Cell Tiss. Res. 161, 239—261 (1975)

Ultrastructure of the Adenohypophysis in the Teleost *Poecilia latipinna*

T. Batten and J. N. Ball

Department of Zoology, The University, Sheffield, S10 2TN, England

M. Benjamin

Department of Cellular Biology and Histology, St. Mary's Hospital Medical School, Paddington, London W.2., England

Received April 14, 1975

Summary. In the sailfin molly, *Poecilia latipinna*, seven morphological endocrine cell-types could be distinguished with the electron microscope. Each of these was identified with one of the seven cell-types distinguished with the light microscope, to most of which endocrine functions have previously been allocated. Corticotrophs and prolactin cells form the rostral pars distalis, and the proximal pars distalis consists of an outer layer of gonadotrophs and an inner zone containing growth hormone cells and thyrotrophs. The pars intermedia contains two cell-types, of uncertain function. Stellate cells (interstitial cells) occur throughout the adenohypophysis, but are most numerous and prominent in the rostral pars distalis. The inner proximal pars distalis contains a cell-type not previously distinguished in this species with the light microscope, the Z-cell, which could be aminergic.

The ultrastructural features of each cell-type are described in detail, and discussed in comparisons with the homologous cells described in other teleosts. There is good agreement for different teleosts in the ultrastructural details of each cell type.

Key words: Adenohypophysis — Teleosts (*Poecilia latipinna*) — Cell types — Ultrastructure.

Introduction

The pituitary gland of the small viviparous cyprinodont *Poecilia latipinna* (sailfin molly, green molly) has been extensively studied at the light microscope (LM) level, and experimentation has allocated specific endocrine function to each of the five cell-types in the pars distalis (Ball, 1965; Ball and Olivereau, 1966; Olivereau and Ball, 1964, 1966; Ball and Baker, 1969). The *rostral pars distalis* (RPD) comprises two cell-types, with the predominant prolactin (PRL) cells in a compact mass and a posterior border of ACTH cells or corticotrophs. The *proximal pars distalis* (PPD) contains dorsally and centrally a mass of growth hormone

Send offprint requests to: Dr. John N. Ball, Department of Zoology, The University, Sheffield S10 2TN/England.

We thank Mr. L. Ethridge, Mr. M. P. Hancock, Mr. D. Hollingworth and Mr. W. Thomson for technical assistance, and Mr. D. Taylor of the Nuffield Institute of the Zoological Society of London for permission to use the Nuffield Institute electron microscope. We are grateful to Dr. Harry Grier, who collected and embedded glands from *P. latipinna* in its natural fresh-water habitat in Florida, U.S.A. T. Batten is in receipt of an S.R.C. Research Studentship.

(GH) cells or somatotrophs, mixed with the scarcer thyrotrophic (TSH) cells; ventrally and laterally the PPD is composed entirely of gonadotrophic (GtH) cells, which form the periphery of this region of the pituitary. The posterior *pars intermedia* (PI) contains two distinct secretory cell-types, one PAS + ve, the other lead haematoxylin + ve (PbH + ve); their functions are still debatable (see Discussion). The distribution of the various cells, as in all teleosts, is strongly regionalised, which greatly facilitates their identification (Fig. 1).

Hopkins (1969) has published a detailed account of ultrastructural changes in the PRL cell of *P. latipinna* following experimental changes in external salinity, but none of the other cell-types in this species has been studied with the electron microscope (EM). Follénius and Porte (1960) gave brief ultrastructural descriptions of the cell-types in the related species *Mollienesia* (*Poecilia*) *sphenops*, *Lebistes reticulatus* (now *Poecilia reticularis*) and *Xiphophorus helleri*, but at that date the cell-types could not be linked to endocrine functions.

The present paper describes the fine structure of the constituent cells of the pars distalis and pars intermedia of *P. latipinna* maintained under standard ("normal") conditions, and forms part of a wider experimental study on the ultrastructure of the hypothalamo-hypophysial system in this fish. A later paper will present a quantitative approach to the basic ultrastructure of the adeno-hypophysial endocrine cells, along the lines of the recent study on *Gasterosteus aculeatus* form *leiurus* by Benjamin (1974).

