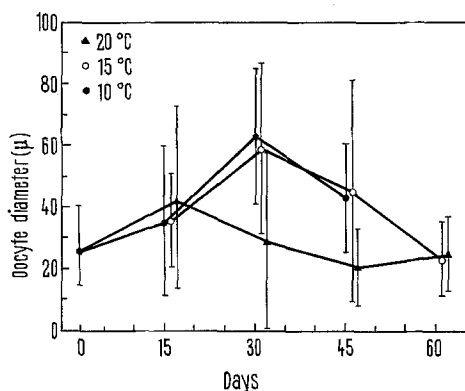


oocyte growth in scallops exposed to different temperatures was examined at intervals to determine the influence of temperature on growth (Figure). On day 15 the oocytes were in the cytoplasmic growth phase ( $34 \mu$ ) at both  $10^\circ\text{C}$  and  $15^\circ\text{C}$ . Oogonia and oocytes in the vitellogenesis growth phase were also present. The oocytes were predominantly in the vitellogenesis growth phase ( $63.03 \pm 21.69 \mu$  at  $10^\circ\text{C}$  and  $59.04 \pm 27.49 \mu$  at  $15^\circ\text{C}$ ) on day 30. However, the oocytes completing the vitellogenesis growth phase were disintegrating at these 2 temperatures. On day 45 oocytes completing the cytoplasmic growth phase were predominant at both temperatures. Oogonia and oocytes in the vitellogenesis growth phase were also present. The oocytes completing vitellogenesis growth phase and dissolution of germinal vesicle were disintegrating. At  $15^\circ\text{C}$ , only oogonia were present on day 60.

The scallops held at  $20^\circ\text{C}$  were completing the cytoplasmic growth phase ( $42.07 \pm 28.37 \mu$ ) on day 15. Oogonia and oocytes in the vitellogenesis growth phase and disintegrating oocytes were observed. On day 45 and 60 at these 2 temperatures only oogonia and oocytes at the beginning of cytoplasmic growth phase were observed.

When summer scallops with oocytes in the cytoplasmic growth phase are exposed to colder temperatures, the oocytes seem to complete vitellogenesis but then disintegrate while additional oogonia develop (Figure). The cycle of oocyte growth and disintegration occurs more rapidly at  $20^\circ\text{C}$  than at the other 2 experimental temperatures.



Oocyte growth response in summer scallops exposed to different temperatures. Vertical lines show the standard deviation from the mean. The decrease of mean oocyte diameter after initial increase is due to disintegration of oocytes completing the vitellogenesis growth phase.

The oocyte growth response in winter scallops indicates that the cytoplasmic growth phase begins when food is present and when temperatures exceed a minimum threshold temperature level<sup>3</sup>. Another threshold temperature, higher than that required for cytoplasmic growth, is necessary for oocytes to reach the stage of fertilizable eggs<sup>5</sup>. In the gonad development of scallops, the initiation of cytoplasmic growth and the maturation of oocytes are apparently 2 control points at which the environment exerts its influence over oogenesis. The response of scallops to these controlling environmental factors regulates the period of oogenesis within the year.

In summer, scallops with oocytes already in the cytoplasmic growth phase, the further development to dissolution of the germinal vesicle seems to occur even though the oocytes are exposed to temperatures below the threshold level for activation of growth. Apparently, the beginning of the cytoplasmic growth phase is controlled by a triggering stimulus. In marine bivalve molluscs, a neurosecretion absent during the neutral state is released at the beginning of oogenesis, reaching a maximum concentration as the oocytes mature<sup>6-8</sup>. It seems likely, that the neurosecretion produced and released when the scallops are exposed to a minimum threshold temperature and to food might stimulate the oogonia to begin the cytoplasmic growth phase. The oocytes, once stimulated cannot be stopped from further development to completion of the vitellogenesis growth phase even though exposed to temperatures below those needed to trigger their growth.

This preliminary report may provide an approach for studying the environmental regulation of neurosecretory activity and its control of oogenesis in marine bivalve molluscs<sup>9</sup>.

**Zusammenfassung.** Temperatur- und Futtereinfluss auf das Gonadenwachstum von Winter- und Sommerkamm-muscheln *Aequipecten irradians* Lamarck.

A. N. SASTRY

Graduate School of Oceanography,  
University of Rhode Island,  
Kingston (Rhode Island 02881, USA), 8 June 1970.

<sup>6</sup> P. LEUBET, C. r. Acad. Sci., Paris 241, 119 (1955).

