of slow waves in the intestine[16-18]. Some authors have suggested that longitudinal propagation of slow waves requires electrical coupling between the longitudinal and circular muscle layers[16,17]. Our results show that each of the muscle layers has the machinery needed for the propagation of current. Also, dye-coupling between the two muscle layers could not be demonstrated in our experiments. It appears, therefore, that an alternative explanation to the question of slow wave generation and propagation has to be sought (see also Szurszewski[18]).

Experiments using the dye injection technique in smooth muscles have been previously performed only in cultured aortic muscle cells[7]. In the present study freshly dissected tissue was used, preserving the original organization of the cells. This experimental approach may open new possibilities for studying intercellular coupling in smooth muscles.

Acknowledgments. We thank Dr G. Gabella for helpful discussions and Prof. P. Hillman for comments on the manuscript.

* To whom all correspondence should be addressed.

1 Beyer, E. C., Kistler, J., Paul, D. L., and Goodenough, D. A., J. Cell Biol. *108* (1989) 595.
2 Dewey, M. M., and Barr, L., Science *137* (1962) 670.
3 Gabella, G., Br. med. Bull. *35* (1979) 213.
4 Daniel, E. E., Daniel, V. P., Duchon, G., Garfield, R. E., Nichols, M., Malhorta, S. K., and Oki, M., J. Membr. Biol. *28* (1976) 207.
5 Gabella, G., and Blundell, D., Cell Tissue Res. *219* (1981) 469.
6 Stewart, W. W., Cell *14* (1978) 741.
7 Blennerhassett, M. G., Kannan, M. S., and Garfield, R. E., Am. J. Physiol. *252* (1987) C555.
8 Hanani, M., Baluk, P., and Burnstock, G., J. auton. Nerv. System *5* (1982) 155.
9 Hanani, M., Chorev, M., Gilon, C., and Selinger, Z., Eur. J. Pharmac. *153* (1988) 247.
10 Hanani, M., Zamir, O., and Baluk, P., Brain Res. *497* (1989) 245.
11 Spray, D. C., and Bennett, M. V. L., A. Rev. Physiol. *47* (1985) 281.
12 Thomas, R. C., J. Physiol. (London) *238* (1974) 159.
13 Miyachi, E.-I., and Murakami, M., J. Physiol. (London) *419* (1989) 213.
14 Bodmer, R., Verselis, V., Levitan, I. B., and Spray, D. C., J. Neurosci. *7* (1988) 1656.
15 Williams, E. H., and DeHaan, R. L., J. Membr. Biol. *60* (1981) 237.
16 Connor, J. A., Kreulen, D., Prosser, C. L., and Weigel, R., J. Physiol. (London) *273* (1977) 665.
17 Bortoff, A., Michaels, D., and Mistretta, P., Am. J. Physiol. *240* (1981) C135.
18 Szurszewski, J. H., in: Physiology of the Gastrointestinal Tract, p. 382. Ed. L. R. Johnson. Raven Press, New York 1987.

0014-4754/90/101002-04$1.50 + 0.20/0


## Functions of the testicular gland of blenniid fish: Structural and histochemical investigations

F. Lahnsteiner and R. A. Patzner

*Zoological Institute, University of Salzburg, Hellbrunnerstr. 34, A-5020 Salzburg (Austria)*
*Received 8 January 1990; accepted 20 March 1990*

*Summary.* We investigated the functions of the testicular glands of blenniid fishes by fine-structural and histochemical methods. These glands function in the differentiation and nutrition of germ cells, in the secretion of sialomucins, in phagocytosis of germ cells, and in lipid storage.
*Key words.* Testicular gland; ultrastructure; histochemistry; blenniid fish; function.

Since its discovery 150 years ago[1], the testicular gland of blenniid fish has been the subject of many structural and functional investigations[2-6]. The gland, which is located adjacent to the testis, was found to store lipids, to produce steroids and acid mucopolysaccharides, and to have lysomatic functions[7,8]. However, the exact role of the testicular gland in the reproduction of blenniid fish is uncertain.