Materials and Methods

Adult male and female *P. latipinna* (weight c. 2 g) were obtained from a dealer in Florida, U.S.A. and acclimatized for at least 4 weeks to standard conditions in the Sheffield laboratory, *viz*: one-third seawater (1/3SW), 9 h illumination per day, 25° C, twice-daily feeding with iodine-enriched Aronson's mixture. The 24 freshwater fish (FW fish), all females carrying late-stage embryos, were collected in the wild in Florida by Dr. Harry Grier, who dissected, fixed and embedded the pituitaries and sent the RPD fragments to Sheffield.

Fixation and Embedding. All the 1/3SW fish were sacrificed between 1400 and 1500 h. They were decapitated and the pituitaries dissected out under fixative. Whole glands or separated segments of RPD, PPD and PI were fixed for 2 h in either 3% glutaraldehyde (GTA) in 0.05 M cacodylate buffer at pH 7.4 and at 5° C, or Karnovsky's (1965) glutaraldehyde in 0.2 M cacodylate at pH 7.2–7.4. GTA-fixed material was post-fixed in 1.33% osmium tetroxide in cacodylate for 3 h after washing overnight in 0.05 M cacodylate + 5% sucrose. Karnovsky-fixed material was washed overnight in 0.2 M cacodylate and then postfixed in Palade's osmium tetroxide for 1 h at 4° C. All material was block-stained in 3% aqueous uranyl acetate for 1 h, dehydrated in graded alcohols, infiltrated with epoxypropylene and embedded in either TAAB resin or Spurr's resin (Spurr, 1969). Pituitaries from FW fish were dissected by Dr. Harry Grier in Florida, and the RPD fragments were fixed in 3% GTA in 0.1 M cacodylate buffer at pH 7.4. They were washed in cacodylate buffer + 5% sucrose, postfixed 1% osmium tetroxide for 30 min, and then dehydrated and embedded in Spurr's resin.

Electron Microscopy. Material was sectioned using a Reichert OM U3 ultramicrotome or a Cambridge Huxley ultramicrotome, and the thin sections stained with 5% uranyl acetate and lead citrate (Reynolds, 1963). They were examined and photographed with a JEOL JEM T7, a Miles MR60C, or an AEI EM 6B electron microscope. For identification of the regions of the gland and the cell-types, adjacent thick (c. 0.5 μm) and thin (silver to grey interference colour) sections were cut. The thick sections were stained with methylene- or toluidine-blue for light microscopy, for comparison with the thin sections under the EM. Identifications

