigas*)

Ultrastructural study of the pirarucu swimbladder (*Arapaima gigas*)

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ABSTRACT

Pirarucu (*Arapaima gigas*), osteoglossoid fish from the Amazon basin in extinction process, comes rising up great economic interest. Due to not very knowledge on histologic and ultrastructural details of this species and because the swimbladder functions is a very important respiratory organ for this fish, an ultrastructural study on its swimbladder was performed. Fragments of several areas of the swimbladder were collected and processed for observation in a transmission electronic microscope. The internal surface of the organ was showed covered by prismatic epithelial cells with countless continuous and intertwined blood capillaries, being also covered by fine cytoplasmic projections of the adjacent epithelial cells. Just below, connective tissue was found, being more cellular in the dorsal portion and more fibrous in the ventral portion of the organ. In the dorsal portion, this tissue joins the swimbladder to the kidney, and in the ventral portion it is continuous to the thick layer of smooth muscle. Externally, this portion is covered by simple pavimentous epithelium. The ultrastructural characteristics found in the swimbladder are suitable with the functions of air exchange suggested for the organ.

Keywords: Ultrastructural, swimbladder, Pirarucu, respiratory.

INTRODUCTION

The Pirarucu (*Arapaima gigas*) is a teleost fish of the family Osteoglossidae and exclusively found in the Amazon basin, Brazil (SILVA et al., 1995). Besides the fact that it is an endangered species, there is great economic interest in it, due to the quality and taste of its meat. According to Cerri (1995), Pirarucu is more lucrative than cattle, since a hectare of extensive cattle breeding yields 203 kg of meat a year, while 4560 kg of meat from Pirarucu can be obtained with the same area.

Since the histology of this species has not been extensively reported in literature, studies on this model are necessary. Concerning the respiration, it is known that this fish is likely to die in the absence of atmospheric air. Therefore, its respiration is not limited to branchial breathing only. In addition, most of its gaseous exchanges occur by means of the swimbladder (CRUZ-HOEFLING et al., 1980; PELLEGRINI et al., 1984; MARTY et al., 1995). As a result, studies regarding this important respiratory organ are much desirable. The aim of this work is to study the ultrastructure of the swimbladder of *Arapaima gigas*.

MATERIAL AND METHODS

Four Pirarucus (*Arapaima gigas*) coming from branches of the Araguaia river in the region of Cocalinhos, state of Mato Grosso, kindly supplied by the "Pirarucu Project" of the Universidade Federal de Uberlândia, were studied. A small number of specimens were used in this study because this species is in extinction process. The same specimens were also used in other experiments.

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Ten fragments of about 1 mm^3 were collected from each specimen: five from the dorsal portion and five from the ventral portion of the swimbladder equally distributed in relation to the long axis of the animal. These fragments were fixed in a 0.1M sodium cacodylate buffered 4% glutaraldehyde solution at pH 7.2 to 7.4. After fixation for 48 hours, fragments were rinsed in cacodylate buffer (0.1M, pH 7.2 to 7.4) twice for 10 minutes and then posfixed for 4 hours in 1% osmium tetroxide and one hour in 1% osmium tetroxide plus 1.25% potassium ferrocyanide. In the end, fragments were embedded in Epon resin and after sectioned in ultramicrotome in a way that ultrathin sections were obtained. Sections were contrasted with uranyl acetate (WATSON, 1958) and lead citrate (REYNOLDS, 1963), and subsequently examined in a Zeiss EM-109 electron microscope.

RESULTS

The swimbladder has two distinct portions, dorsal and ventral, with the first presenting only an inner surface since its dorsum is already in direct contact to the kidney, and the second presenting inner and outer surfaces. Boundary

The inner surface of the swimbladder, in both dorsal and ventral portions, appears covered by prismatic epithelial cells, which have a great number of organelles, such as granular and smooth endoplasmic reticulum, Golgi apparatus, mitochondria and rare granules (Figure 1). A number of continuous blood capillaries, with no pores and tight endothelial cells are found in nearly all the inner surface, being attached between the epithelial cells and coated only by thin cytoplasmic projections of adjacent epithelial cells. Joining the epithelial cells and also their supracapillary projections, the tight junction and desmosomes are found (Figure 2). By separating the air inside the swimbladder and the blood from superficial capillaries, only the generally thin, epithelial cytoplasmic projections, basal lamina and very thin capillary endothelium are found (Figure 3). The epithelium of the inner surface occasionally presents small digit-shape projections into the organ.

