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Morphology of the Ultimobranchial Body in Cockerels

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Abstract

The morphology of the ultimobranchial body in 12-week-old cockerels was compared with published descriptions of that body in chickens and laying hens. In the cockerel the body is a smaller, more compact gland than that reported in hens and may be obscured by thymic tissue. Wide variability in the vesicular component of the cockerel ultimobranchial body was noted and three distinct patterns were described. Two distinct types of secretory cell in the connective tissue surrounding the vesicles were identified with respect to their granule populations and stereological investigations on transmission electron micrographs confirmed the presence of two distinct granule populations.

Extra keywords: ultimobranchial vesicles; calcitonin cells (C cells); calcitonin granules.

Introduction

The histology and ultrastructure of the avian ultimobranchial bodies have been studied (Sehe 1965; Hodges 1970, 1971; Bélanger 1971; Gould and Hodges 1971), but there are no specific descriptions of the cockerel ultimobranchial bodies. The purpose of this study is to describe the morphology of that body and to compare it with published descriptions of ultimobranchial bodies in chickens and laying hens (Dudley 1942; Malmqvist *et al.* 1968; Hodges 1970, 1979; Isler 1973; Chan 1977, 1978; French and Hodges 1977).

Materials and Methods

Twenty 12-week-old Rhode Island Red–Australorp hybrid cockerels were used. The ultimobranchial bodies were dissected out by following the illustrations of their location in the fowl (Hodges 1974). Tissues were fixed by perfusion of the common carotid artery for 5–8 min at a perfusion pressure of 133 kPa with 2% (v/v) glutaraldehyde (Ladd Research Industries) and 2% (w/v) paraformaldehyde (BDH) in 0·1 M sodium phosphate buffer, pH 7·3, under intraperitoneal sodium pentobarbital anaesthesia. Following perfusion, tissues were further fixed in the same solution for 5 h, post-fixed in 1% (w/v) OsO_4 buffer with 0·1 M sodium phosphate at pH 7·4 for 2 h, dehydrated and embedded in an Epon-Araldite mixture. Ultrathin sections were cut with a diamond knife (Diatome) on an LKB UMI ultramicrotome and mounted on bare copper grids. Each grid was post-stained with 2% (w/v) uranyl magnesium acetate and Reynold's lead citrate (Reynold 1963) and viewed on a Hitachi HUIIE-S electron microscope operated at 50 kW.

Electron micrographs were taken of all secretory cells where the nucleus was clearly visible and cell boundaries were well defined. These were then divided into two groups, resulting in 15 secretory cells with small granules and 20 secretory cells with larger granules. All electron micrographs were taken at × 5600 and prints were made at a constant magnification (× 9·8). Stereological investigations on the transmission electron micrographs of secretory cell granule populations were carried out using a digital image analyser (MOP-I). Using the image analyser the cytoplasmic area of each cell was determined, the granules in each cell counted and the cross-sectional area of the granules calculated.