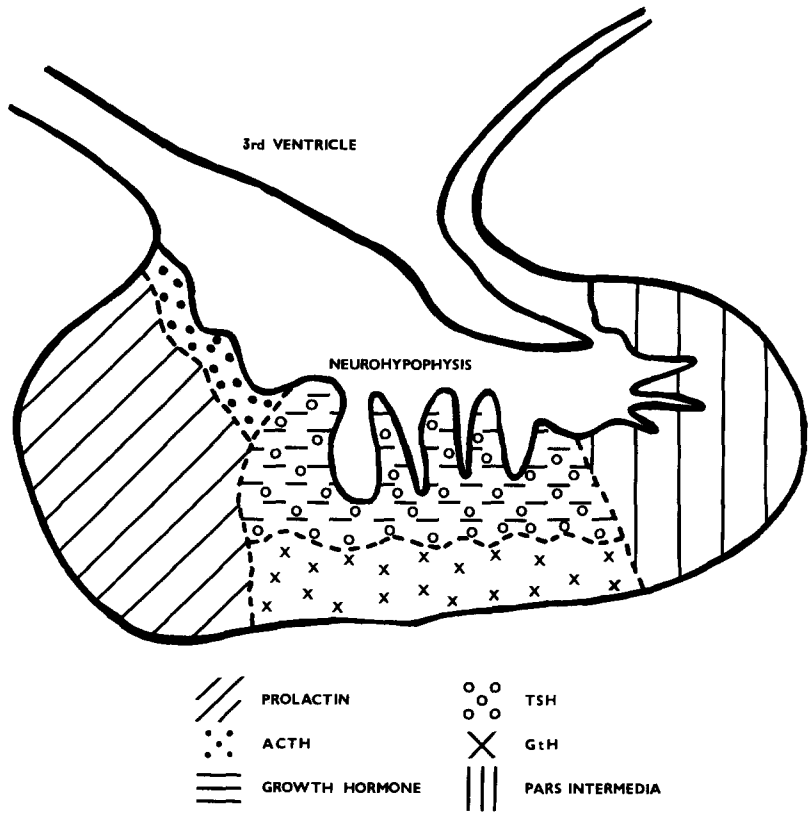


Fig. 1. Diagrammatic sagittal section through *P. latipinna* pituitary, to show the distribution of the various cell-types. Anterior to the left

were facilitated by examination of fragments of different regions processed separately, and by comparisons with conventional LM sections stained by techniques standard for the pituitary (Ball and Baker, 1969).

The diameters of secretory granules were measured on micrographs with a final print magnification of 100000, using a centimetre scale and measuring to the nearest 1 mm division. Before taking the micrographs for this purpose the microscope (JEM T7) was accurately calibrated using a "Polaron" beef liver catalase crystal with a spacing of 2.17 nm. Measurements were made to the outside of the limiting membrane or to the edge of the dense core if no membrane was visible. In the case of elliptical or irregularly-shaped granules, measurements were made along two perpendicular axes and the average was taken. Two micrographs were taken of each cell-type in each of five females (ovary stage 2, Ball and Baker, 1969) adapted to 1/3SW; 30 recognisable granules were randomly chosen and measured in each micrograph, giving a total of 300 measurements for each cell-type.

This account derives from observations on a total of 68 adult fish. Eighteen females adapted to 1/3SW provided the basic material. Nine males adapted to black background and 9 males adapted to white background, all in 1/3SW, were used to provide standard pars intermedia material. Eight females adapted to 1/3SW and with oocytes in late vitellogenesis (Stage 4, Ball and Baker, 1969) were used for the account of the GtH cells. RPD fragments from 24 pregnant females caught in FW in the wild in Florida were examined to assess the effects of external salinity on the PRL and ACTH cells.

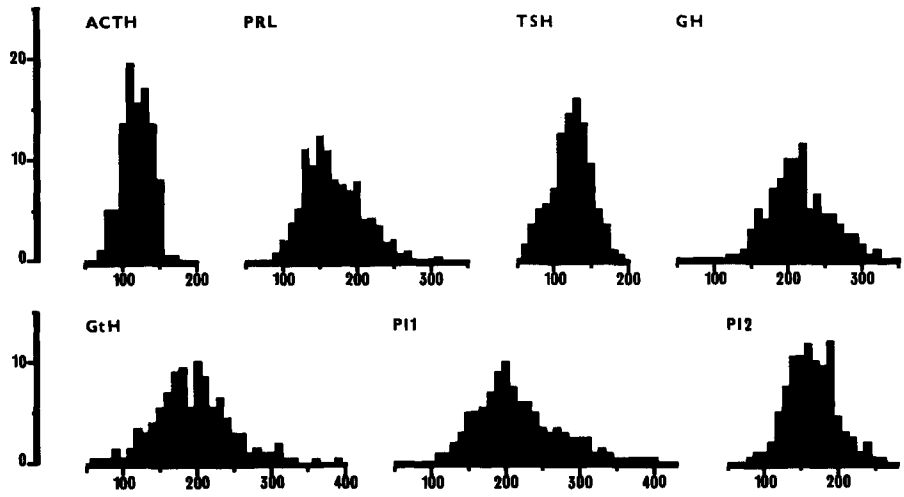


Fig. 2. Percentage frequency distributions of the profiles of secretory granule diameters in the cell-types of the pituitary. Ordinate, percentage frequency; abscissa, granule diameter (*nm*)

