

# The resorptive vesicle of *Temnocephala jheringi* (Temnocephalida)

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**ABSTRACT.** The wall of the resorptive vesicle is a merocrine secretor and a phagocytic syncytium, and its free plasma membrane shows deep and intertwined invaginations. Redundant foreign spermatozoa are engulfed by these invaginations, digested and incorporated into the individual's metabolism. The observations presented in this paper lead us to conclude that, during copulation, the resorptive vesicle acts as a temporary receptive organ for the foreign male gametes (bursa copulatrix role).

**KEY WORDS:** Platyhelminthes, *Temnocephala*, resorptive vesicle, reproductive biology, ultrastructure, epithelia

## INTRODUCTION

To date there is no ultrastructural information available on bursal organs of *Temnocephala* (RIEGER et al. 1991). The resorptive vesicle was originally called receptaculum seminis by HASWELL (1888) and WEBER (1889) who assigned it a function as a reservoir for spermatozoa. Later, HASWELL (1924) changed his mind and called it vesicula resorbiens following MERTON's (1914) identification of the true receptaculum seminis, and in view of the fact that it contained yolk, and no spermatozoa. According to FYFE (1942), the vesicula resorbiens "is a reservoir of superfluous reproductive material which can be absorbed at intervals into the gut".

Recently, WILLIAMS (1994) using light microscopy (with haematoxylin/eosin and Mallory's trichrome staining) claimed that it functions in the digestion of excess spermatozoa.

In the present work we postulate, on the basis of our studies, and in accordance with HYMAN's physiologic concept (HYMAN, 1951), that the resorptive vesicle of *Temnocephala jheringi* acts as a bursa copulatrix. We also postulate that phagocytic activity takes place in the epithelium of the resorptive vesicle, and remnant foreign sexual products are digested.

## MATERIAL AND METHODS

Specimens of *Temnocephala jheringi* (Haswell, 1893) were extracted from the paleal cavity of *Pomacea caniculata* (Mollusca) from the marshy coastal waters of the River Plate, just in front of the University of Buenos Aires Campus (Ciudad Universitaria). Sexually active specimens were fixed in Bouin-Hollande fixative and embedded in paraffin wax.

Longitudinal oblique and transversal serial sections of 6-8  $\mu\text{m}$  thickness were obtained. The Mann-Dominici's method, Masson's trichomic and PAS reaction (GABE, 1968) were employed for staining.

For transmission electron microscopy (TEM) specimens were fixed in 3% glutaraldehyde in sodium cacodylate at pH 7.3, post-fixed for a one hour in 1% osmium tetroxide in 0.25 M cacodylate with 0.12 M sucrose, and embedded in Spurr's resin. Following staining with uranyl acetate and lead citrate, thin serial sections were scanned by means of a JEOL-JEM-100C apparatus. Light microscopy was performed to study 1  $\mu\text{m}$  sections after staining with Loeffler's alkaline methylene blue (GRAY, 1975).

## RESULTS

*Temnocephala jheringi* individuals are hermaphroditic. The female system includes an ovovitelline duct, paired

seminal receptacles, and a resorptive vesicle. The first leads to the common genital atrium, and in the post-copulation stage its anterior end acts as a sphincter. The other end narrows and opens into the resorptive vesicle. The resorptive vesicle has the shape of a hollow hemisphere of diameter approximately 300  $\mu\text{m}$ . The connection to the ovovitelline duct lies centrally in its base. The seminal receptacles are connected to the ovovitelline duct through openings that are contiguous with the entrance to the resorptive vesicle; each is approximately 80  $\mu\text{m}$  long with muscle fibers at the entrance. In sections, they are seen as notches over the wall of the duct.

No connection of any type (permanent or transitory) was observed between the resorptive vesicle and the neighboring intestine. No female gametes, no vitelline cells, and no characteristic vitellus were seen amongst the contents of the vesicle.

The epithelium of the resorptive vesicle shows a basal or proximal part and an apical or distal part (Fig. 1). The basal part is pierced by dorso-ventral and askew oriented muscular fibers.