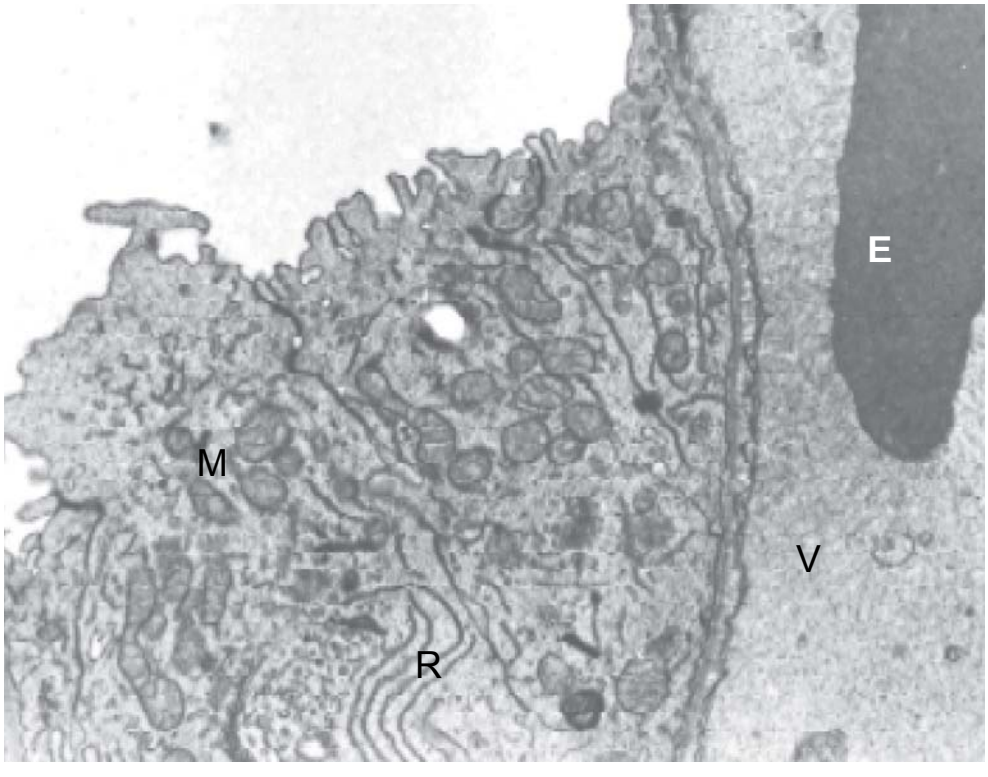

Figure 1. Inner surface of the swimbladder in *A. gigas*, showing prismatic epithelial cells with presence of many mitochondria (M), and laterally, a vessel (V) containing an erythrocyte (E). endoplasmic reticulum (R). (Magnification 32,900X).