Results

Rostral Pars distalis

ACTH Cells (Corticotrophs). The ACTH cells formed a discontinuous layer along the junction of the RPD and the neurohypophysis, often only 3 or 4 cells deep and separated from the neurohypophysis by a continuous double basement membrane (Fig. 1). The basement membrane was in direct continuity with that of adjacent capillaries, and in places it produced extensions which passed inwards as far as the prolactin cells, penetrating between ACTH cells and stellate cells. The space within the double basement membrane contained an amorphous, finely-granular material, apparently identical with that in pericapillary spaces (Fig. 3).

Corticotroph nuclei were frequently indented, and were generally elongated with the long axis parallel to the basement membrane. The cytoplasm was usually sparse, typically forming only a narrow rim around the central nucleus. The secretory granules, round or rod-shaped, were characteristically "haloed", consisting of an electron-dense core separated from the limiting membrane by a clear wide space (Fig. 3); their diameter (Fig. 2) was measured to the edge of the limiting membrane. The smaller granules occurred in the Golgi region, and are assumed to be immature stages. Ordered rough endoplasmic reticulum (RER) was rare, and when present it consisted of only a few pieces. The Golgi region, of variable development in different fish, consisted of stacks of flattened cisternae with numerous small vesicles and a few larger dilated vacuoles. The small vesicles were also scattered throughout the cytoplasm, and near the Golgi zone some of them contained electron-dense material, suggestive of a graduation from small empty Golgi vesicles to fully-formed secretory granules. Microtubules were scattered throughout the cytoplasm, and seemed to be more numerous than in other cell-types. Ribosomes, usually grouped as polyribosomes, were freely distributed in all parts of the cell. The plentiful mitochondria varied in shape from