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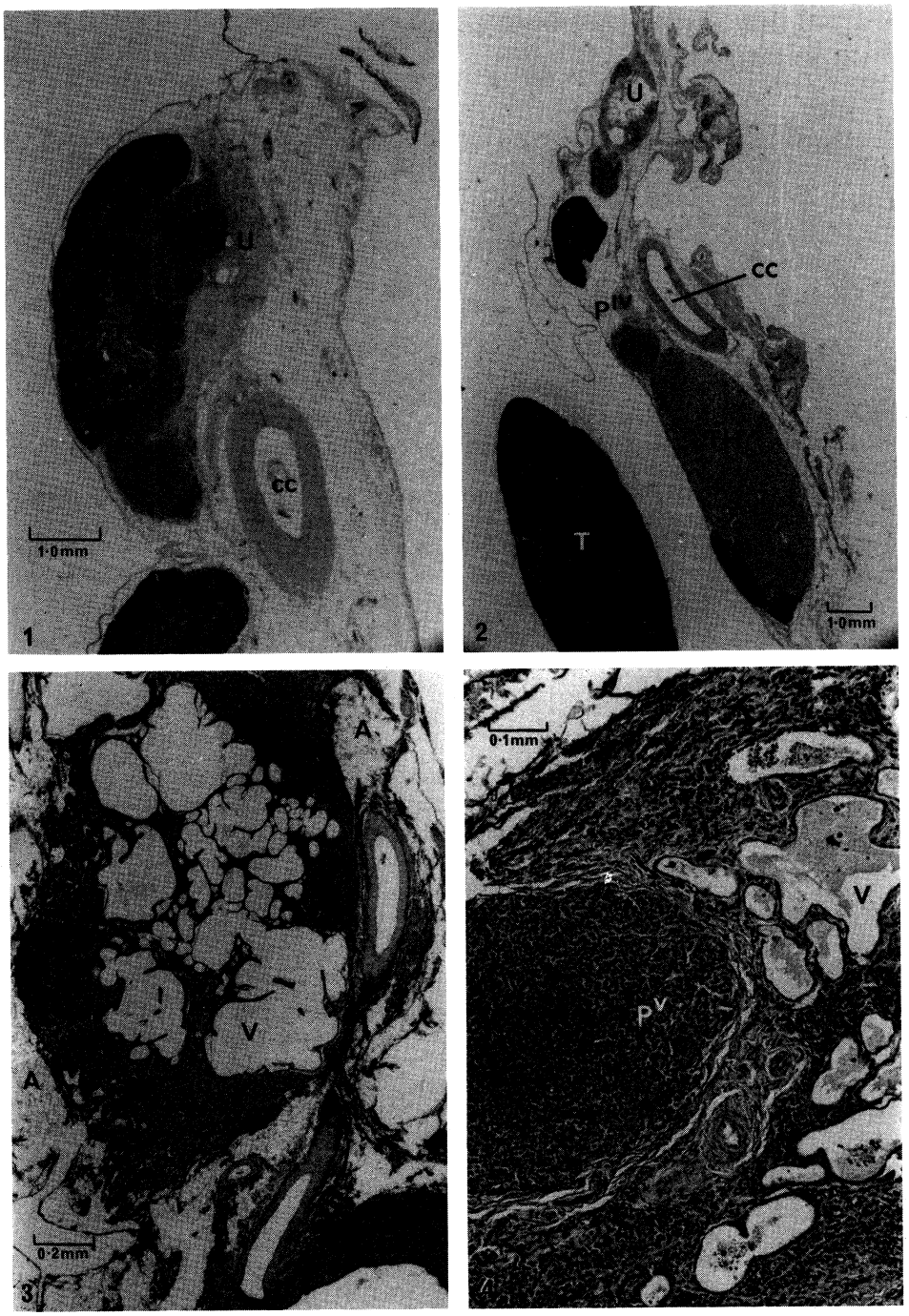


Figs 1–4. Ultimobranchial bodies of cockerels; formalin fixation, haematoxylin and eosin stain. **1**, Relationship of the ultimobranchial body (*U*) to the common carotid artery (*cc*) and thymic tissue (*T*). **2**, Relationship of the thyroid (*TH*); parathyroid III, IV, V (P^{III}, P^{IV}, P^{V}); thymus (*T*) and common carotid artery (*cc*) to the ultimobranchial body (*U*). **3**, Vesicular pattern of the ultimobranchial body (*U*) and the surrounding adipose tissue (*A*). *V*, vesicle. **4**, Encapsulated nodule of parathyroid *V* (P^{V}) within the body of the ultimobranchial body (*U*) and close to the vesicles (*V*).

The number of granules per unit volume, N_v, was then calculated from the relation (Gemmell *et al.* 1983):

$$N_v = N_a/\overline{D} ,$$

where N_a is the number of granules per unit area (1 μm^2) and $\overline{D}$ their mean diameter. The mean area of 200 randomly selected small granules and 200 randomly selected larger granules was used to calculate the mean diameter $\overline{d}$ of granule profiles. $\overline{D}$ was calculated from the relation (Weibel 1969; Gemmell *et al.* 1983):

$$\overline{D} = 4\overline{d}/\pi .$$

Tissue removed for light microscopy was fixed in 10% (v/v) neutral buffered formalin, processed in an automatic tissue processor through graded ethanol, toluene and then embedded in paraplast wax (56°C). Sections were stained with either haematoxylin and eosin, or toluidine blue.

Results

In the present study, the ultimobranchial body was always found to be a pale pink structure (1–2 mm wide by 2 mm long), irregular, lenticular or ovoid in shape and with a very soft texture. Small segments of the thymus often continued past the thyroid gland and sometimes obscured the location of the parathyroid gland and the ultimobranchial body (Fig. 1).