The few nuclei are in the basal part; they are irregular and their main axis lies parallel to the cellular limit. They measure 20 $\mu\text{m}$  x 24 $\mu\text{m}$ , have a 7 $\mu\text{m}$  nucleolus, and, in some cases, have an extra one that is smaller in size. The nuclear membrane shows small internal indentations that limit the nuclear pores and constitute a prickly surface. In this zone there are abundant mitochondria and dictyosomes. The latter produce a secretion that is discharged through the plasma membrane at the distal zone (merocrine extrusion). The distal or apical region is constituted by a tubular system and cisterns with vacuolated cytoplasm.

The parietal cytoplasm appears differently colored depending on the staining used; with the Mann-Dominici's method, the basal part stains light violet (basophilic) and the apical one that limits the vesicular lumen stains pinkish (acidophilic). With Masson's trichromic, the apical part stains lilac, and the basal one red. With PAS technique, the apical is positive. No cellular junction structures were detected at the vesicular wall level.

After copulation, the resorptive vesicle holds foreign spermatozoa, which are free, together with prostatic secretion ejaculated by the sexual partner. Bundles of sperm cells lie between the seminal receptacles and the lumen of the resorptive vesicle (Fig. 2). A viscous fluid accompanies the moving sperm cells bundles. The fluid fills the lumen of each receptacle and, in some cases, protrudes into the ovovitelline duct cavity.

In the post-copulation stage, the apical zone of the resorptive vesicle shows a system of tubules and cisterns formed by deep invaginations of the distal plasma membrane of the vesicular syncytium (apical labyrinth). The apical labyrinth is connected to the vesicular lumen. The cytoplasm of the apical zone contains vacuoles and mito-

chondria. Circumscribed areas from the apical labyrinth may protrude into the vesicular lumen. The remnant gametes that lie in juxtaposition to the parietal epithelium free edge become engulfed within vesicles through a phagocytic process (Fig. 3). When few spermatozoa remain into the resorptive vesicle, there are lytic products from the digestion of remnant spermatozoa and remains of their cilia near the nuclei (Fig. 4).