The testicular gland of five species of adult, male Mediterranean blennies (8 *Salaria pavo*; 8 *Lipophrys dalmatinus*; 5 *Lipophrys adriaticus*; and 6 *Aidablennius sphynx*) was investigated with routine transmission electron microscopy (fixation: 4.5% paraformaldehyde, 2.25% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.5); postfixation: 1% $OsO_4$ in 0.1 M cacodylate buffer) and with histochemical techniques. Glycogen was demonstrated on paraffin sections (7 µm) by periodic-acid-Schiff (PAS) staining (McManus)[9] and Best's glycogen detection[9]. Sulfated and non-sulfated mucosubstances were stained with alcian blue at pH 1 and pH 2.5[9] on 7-µm-thick paraffin sections and on 10-µm-thick cryostat sections. The non-sulfated mucosubstances were shown to be sialomucins by the method of neuraminidase extraction[9] (neuraminidase from *Clostridium perfrigens*, activity: 0.5–1.3 units/mg NAN-lactose). Lysosomal activity in the testicular gland was demonstrated with Gomori's lead phosphatase method[9] and steroid production with the method of 3β-steroid dehydrogenase activity (substrate: dehydroepiandrosteron)[10]. Both reactions were carried out on 10-µm-thick frozen sections.

Most Mediterranean blenniid fish have an annual spawning season lasting from the beginning of June until the end of July. The mature testes of blennies do not contain any spermatozoa; spermiogenesis in the testis progresses only up to the stage of spermatidal development. Spermatids are then released into the testicular gland (figs 1 and 2). During the spawning period the cells of this gland are characterized by an abundance of smooth endoplas-

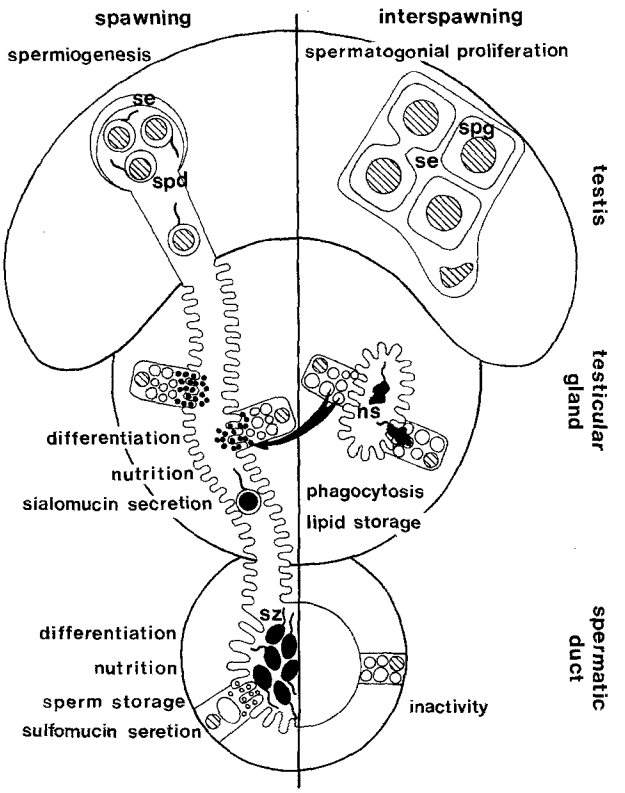


Figure 1. The male gonads of blenniid fish and their functions in reproduction during spawning (left) and during the interspawning period. Cross section. ns, necrotic spermatid; se, Sertoli cell; spd, spermatid; spg, spermatogonium; sz, spermatozoon.

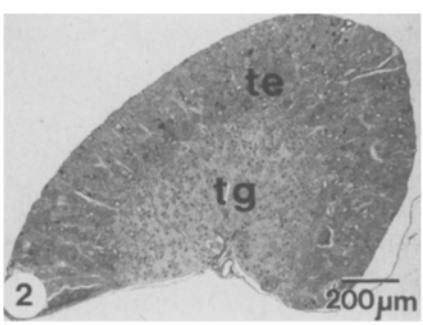


Figure 2. Testis (te) and testicular gland (tg) of *Aidablennius sphynx*. Paraffin cross section.