Although the relationship of the thyroid, parathyroid and ultimobranchial body varied from left to right sides, the overall arrangement was very constant, viz. thyroid gland, parathyroid III, parathyroid IV, and finally the ultimobranchial body which sometimes contained parathyroid V or was in close contact with it (Fig. 2).*

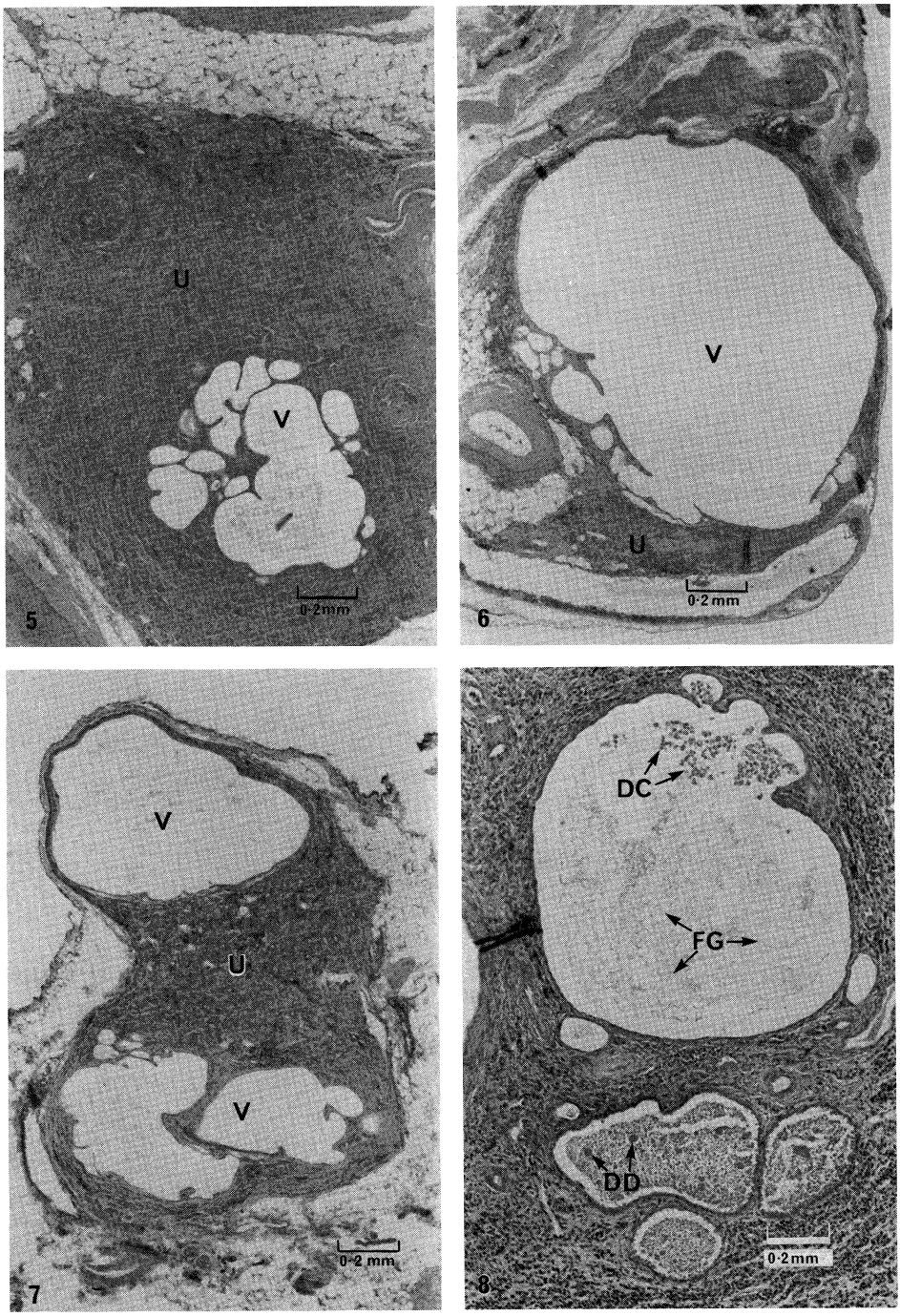
The overall histological appearance of the ultimobranchial body was of a small, oval to round structure (2 by 1·5 mm) with a periphery of randomly arranged calcitonin-secreting cells (C cells), islands of parathyroid cells and lymphoid tissue, and a core of vesicles of varying size and shape which formed an irregular labyrinth (Fig. 3). The ultimobranchial body was often not encapsulated but because of its cellularity could be distinguished from the surrounding connective tissue which was usually rich in adipose tissue (Fig. 3).