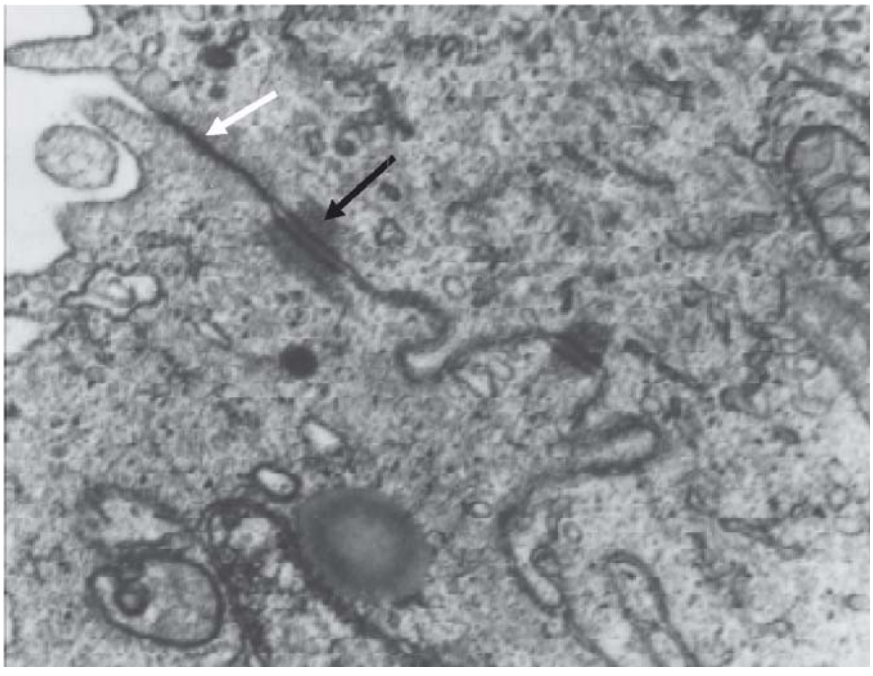

Figure 2. Boundary between epithelial cells showing desmosome (filled arrow) and tight junction (empty arrow). (Magnification 94,000X).

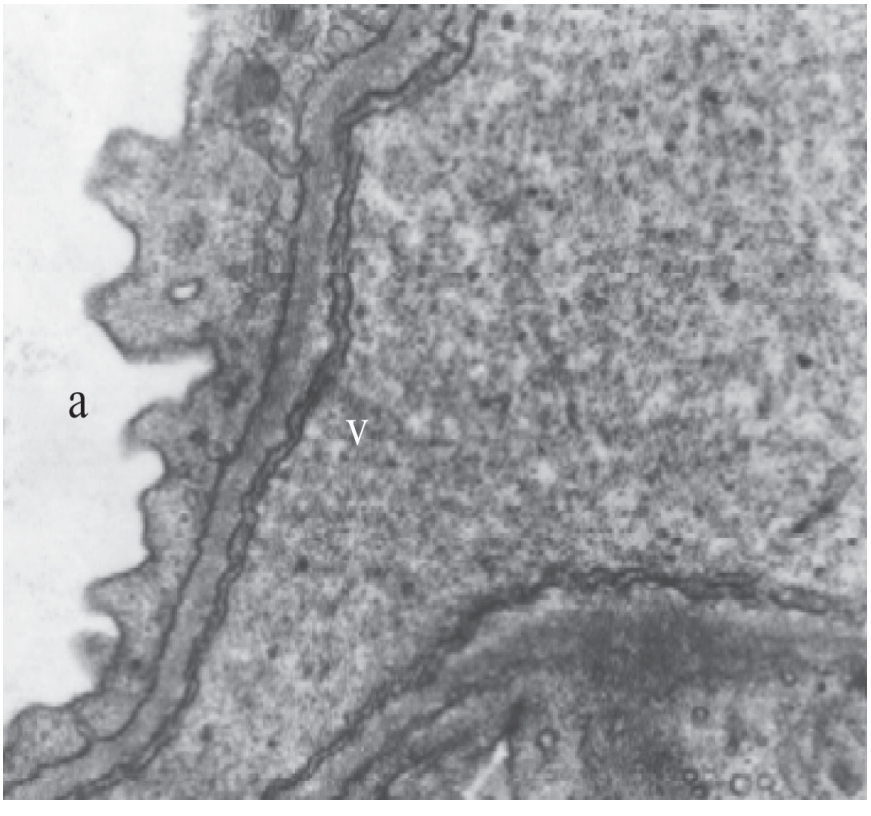

Figure 3. Barrier between the air inside the swimbladder (a) and the blood from the superficial capillaries (v). (Cytoplasmic projections of the epithelial cells, basal lamina and capillary endothelium). (Magnification 94,000X).