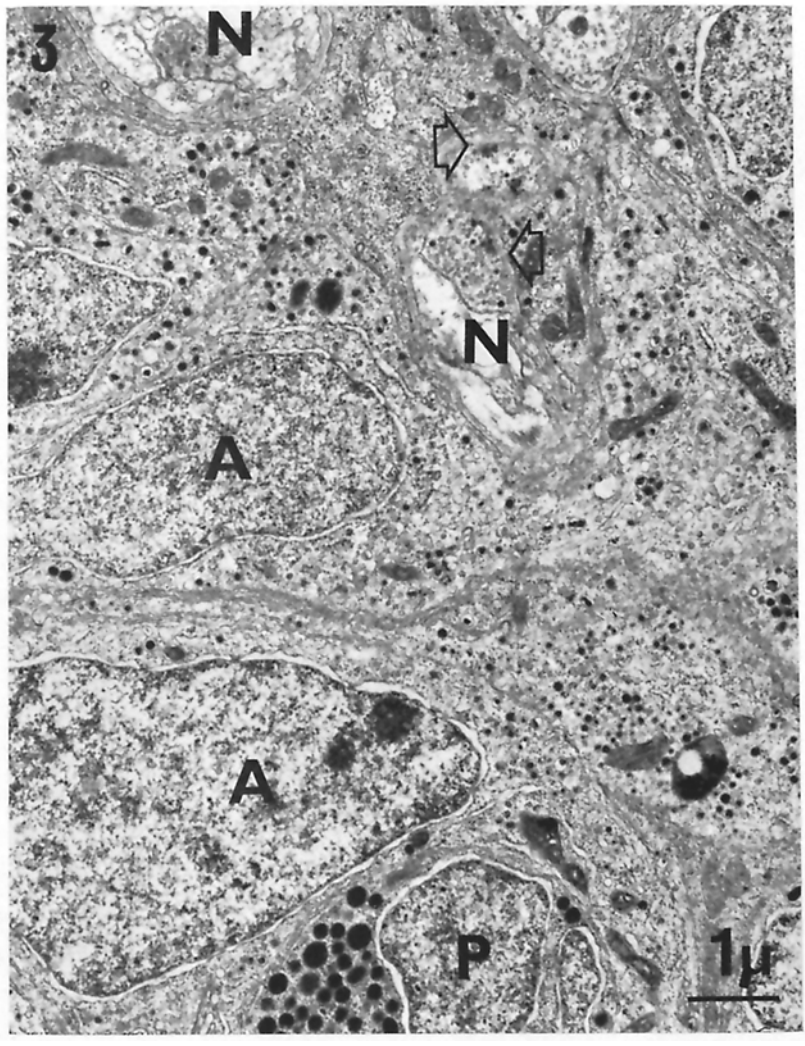


Fig. 3. ACTH cells (*A*) of a 1/3SW fish, lying between the prolactin cells (*P*) and the neurohypophysis (*N*). Small haloed granules, lack of parallel RER, numerous small vesicles. Note the type B nerve fibre terminating on the basement membrane (arrow). × 12000

small rounded bodies to rod-shaped structures, and contained distinct but randomly-arranged cristae. Other inclusions were uncommon, though larger granule-like bodies (300–400 nm diameter), of low electron density were occasionally seen, and are presumed to be lytic; large lytic bodies with lipid droplets, such as seen in Fig. 3, were extremely rare. There were no obvious differences between the ACTH cells of fish adapted to FW and 1/3SW.

Prolactin Cells (PRL Cells). Forming a compact mass occupying most of the RPD (Fig. 1), the PRL cells were usually rounded or oval, with a central rounded nucleus that was usually deeply indented (Fig. 4). The nucleus con-