The C cells were the major epithelial component. They were arranged in cords and islands of approximately 3–10 cells and were irregularly dispersed throughout the connective tissue stroma. The C cells had a large, round to oval, eccentrically placed nucleus, with dispersed chromatin and one or two prominent nucleoli (seen only with toluidine blue stain). They lay in close proximity to an intense network of anastomosing capillaries.

Parathyroid V was sometimes present partly or completely enclosed within the ultimobranchial body, but invariably it was well encapsulated (Fig. 4). Additional islands or cords of parathyroid cells were sometimes seen closely associated with the epithelial lining of the vesicles. Nodules of lymphoid tissue were commonly seen at the periphery of the body.

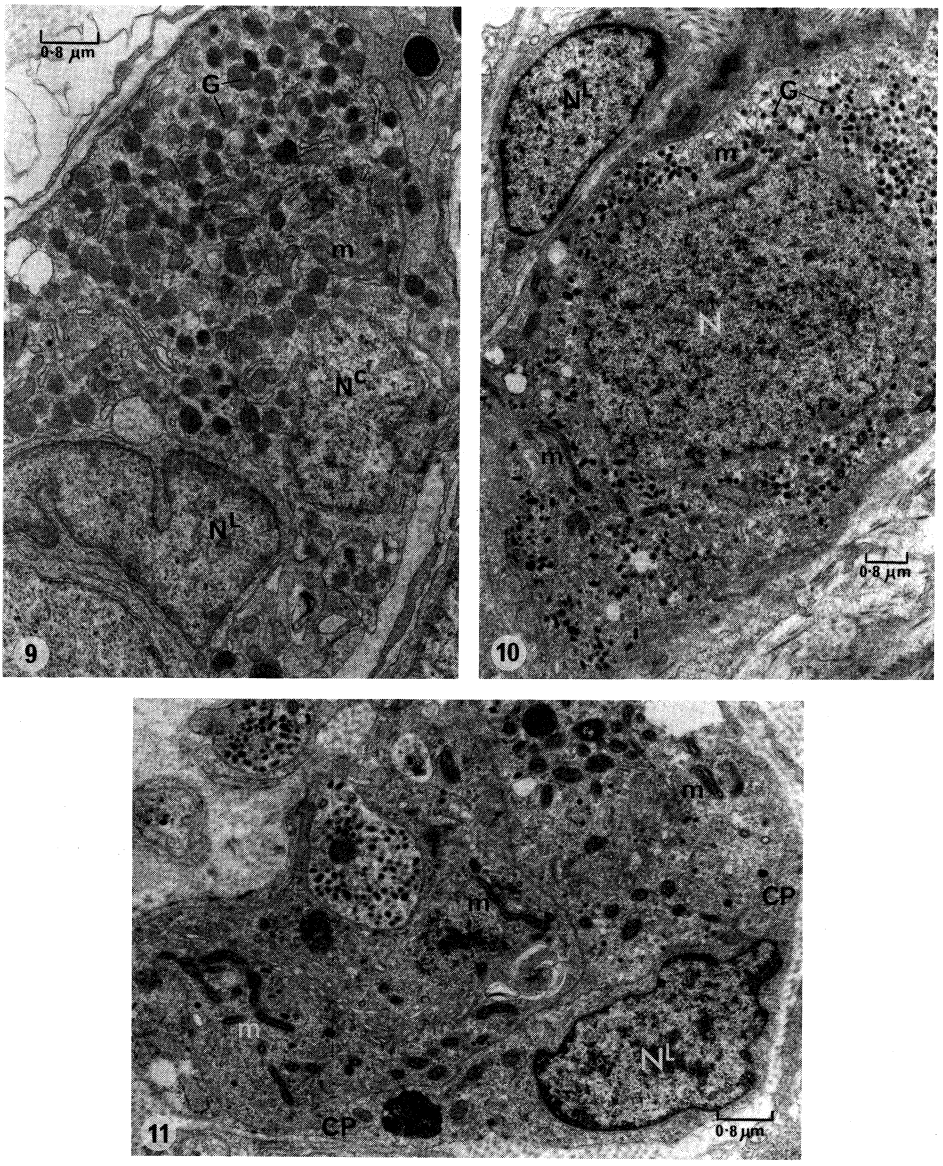
A detailed study of serial 5 μm sections established that approximately one-half of the gland was occupied by vesicles varying in diameter from 0·02 to 1·7 mm. These vesicles could be grouped into three classes with respect to size, shape and number. Most commonly (pattern 1) the vesicles were found in the centre of the gland (Fig. 5) and formed a labyrinth which at its greatest diameter almost completely filled the gland (Fig. 3). In the next most common morphology (pattern 2), the vesicles were found as very thin-walled, oval to circular cavities (Fig. 6). Some labyrinth pattern was often present, but the dominant appearance was a thin-walled, oval to circular cavity. The third pattern seen (pattern 3) contained two separate and distinct sacs within the one ultimobranchial body (Fig. 7).