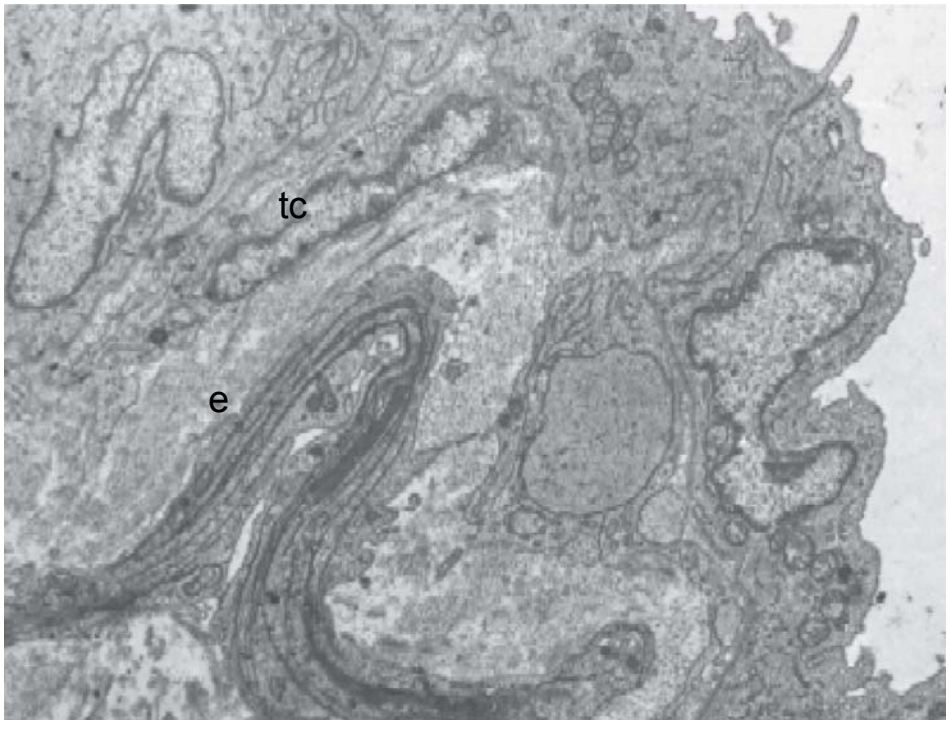

Figure 4. Portion of the swimbladder of *A. gigas* in its inner surface, showing connective tissue (tc) and epithelium (e) with absence of blood capillaries (Magnification 20,700X).

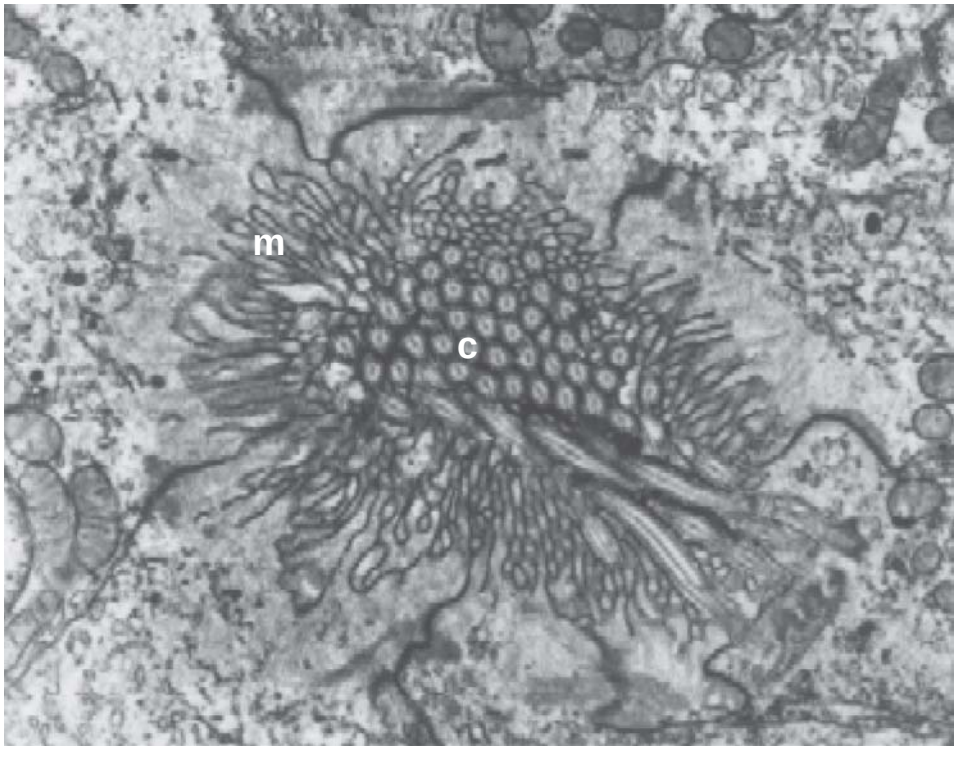

Figure 5. Apical portion of the renal tubule cells continuous to the subepithelial connective tissue at the dorsal portion, showing cilia (c) and microvilli (m) (Magnification 32,900X).