<sup>7</sup> R. NAGABHUSANAM, Ind. J. exp. Biol. 7, 161 (1963).

<sup>8</sup> M. GABE, Arch. Anat. microsc. 54, 371 (1965).

<sup>9</sup> Supported by National Science Foundation Grant No. GB-1356. Performed at the Duke University Marine Laboratory, Beaufort (North Carolina, USA).

## Tonofilament Aggregations in Ultimobranchial Gland Cells of *Rana temporaria* L.

Recently it has been suggested that the anuran ultimobranchial gland may be implicated in water drive phenomena associated with the breeding period<sup>1</sup>. Owing to the paucity of published ultrastructural observations on anuran ultimobranchial glands, which have been restricted to 2 species, viz.: *Rana pipiens*<sup>2-4</sup> and *Xenopus laevis*<sup>5</sup>, an investigation was performed to determine the 'normal' ultrastructure of ultimobranchial (UB) secretory cells of some common British frogs and toads during and following the breeding season. During the course of this study, which will be reported in detail elsewhere<sup>6</sup>, very large volumes of tonofilaments were encountered in the frog UB secretory cells. In view of the current widespread interest in calci-

tonin and with the strong possibility that these cells may be producing a calcitonin-like factor, the presence of large volumes of tonofilaments within these cells is of especial interest.

**Material and methods.** Untreated adult *Rana temporaria* L. and *Bufo bufo* L. were obtained from a commercial

<sup>1</sup> D. BOSCHWITZ, Israel J. Zool. 18, 277 (1969).

<sup>2</sup> D. R. ROBERTSON and A. L. BELL, Z. Zellforsch. 66, 118 (1965).

<sup>3</sup> D. R. ROBERTSON, Z. Zellforsch. 67, 584 (1965).

<sup>4</sup> D. R. ROBERTSON, Z. Zellforsch. 85, 453 (1968).

<sup>5</sup> R. COLEMAN, in *Calcitonin 1969*. Proc. Second Int. Symp (Ed. S. TAYLOR; Heinemann Medical Books, London 1970), p. 348.

<sup>6</sup> R. COLEMAN, Z. Zellforsch., in press.

source<sup>7</sup> over the period from the beginning of March to mid-June. UB glands were dissected from the frogs and toads and fixed in ice-cold 3% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2) followed by secondary fixation in veronal acetate-buffered 1% osmium tetroxide prior to embedding in Epon 812. Sections were stained briefly with lead citrate and uranyl acetate prior to examination in an AEI EM6B electron microscope.

**Results and discussion.** The glands contain several follicles, the main cells of which are characterized by the presence of large numbers of cytoplasmic, membrane-bound, densely-staining secretory granules of about

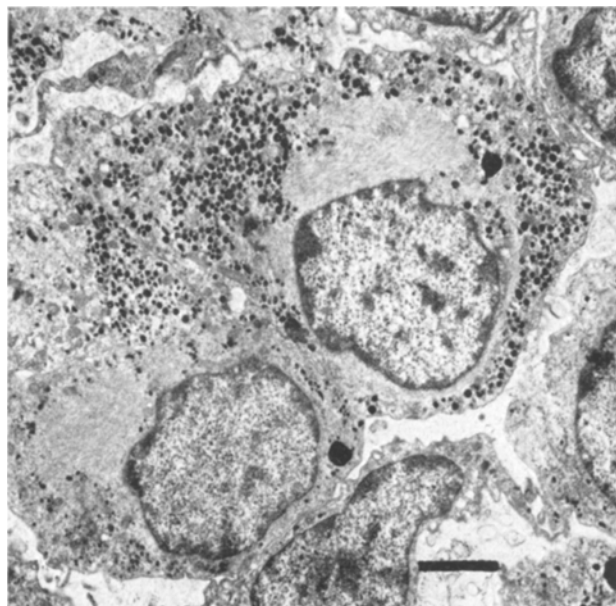


Fig. 1. Typical ultimobranchial cells of untreated male frog in mid-March showing juxtanuclear aggregations of tonofilaments and the characteristic secretory granules. Scale, 2  $\mu$ m.