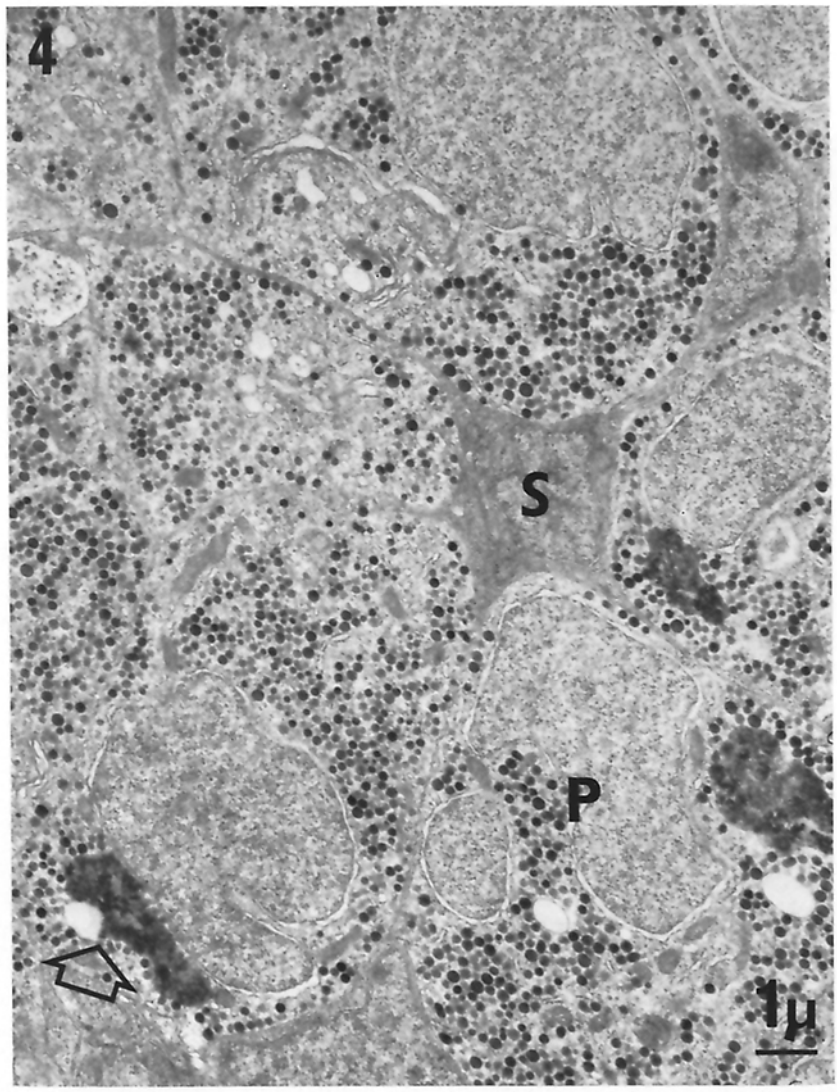


Fig. 4. Prolactin cells (*P*) of a 1/3SW fish surrounding stellate cells (*S*). Note dense granulation and sparse RER, large lytic body (arrowed) containing a clear droplet. ×8000

tained one or two nucleoli, but these were commonly small and indistinct. The mature secretory granules had an electron-dense core to which the limiting membrane was tightly adherent (Fig. 3). There were also sometimes present smaller and more translucent granules, perhaps immature forms, distributed mainly in the Golgi region of active cells. The cytoplasm varied greatly with the secretory activity of the cell. In *fish adapted to 1/3SW* the prolactin cells were relatively inactive, containing many secretory granules but only a little RER, mainly in the form of small non-dilated pieces scattered throughout the

cytoplasm. Mitochondria, round or rod-shaped, were also scarce, and the Golgi apparatus consisted of scattered arrays of flattened cisternae and small vesicles, associated with numerous acanthosomes. Multivesicular bodies were sometimes present, and most cells contained a large electron-dense lytic body, often including clear (lipid ?) droplets and sometimes juxta-nuclear (Fig. 4). *Fish adapted to FW* displayed more active PRL cells, much less heavily granulated and with extensive RER systems in the form of parallel perinuclear arrays, and isolated strands with dilated cisternae throughout the cytoplasm. Very active cells had extensive RER whorls. Mitochondria were more numerous than in 1/3SW fish, with greater variation in shape and with more distinct cristae. Golgi profiles were more prominent, and included large swollen vacuoles in addition to flattened cisternae and small vesicles; the juxta-Golgi acanthosomes seen in 1/3SW fish were rare or absent in FW fish. Granular lytic bodies were commonly present, showing great variations in texture and size, some attaining diameters of about 2–3 μm. Multivesicular bodies also frequently occurred. These active PRL cells also displayed polyribosomal aggregations scattered generally, nuclei with more extensive indentations, and a higher proportion of nuclei with a distinct nucleolus.

In some individuals the PRL cells seemed to occur in two distinct forms: cells with large granules and extensive heavily granulated cytoplasm, often centrally placed in the RPD; and smaller cells, with smaller granules and more indented nuclei, often at the periphery of the RPD.

Stellate Cells. Non-secretory stellate cells (chromophobes, interstitial cells) were present throughout the RPD, each cell having long narrow processes which extended between the PRL cells and contacted processes from other stellate cells (Fig. 4). This network of stellate cells also contacted the juxta-neurohypophysial basement membrane and its inward extensions, and also the bounding membrane of pericapillary spaces. Although stellate cell bodies were found only rarely between the ACTH cells, their processes penetrated freely between them. The stellate cell nucleus was usually flattened with an indistinct nucleolus, and the cytoplasm was generally clear, although in some cells it contained pieces of RER and scattered ribosomes, often aggregated, in the perinuclear area. Also present were microtubules, microfibrils and Golgi-like vesicles, and in some stellate cells the perinuclear cytoplasm was packed with round or oval mitochondria which contained randomly-arranged tubular cristae. In FW-adapted fish the stellate cells were more prominent and the system of processes between the PRL cells was more extensive. PRL granules often appeared to be released by exocytosis into the space between a stellate cell and the PRL cell.