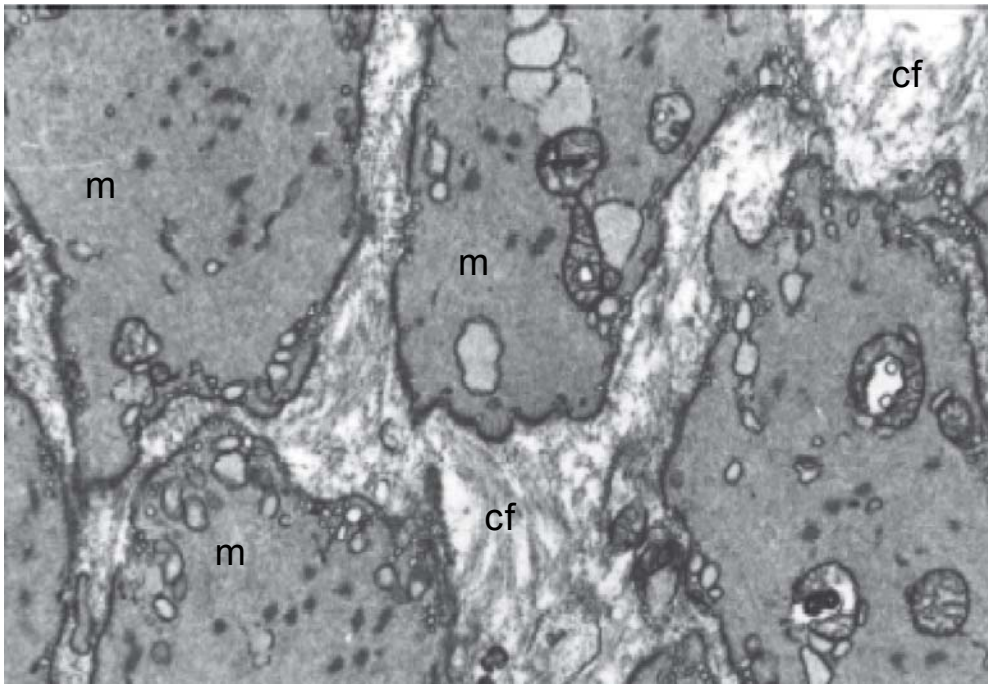

Figure 6. Cross-section of the muscular layer, showing cells (m) separated by collagen fibers (cf) (Magnification 32,900X).