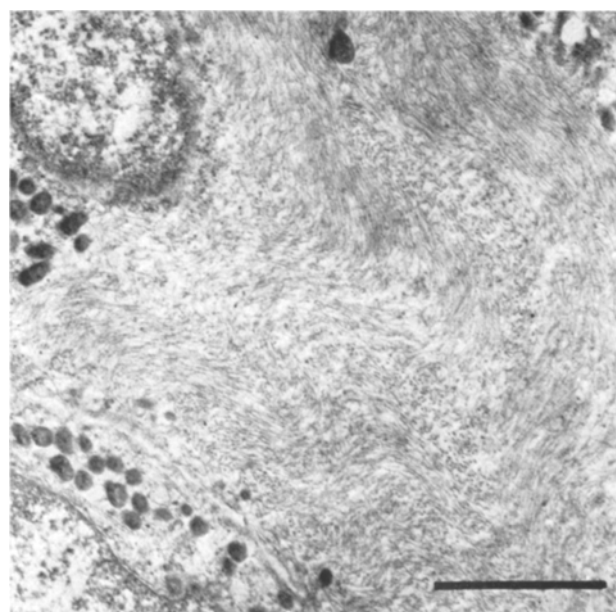


Fig. 2. Portion of a typical tonofilament aggregation in an untreated female frog in mid-March; characteristic secretory granules with associated microtubules can be observed in the bottom left region of the micrograph. Scale, 1  $\mu$ m.

100 nm diameter. In addition, much larger lipid-like droplets, up to 2  $\mu$ m in diameter, are also found but in much sparser numbers. Virtually every UB secretory cell in *R. temporaria* contains large aggregations of tonofilaments in juxtanuclear locations (Figures 1 and 2). The tonofilaments are very uniform in size being approximately 6 nm thick and at high magnifications seem to possess a delicate beaded appearance along their length. The tonofilaments seem to be organized in sinuous bands (Figure 2). Some of the characteristic secretory granules are found embedded within the tonofilament masses as are the rarer lipid-like inclusions on which tonofilaments seem to terminate or originate.

The volume of tonofilaments in the frog UB cells did not change appreciably during the period of the investigation; moreover, no sex differences could be detected. ROBERTSON and BELL<sup>2</sup> described the presence of tonofilaments in UB gland cells of adult *Rana pipiens* examined in October, but did not show the enormous volume of tonofilaments described here in *R. temporaria*. It is possible the numbers or volume of these tonofilaments may be related to seasonal activity of the glands together with correlated changes in metabolic activity. In *Bufo bufo* UB gland cells tonofilaments were only occasionally found and remained fairly inconspicuous. There would thus appear to be quite distinct species differences in the amount and possible metabolic role of tonofilaments in anuran UB cells.

PEARSE<sup>8</sup> has recently suggested that one of the ultrastructural characteristics of C cells is their tendency to produce fine protein microfibrils. The observations described here on *R. temporaria* UB cells give added support to the hypothesis that these are in fact C cells and homologous with mammalian C cells though the function of tonofilaments in such cells remains to be established.

FAWCETT<sup>9</sup> briefly reviews the presence of similar tonofilament aggregations in a variety of cell types and considers such filaments may represent a class of fibrous proteins, though from the various studies cited little can be deduced as to their functional significance. This is exemplified by one of the cell types cited, the interstitial cells of pigeon testis, which are specialized for the production of androgenic hormones and also contain tonofilament aggregations. Such cells are not apparently mobile and have no apparent need for a specially developed cytoskeletal filamentous system. Thus, one is led to conclude that, as yet, there is extremely little indication as to the structural or physiological significance of tonofilament aggregations in cells and this is especially true of UB cells<sup>10, 11</sup>.

**Résumé.** Observations ultrastructurales sur les corps ultimobranchiaux des grenouilles adultes (*Rana temporaria*) au printemps ont montré des accumulations de microfibrilles cytoplasmiques.

R. COLEMAN

Department of Zoology, Bedford College,  
University of London, Regent's Park,  
London N.W.1 (England), 8 June 1970.

<sup>7</sup> Frogs and toads were supplied by L. Haig & Co., Newdigate, Surrey (England).

<sup>8</sup> A. G. E. PEARSE, in *Calcitonin 1969*. Proc. Second Int. Symp. (Ed. S. TAYLOR; Heinemann Medical Books, London 1970), p. 125.

<sup>9</sup> D. W. FAWCETT, in *The Cell, An Atlas of Fine Structure* (W. B. Saunders Co., Philadelphia 1966), p. 244.

<sup>10</sup> This work was assisted by apparatus supplied by the Central Research Fund of London University.

<sup>11</sup> I am grateful to Mr. R. L. JONES for his expert technical assistance.