The Development and Fine Structure of the Ultimobranchial Glands in Larval Rana temporaria L.

Raymond Coleman and Alan D. Phillips*
Department of Zoology, Bedford College, University of London, England

Received September 10, 1973

Summary. The fine structure of ultimobranchial (UB) gland cells from Rana temporaria larvae 48 h after hatching until the completion of metamorphosis is described. A single UB cell type is present, believed to be the characteristic C cell, in which secretory granules are first detectable in 8 day post-hatching larvae. These secretory granules show an intimate association with lipid droplets. Unusual membranous and crystalloid inclusions, which may represent yolk platelets, are found in UB glands of very small larvae. The significance of a range of UB organelles is discussed and some scanning electron micrographs presented. This report is believed to be the first published ultrastructural and scanning electron microscope study of larval anuran UB glands.

Key words: Ultimobranchial glands — Anuran larvae — Metamorphosis — Ultrastructure — Scanning electron microscopy.

Introduction

It is now becoming well established that the homologues of the calcitonin-producing cells in the mammalian thyroid, the "parafollicular" or C cells, are found in the lower vertebrates within discrete ultimobranchial (UB) glands, the endocrinology of which has recently been reviewed in amphibians (Robertson, 1971a). Most of the recent experimental studies on amphibian UB glands have been performed on anuran adults and as yet, as far as we are aware, there have been no published ultrastructural reports on the changes in the anuran UB glands during larval development in spite of the fact that optical microscope studies indicate that they are especially active during metamorphosis (Boschwitz, 1960; Robertson and Swartz, 1964; Robertson, 1971b; Saxen and Toivonen, 1955; Watzka, 1933).

We have now investigated the development of the UB gland in tadpoles of the common British frog, Rana temporaria, in an attempt to see if we could establish by ultrastructural methods in normal untreated larvae when the UB glands first show signs of secretory activity and to examine spontaneous changes occurring in the glands during metamorphosis. By so doing we hoped to determine a normal baseline against which future experimental studies on this species may be compared and contrasted.

* We are indebted to the Science Research Council for an award making this research possible. We would also like to thank Mr. R. L. Jones, Mr. Z. Podhorodecki and Miss V. Cooper for technical assistance.
Material and Methods

Our study was performed in two successive years. In early March 1972 four pairs of adult frogs (*Rana temporaria* L.) from a batch purchased through a commercial supplier produced our supply of fertile spawn. The following year spawn was collected from garden ponds in the London area or bought commercially towards the end of March and the beginning of April. The spawn was hatched in aerated tapwater in plastic or glass aquaria at room temperatures. Initially the larvae were fed on nettle powder and later with slices of lamb liver or ox heart.

UB glands were dissected from larvae immersed in fixative under a binocular dissection microscope by means of fine watchmakers forceps. The site of the paired glands has been described by Greil (1905). A rapid procedure to locate the glands is to dissect out the entire internal gill system together with the heart and then the glands can be found from the ventral side in the most posterior branchial pouch adjacent to the pericardial region. This flap of tissue containing the UB gland can then be removed and processed for electron microscopy (see Figs. 17, 18). After metamorphic climax with the associated changes in the internal anatomy, in particular regression of the branchial system, the UB glands become more difficult to find, but can be located on muscle on either side of the opening to the larynx.

We removed UB glands from tadpoles 48 h after hatching (total length c. 13 mm) and at successive size and time intervals throughout metamorphosis for optical and transmission electron microscopy. In total a detailed examination of 92 UB glands was made using these methods.

**Electron microscopy.** UB glands were fixed briefly (5–30 mins) in ice-cold 3% glutaraldehyde in 0.1% sodium cacodylate buffer, pH 7.2, rinsed briefly in this buffer containing 7.5% w/v sucrose and post-fixed for 1 h in 1% osmium tetroxide in veronal acetate buffer at the same pH. The glands were then rinsed in veronal acetate buffer, dehydrated in graded ethanols, treated with propylene oxide and embedded in Epon 812. Sections (60–90 nm), cut on a Cambridge (Huxley Mk. 1) ultramicrotome, were mounted on uncoated copper grids, stained briefly in lead citrate and examined in either an AEI EM6B or AEI Corinth 275 transmission electron microscope at 60 kV.

Thick (0.5 μm) epon sections, cut on the ultramicrotome, were stained in 0.1% toluidine blue in 1% borax for correlated optical microscopy.

Surface structure of selected UB glands from premetamorphic larvae was examined by scanning electron microscopy. Pieces of branchial tissue containing the UB gland were dissected in glutaraldehyde fixative as above, placed directly in a colloidon-covered stub and air dried. The specimens were then thinly coated with gold-palladium prior to examination in the Cambridge S4-10 Stereoscan electron microscope of London University Board of Studies in Zoology at Bedford College.

Stages of development are given in terms of days after emerging from the egg (hatching) or total length (front of head to end of tail).

Observations

We could remove UB glands consistently from larvae at 48h post-hatching, by which time the external gills are rapidly regressing and the internal gill system has its basic form. At this stage the UB glands are fairly compact spherical or ovoid bodies, typically about 56 × 40 μm in section, which progressively increase in size during larval development and tend to become more ellipsoid or elongate (Figs. 1–6).

The UB glands are composed of epithelial cells, the most peripheral of which can be seen in electron micrographs to lie on a typical basal lamina. The increase in UB volume during development results mainly from mitosis, especially in premetamorphic larvae, although mitotic figures are found in UB glands right throughout metamorphosis, usually in central rather than peripheral sites. Occasionally UB glands of young larvae up to 8 days post-hatching are found that are still connected to the pharynx by a solid stalk of cells.