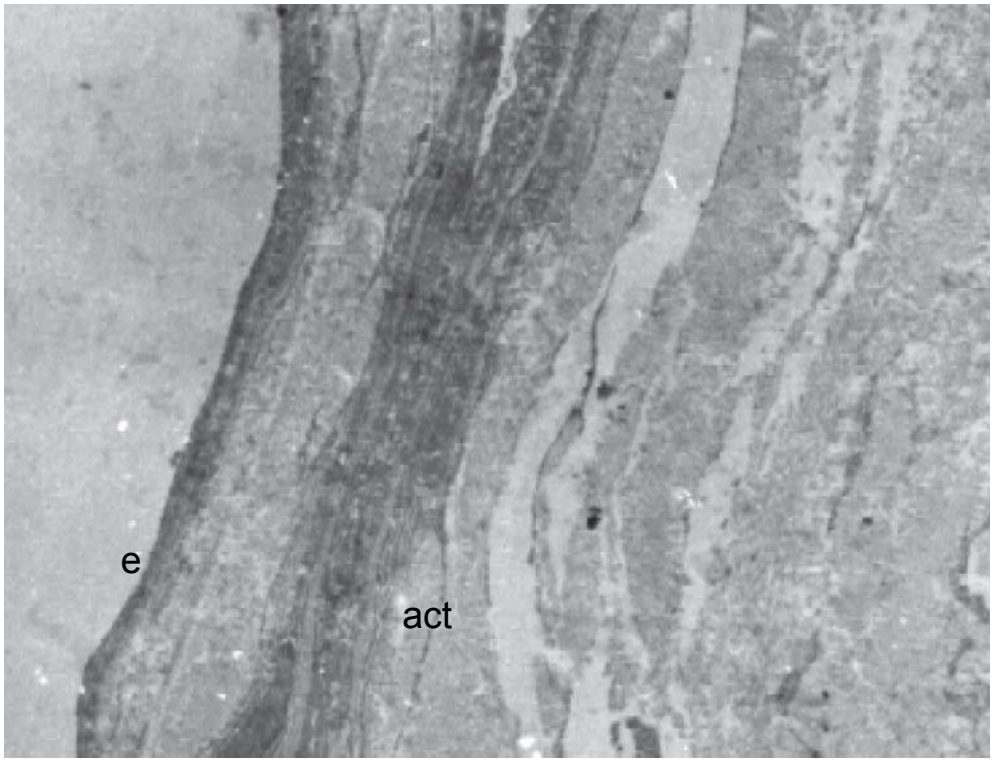

Figure 7. Ventral portion of the swimbladder in *A. gigas* covered by pavimentous simple epithelium (e) and adjacent connective tissue (act) (Magnification 14,100X).

The presence of micropinocitosis vesicles was also noticed all along the epithelium. There are portions of the swimbladder in its inner surface where the presence of superficial blood capillaries was not noticed; only epithelium was found (Figure 4).

The connective tissue is found right below the epithelium and the blood capillaries. In this subepithelial connective tissue are cells abound in the dorsal portion (Figure 5), although it is more fibrous at the ventral portion. Various cell types were found in this tissue, especially granular eosinophill cells and elongated fibroblasts. At the dorsal portion this connective tissue continues with the renal tubules, presenting no defined limit between the swimbladder and the kidney (figure 6). In contrast, at the ventral portion, this connective tissue is followed by a typical, thick smooth muscle layer.

The cells of the muscular layer are not generally close, but separated by collagen fibers and present many micropinocitosis vesicles and mitochondria (Figure 7).

On the outside, the ventral portion of the swimbladder is covered by pavimentous simple epithelium and proper lamina of connective tissue rich in collagen fibers.

DISCUSSION

The ultrastructural characteristics of the inner surface of the swimbladder in *Arapaima gigas* are typical of this species, showing few similarities to other ones (CRUZ-HOEFLING et al., 1980; PELLEGRINI et al., 1984; MARTY et al., 1995).

The capillaries close to the surface and separated from the air inside the organ only by thin epithelial cytoplasmic projections, proper lamina and thin endothelium are morphological characteristics very similar to those of the lung in higher mammals (BANKS, 1991; DELLMANN; BROWN 1982). The presence of desmosome and tight junction between the epithelial cells ensures a strong adhesion between them, preventing from the transference of substances in these spaces and having propagation only inside them. Thus, high selectivity between air and blood is ensured.

Several characteristics of the epithelial cells in the inner surface of the swimbladder, such as a great amount of smooth and granular endoplasmic reticulum, Golgi apparatus and presence of some granules, suggest that these cells probably have a secretory function. Once typical surfactant secretory cells were not found, it is likely that the epithelial cells could secrete substances that may help the gaseous exchanges by reducing the surface tension. Also, there are portions of the inner surface where no vessels were found, suggesting the absence of gaseous exchange in this region.

The presence of thick smooth muscular layer probably helps aspirating and expiring the air from the swimbladder, in contrast to the reports of Greenwood; Liem (1984) who stated that this function is exerted basically by an oropharyngeal pump.

The morphological characteristics of this organ suggest the function of gaseous exchanges due its similarity with mammal lungs. Dzhumaliyev (1977) does not exclude the possibility of cellular secretion of gases inside the swimbladder in members of some families of fishes. In addition, this fish, once adult, can reach great dimensions, thus demanding from its organism a higher supply of oxygen, since it would not supply its demands only by means of branchial respiration. According to that, gaseous exchanges through a modified swimbladder represent a resource achieved by evolution of this species as well as of other fishes. Berra et al., (1989) states that the histological structure of the swimbladder of *Lepdogalaxias salamandroides* appears to demonstrate an accessory respiratory function associated to the branchial chamber.

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