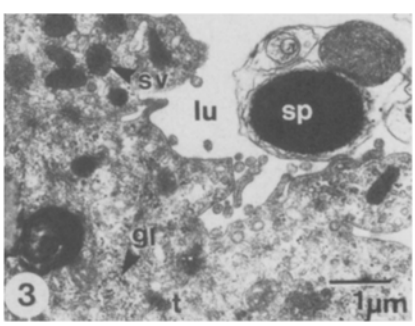


Figure 3. Testicular gland cell of *Salaria pavo* during the spawning period. Transmission electron micrograph. gl, glycogen granula; lu, lumen of a duct of the testicular gland; sp, spermatid; sv, secretory vesicle; t, tight junction.

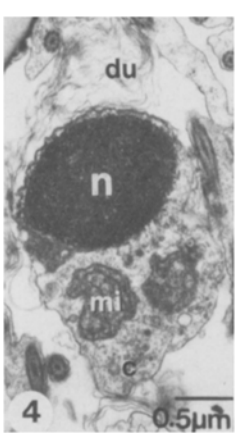


Figure 4. Spermatid of *Aidablennius sphynx* in a duct of the testicular gland (du). Transmission electron micrograph. mi, mitochondria. Note that the nuclear material (n) is almost completely condensed. The caudal pole of the cell still contains large amounts of cytoplasma (c).

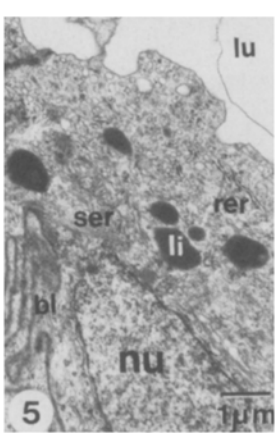


Figure 5. Testicular gland cell of *Salaria pavo* at the beginning of the postspawning period. Transmission electron micrograph. bl, basal lamina; lu, lumen of a duct of the testicular gland; nu, nucleus; ser, smooth endoplasmic reticulum. Note the formation of lipid vacuoles (li) and the increase in rough endoplasmic reticulum (rer).

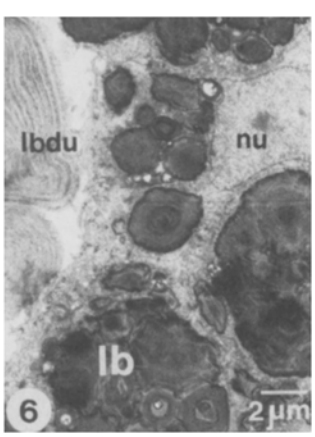


Figure 6. Testicular gland cell of *Lipophrys dalmatinus* during interspawning period. Transmission electron micrograph. lb, lamellar body consisting of phospholipids; nu, nucleus. Note lamellar bodies in the duct of the testicular gland (lbdu) originating by necrosis of spermatids and of remaining testicular gland secretion.

mic reticulum and tubular mitochondria, and by a layer of microvilli (fig. 3). The cells secrete glycogen and sialomucins into the glandular ducts as an apocrine secretion. Within the testicular gland, spermatids undergo further differentiation (fig. 4) and are nourished with glycogen, which they take up by pinocytosis. The final differentiation of spermatids into spermatozoa occurs in the spermatic ducts, which also function in the nutrition and storage of spermatozoa and in the secretion of sulfomucins (fig. 1). During the height of the spawning period male blennies spawn almost every day with a preference to early morning hours[11]. This timing of activity indicates that there is a diurnal cycle of differentiation of spermatids in the testicular glands and spermatic ducts. The sialomucins of the testicular gland and the sulfomucins secreted by the spermatic ducts are thought to increase the viscosity of the seminal fluid as do the sialomucins of the bulbourethral glands of mammals[12]. In blennies, a seminal fluid of high viscosity and agglutination may be important to cause layers of sperm to adhere to the substrate on which the eggs then are deposited. The eggs of blenniid fishes adhere to the substrate with the micropylar region[13]; however, the precise mode of fertilization is still unknown[14].