*The nomenclature parathyroid III, IV and V follows the practice of Hodges (1974) and identifies their embryological development by pharyngeal pouch number.



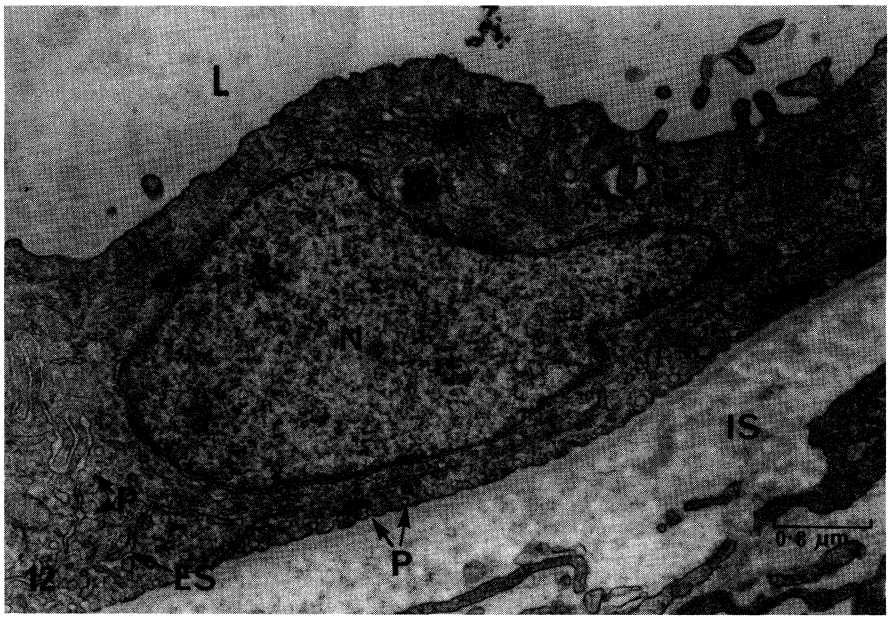
Figs 5–8. Ultimobranchial bodies of cockerels, formalin-fixed, haematoxylin and eosin stain. **5**, Pattern 1: the vesicles (*V*) start within the structure of the ultimobranchial body (*U*). **6**, pattern 2: a very thin-walled vesicle (*V*) starting to reveal the labyrinth pattern. **7**, pattern 3: showing two separate areas of vesicles (*V*) formation separated by the C cell component of the ultimobranchial body (*U*). **8**, Vesicle contents: finely granular material (*FG*), densely staining droplets (*DD*) and desquamated cells (*DC*).