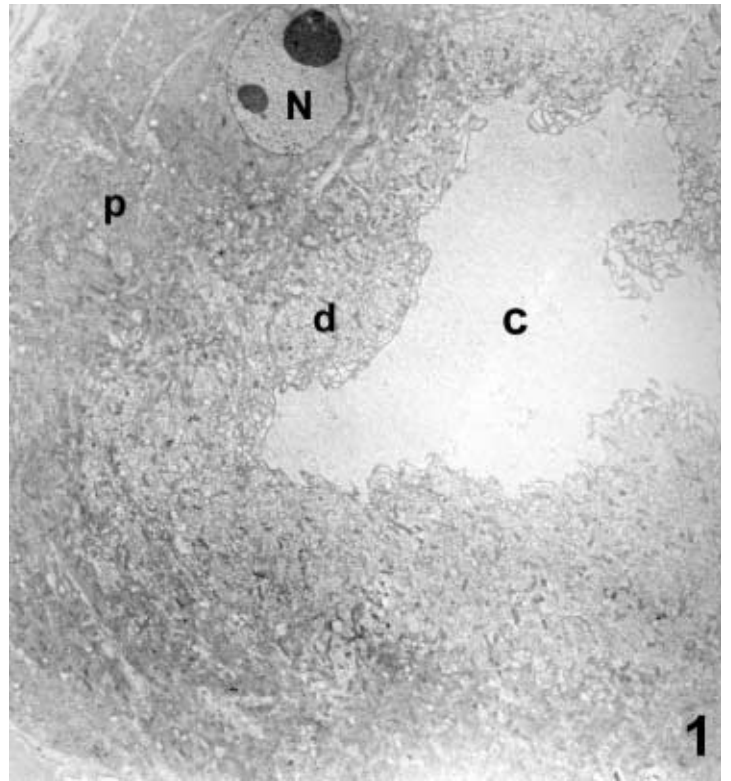


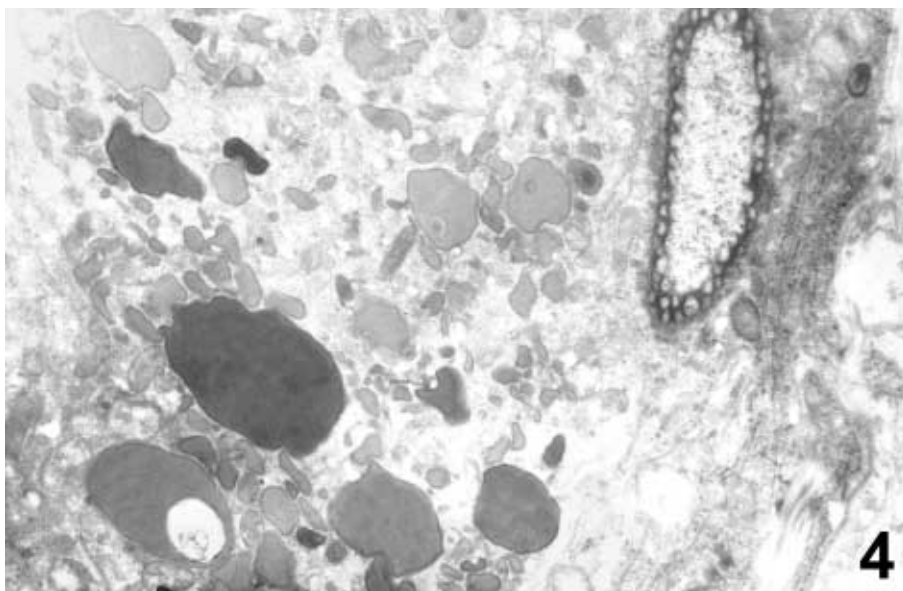
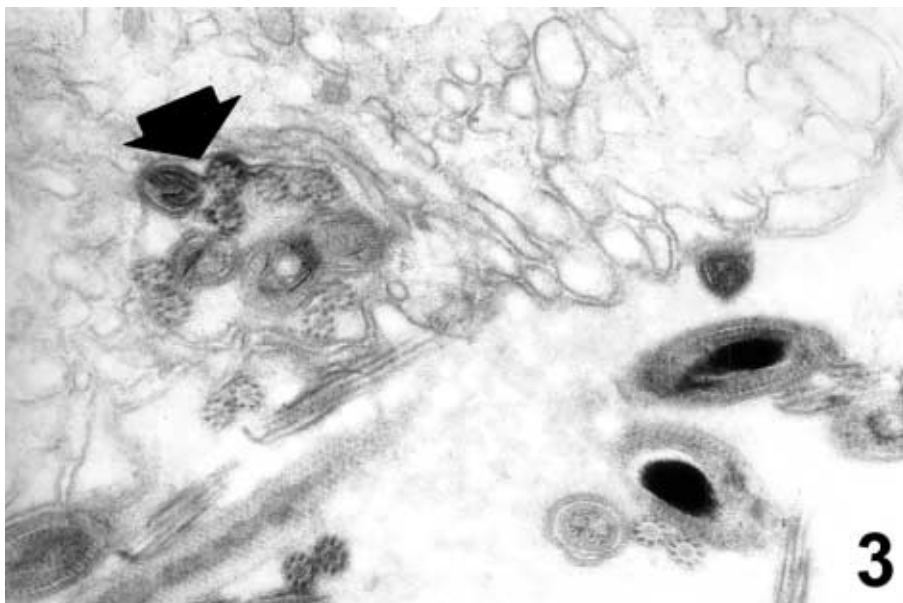
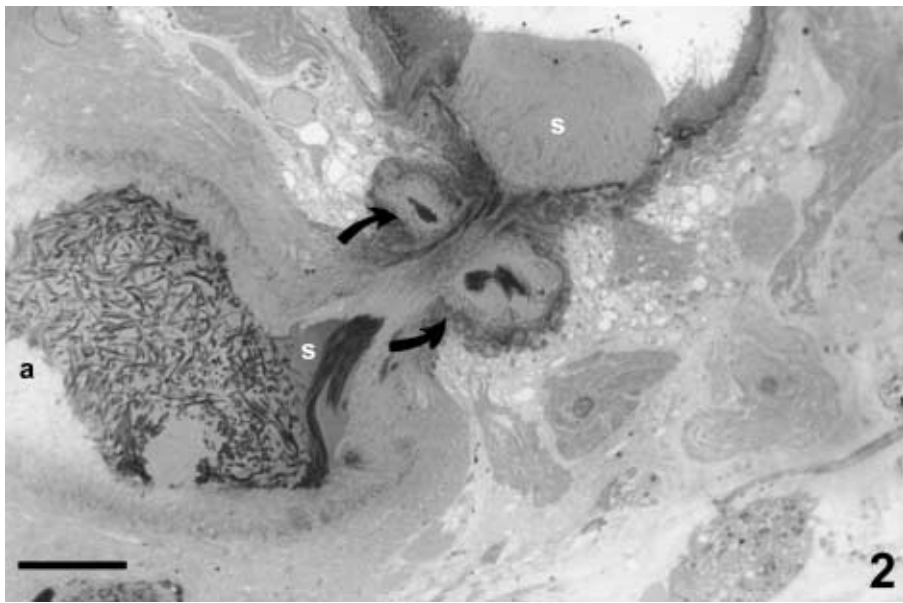
Fig. 1. – Oblique section of resorptive vesicle. The two regions of its epithelium. p = proximal (or basal) region with nucleus (N); d = distal (or apical) region; c = vesicular cavity. TEM. (X 2000)