During the postspawning period (beginning of August until the end of November) the amount of rough endoplasmic reticulum increases remarkably in the testicular gland cells (fig. 5), as does the number of lysosomes. The remaining spermatids degenerate and are resorbed into the testicular gland cells. Here they are transformed into phospholipids and lipids greatly increasing the levels of these substances in the testicular gland cells (fig. 6). During the postspawning and interspawning periods (beginning of December until the end of February) stored lipids and phospholipids are re-transformed into the testicular gland secretion for the prespawning period (beginning of March until the end of May).

Steroids are not synthesized by the testicular gland cells themselves, as reported by former authors[5,8], but by interstitial cells that are homologous to the Leydig cells described in the testes of other fishes[15].

The testicular gland is involved in the following five functions: a) regulating the differentiation processes in spermatids, b) nutrition of spermatids, c) secretion of sialomucins, d) phagocytosis of remaining germ cells, and e) storage of lipids and phospholipids. In (a), (b) and (c) the testicular gland has similar functions to the Sertoli cells of other fishes[15] and therefore, the gland may be considered to have taken over testicular functions to some extent. This is clearly seen in *Lipophrys* spp., where the testes are smaller in relation to body weight than in other blennies, while the testicular glands are very voluminous. In *Salaria pavo*, the volume of the testicular gland amounts to 30% of the testicular volume, but in *L. dalmatinus* it is 250% of the testicular volume (unpublished observation). In the spermatids of *Lipohrys* spp. which are released into the testicular gland, nuclear condensation is less advanced than in species having a voluminous testis, and the spermatids contain larger amounts of cytoplasm and have an undifferentiated flagellar sheet. This observation suggests that in *Lipophrys* spp. spermatids are released into the testicular gland at an earlier stage of maturation than in other blenniid species which have a large testis.

Acknowledgments. The research was supported by the Austrian Fonds zur Förderung der wissenschaftlichen Forschung (grant no. 5338) and by the Austrian National Bank (project no. 1568). Graphical work was done by Mrs Petra Lahnsteiner-Oberndorfer.

1 Rathke, H., Archs Anat. Physiol. (1836) 170–186.
2 Eggert, B., Z. wiss. Zool. *126* (1931) 249–558.
3 Champy, C., and Gley, P., Bull. Soc. zool. France *47* (1922) 199–208.
4 Sperotti, G., Atti R.I.V. di S.L.A.-T.C. (1940) 117–147.
5 Chieffi, G., and Botte, V., Boll. Zool. *31* (1964) 471–477.
6 Bluem, V., J. exp. Zool. *181* (1972) 203–216.
7 Seiwald, M., and Patzner, R. A., Zoomorphology *107* (1987) 26–32.
8 Seiwald, M., and Patzner, R. A., J. Fish Biol. *35* (1989) 631–640.
9 Pearse, A. G. E., Histochemistry. Theoretical and Applied, Analytical Technology. Churchill Livingstone, Edinburgh London/Melbourne 1985.
10 Baillie, A. H., and Ferguson, M. M., Developments in Steroid Histochemistry. Academic press, New York 1966.
11 Patzner, R. A., Seiwald, M., Adlgasser, M., and Kaurin, G., Zool. Anz. *216* (1986) 338–350.
12 Mann, T., The Biochemistry of Semen and the Male Reproductive Tract. Methuen and Co., London 1974.
13 Patzner, R. A., Zool. Anz. *213* (1984) 44–50.
14 Gibson, R. N., A. Rev. Oceanogr mar. Biol. (1984) 376–410.
15 Grier, H. J., Am. Zool. *21* (1981) 345–357.

0014-4754/90/101005-03$1.50 + 0.20/0