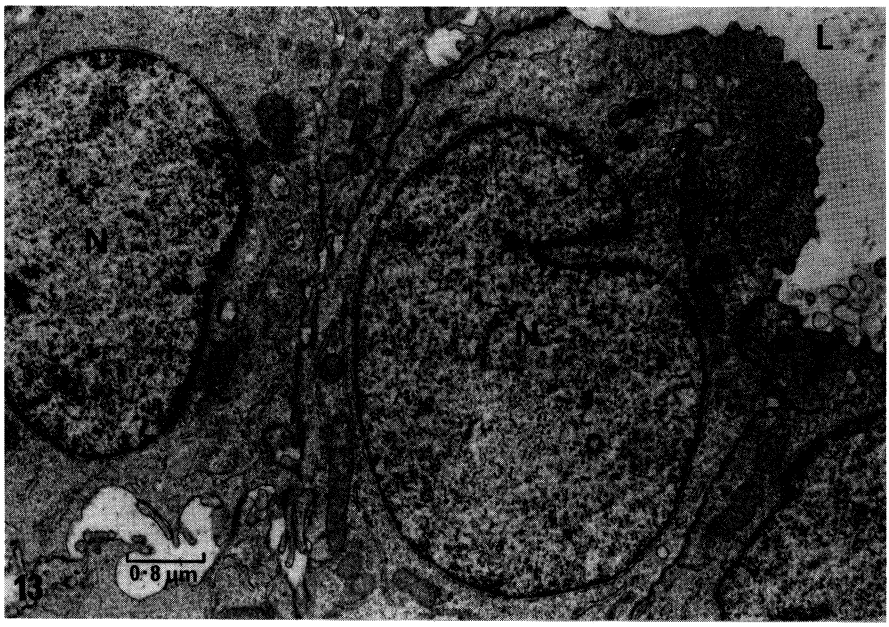
The epithelium lining the vesicle appeared to be variable. In general, the smaller vesicles were lined with cuboidal epithelium. Some vesicles appeared empty in sections, but in others there were desquamated cells, finely granular material or droplets of densely staining material which appeared to be confined mainly to the smaller vesicles (Fig. 8).



Figs 9–11. Cockerel ultimobranchial body; glutaraldehyde–paraformaldehyde–osmium tetroxide fixation. **9**, Secretory cell type with large granules (*G*) and characteristic mitochondria (*m*) with longitudinal cristae; Nucleus of secretory cell (N^C) and nucleus of lining cell (N^L) also indicated. **10**, Secretory cell with small electron-dense granules (*G*) and mitochondria (*m*); nucleus of secretory cell (*N*) and nucleus of lining cell (N^L) also indicated. **11**, Lining cell nucleus (N^L) and its cytoplasmic projections (*CP*) partly surrounding several secretory cells whose characteristic mitochondria (*m*) are visible.

Apart from the normal supporting cells and immune-system cells, three distinctly different types of cell were detected in electronmicrographs: two types of secretory cells and a lining-type cell associated with them. The secretory cell which occurred in greatest





Figs 12 and 13. Cockerel ultimobranchial body; glutaraldehyde–paraformaldehyde–osmium tetroxide fixation. **12**, Vesicle lining cell with prominent nucleus (*N*), pinocytotic activity (*P*) and ergastoplasmic sacs (*ES*). *IS* denotes a wide intercellular space and *L* the lumen of the vesicle. **13**, Cells lining a vesicle within parathyroid tissue in the ultimobranchial body. Their apical borders bulge into the lumen (*L*) and two prominent nuclei (*N*) are present.

numbers was generally arranged in cords and these cords were usually located close to vesicles. In this cell the nucleus was always large and placed eccentrically. It had finely dispersed chromatin, one or two nucleoli and a prominent double nuclear membrane. In the cytoplasm of this type of secretory cell there were numerous membrane-bound secretory granules which were predominantly spherical in shape, and with a mean diameter (±s.e.) of 407 ± 100 nm (Table 1). The granules were often pale, their limiting membrane appeared disrupted and they almost filled the cytoplasm (Fig. 9). Rough endoplasmic reticulum, a well-developed Golgi complex and narrow elongated mitochondria with longitudinally orientated cristae were always present.

Table 1. Comparison of the mean diameter, area, number per unit area and density of granules from two types of secretory cell in the ultimobranchial body of the cockerel

Values given are means ± s.e. of counts. Significance of difference between measurements of granules from the two types of secretory cell is indicated as follows: $^{**}P < 0.01$; $^{***}P < 0.001$

Size of granule	Mean diameter ±s.e. (nm)	Mean area ±s.e. (μm^2)	Mean No. per unit area ($=1\mu m^2$) ± s.e.	Mean No. per unit volume ($=1\mu m^3$) ± s.e.
Small	143 ± 49	0·010 ± 0·001	4·88 ± 0·59	34·16 ± 4·13
Large	407 ± 100**	0·080 ± 0·005***	3·55 ± 0·51	8·73 ± 1·24***

The second type of secretory cell, which was usually but not uniformly seen in close association with the first type, was characterized by having much smaller secretory granules, with a mean diameter (±s.e.) of 143 ± 49 nm (Table 1). These granules were very electron-dense and were membrane-bound, with a zone of lower density separating the membrane from the core of the granule (Fig. 10). The nucleus was usually large in relation to the cell, centrally placed and housed one to three nucleoli. As with the first type of cell the mitochondria had unusual longitudinally oriented cristae. Although the Golgi complex was well-developed, rough endoplasmic reticulum was not a prominent feature. A comparison of the granules present in both types of secretory cell is shown in Table 1. The difference in granule density per unit area (1 μm^2) between the two cell types was not significantly different, but there were highly significant differences within the other measurements, viz. the density per unit volume and the mean granule areas ($P < 0.001$).