#### Legends to the figures (see opposite page)

Fig. 2. – Post-copulation stage. Bundles of spermatozoa going into the seminal receptacles (arrows). Note the secretion (s) accompanying the spermatozoa and filling the seminal receptacle region. The apical labyrinth (a) of the resorptive vesicle engulfs spermatozoa. Longitudinal section (1 $\mu\text{m}$ ). Loeffler's methylene blue. Scale bar: 20 $\mu\text{m}$ .

Fig. 3. – Some spermatozoa engulfed by the apical labyrinth. (arrow) TEM, 20000 X.

Fig. 4. – Vesicles containing lytic products from the digestion of spermatozoa in the proximal (basal) epithelial zone of the resorptive vesicle. TEM, 8000 X.



## DISCUSSION AND CONCLUSION

According to WILLIAMS (1994), insemination in *Temnocephala genoma* would occur through the gonopore into a ventral expansion of the ovovitelline duct (a particular characteristic of that species), which would act as bursa copulatrix. The resorptive vesicle's only function in that species would be digestion of the excess foreign sperm cells. As degenerated spermatozoa were observed intraepithelially, she concluded that at least some part of the digestive process occurs within the epithelium.

According to Hyman's criterion (HYMAN, 1951), however, the resorptive vesicle of *Temnocephala jheringi* may be regarded as a bursa copulatrix, because it is a temporary holder of the foreign male gametes during copulation. In the post-copulation stage the foreign sperm cells would be selected on the motility of the bundles in which they are grouped and for their capacity to escape from the bursa to the seminal receptacles. The fluid that accompanies the sperm cells and fills the region of the seminal receptacles may be protective, and may originate in the epithelium of this region. This extrusion is stained with Loeffler's alkaline methylene blue.

Remnant and redundant bundles would be destroyed within the bursa, their spermatozoa lysed, and the resulting products incorporated into the individual's metabolism. These findings are consistent with what we have previously observed in other species of South American Turbellaria (MORETTO, 1996). After copulation, the bursa retains the foreign male sexual products for a short time. We therefore suggest that the term bursa seminalis proposed by FISCHLSCHWEIGER & CLAUSNITZER (1984) is not suitable in Turbellaria.

In *Temnocephala*, the remnant spermatozoa are phagocytized by a pseudopodial activation at the distal zone of the bursal epithelium.

Under a static vision, the apical or distal labyrinth observed in the bursal epithelium of *T. jheringi*, in the post-copulation stage, would correspond to the smooth endoplasmic pseudo-reticulum that can be found in fish gills cells. This constitutes a tubule system that connects directly to the cellular surface. This surface is formed by deep and wide invaginations of the plasma membrane (WELSCH & STORCH, 1976).

In conclusion, during copulation the resorptive vesicle of *Temnocephala jheringi* receives the foreign sexual products. In the post-copulation stage, the bursal epithelium becomes a merocrine glandular syncytium and lyses all the spermatozoal bundles that do not escape in time

from within the bursa, and the resulting products are incorporated into the metabolism of the individual.

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