The third type of cell, when visible, was in close apposition to both types of secretory cells and appeared to share a common basement membrane with them. Thin, cytoplasmic processes from these cells partly surrounded the secretory cells (Fig. 11). Their cytoplasm was usually more electron-dense than the secretory cells, and contained a variable number of round to oval mitochondria, rough endoplasmic reticulum and a well-developed Golgi complex. No granules were visible in the cytoplasm of these cells. The nucleus was irregular, often thin and tapering, and the nuclear membrane was coated with a thick patchy layer of chromatin with streams of chromatin projecting into the nucleus. The ultrastructure of the vesicular component is similar to that described by Chan (1978) and Hodges (1979). The main features are shown in Fig. 12.

Accessory parathyroid nodules consisted of typical parathyroid tissue which occasionally contained small vesicles. These vesicles were lined by cuboidal to low columnar cells, whose apical border bulged into the lumen of the vesicle (Fig. 13). Their cytoplasm contained well-developed rough endoplasmic reticulum, long narrow mitochondria and free ribosomes but no secretory granules were visible.

Discussion

The anatomical location of the ultimobranchial body in cockerels was similar to its location in laying hens (Dudley 1942; Hodges 1970, 1974; Abdel-Magied and King 1978).

Apart from minor variations between left and right sides, the ultimobranchial body always lay posterior to parathyroid IV. Little mention has been made in previous studies of the very close relationship of thymic tissue to the ultimobranchial body. However, this study has shown that thymic tissue was not only closely associated, but was occasionally intermingled, with it, with no separating capsule (Fig. 1). The overall size of the ultimobranchial body was considerably smaller in the cockerel (greatest length 1–2 mm) than in the hen (greatest length 3–4 mm) (Nickel *et al.* 1977). This is probably correlated with the physiology of the hen as hens do have a much greater turnover of calcium and phosphate which are involved in egg production. Differences were also recorded between fowl and cockerel in the size and distribution of the vesicles in the gland. Hodges (1970) reported that large vesicles in hens had a diameter of up to 500 μm. Although the overall size of the cockerel ultimobranchial body was less than that of hens, the vesicular component occupied a much larger proportion of the whole body in cockerels.

A wide variation in the appearance of the vesicular component of the ultimobranchial body in cockerels, chickens and hens is common. However, no previous reference has been made to distinct classes of vesicles of the avian ultimobranchial body as was found in this study. Any functional significance of this morphology is not known. It has been suggested that the cells lining the vesicles of the fowl ultimobranchial body secrete a mixture of glycoproteins (Sehe 1965; Hodges 1970, 1971).

Although the ultimobranchial body was not encapsulated in the cockerel the cellular components were well-defined and compact. This contrasted with published reports (Stoeckel and Porte 1970; Hodges 1974) on their histology in the laying hen, which noted that the ultimobranchial body in the hen is less well-defined, with its cellular components not compact, and merging with the surrounding connective tissue.

In this present study, two distinct types of secretory cell were identified in the cockerel ultimobranchial body, one with small granules (143 ± 49 nm diameter) and the other with large granules (407 ± 100 nm diameter). The data thus confirm the observations on ultimobranchial bodies in hens by Stoeckel and Porte (1969, 1970) who described more than one type of secretory cell, with granules of different sizes. These authors were unable to determine whether the different granules were associated with different cell types or if they were merely functional variations of the same cell. If these different granules represent different functional states of the same cell, it would be reasonable to expect to find intermediate cell forms containing both granule types, but in this present study intermediate cell types were not seen.

The concept of two distinct types of secretory cell is also supported by the data in Table 1. The granules seen in both types of cell were significantly different in area ($P < 0{\cdot}001$). It is possible that the two types of cell are different populations of cells with different functions, viz. secreting different substances. Further studies of their possible functional significance would seem warranted. One cell, for example, may be secreting calcitonin and the other, substances like somatostatin or 5-hydroxytryptamine. Their proximity would suggest the secretion of one cell type exerting a control on the secretory activity of the other.

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