Proximal Pars distalis

Gonadotrophin (GtH) Cells, Gonadotrophs. Because the GtH cells show pronounced structural changes related to gonad state (cf. Ball and Baker, 1969), this account is based on 8 female fish containing oocytes in late vitellogenesis (Stage 4 of Ball and Baker, 1969) and adapted to 1/3SW. It should be emphasized that in any one individual fish all the GtH cells lay within fairly narrow limits of structural variability and furnished no evidence of belonging to more than one category.

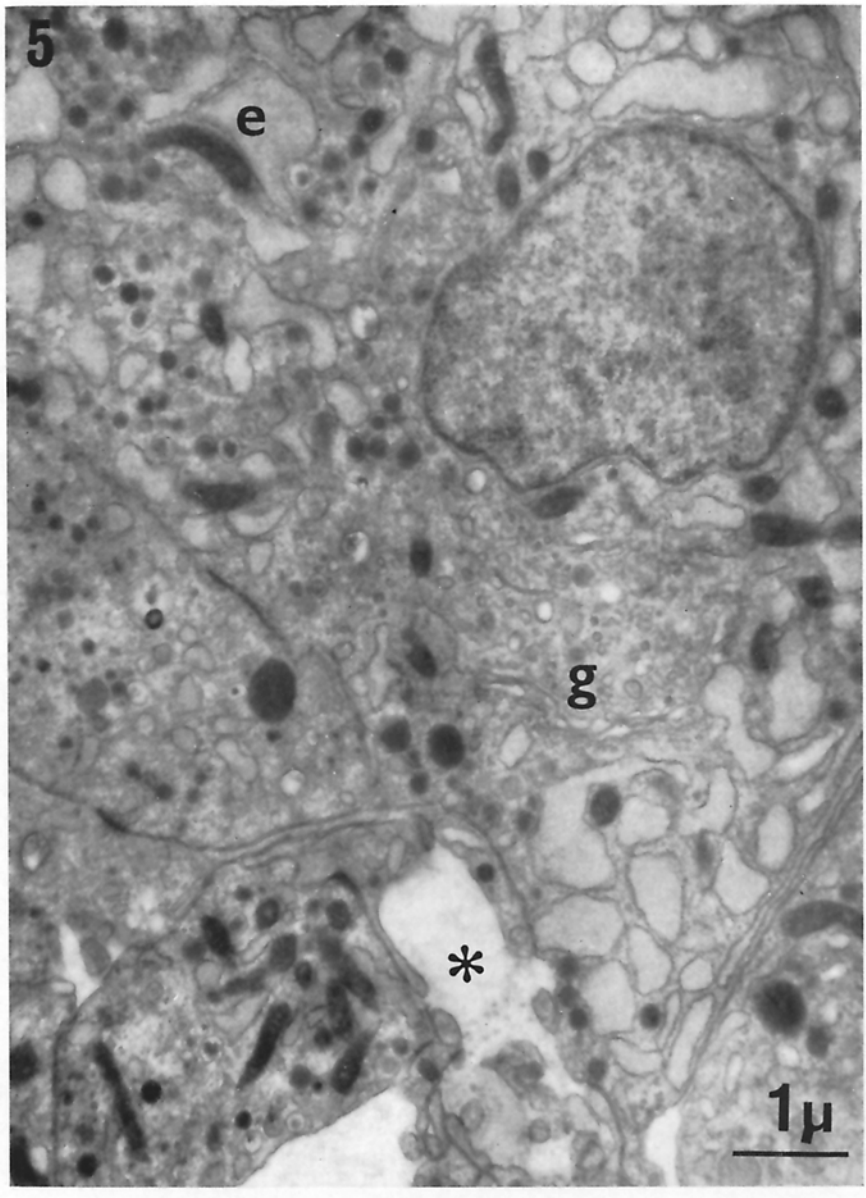


Fig. 5. Gonadotroph (GtH cell) of mature female in 1/3SW. Note dilated RER cisternae (*e*), heterogeneous granulation and large Golgi apparatus (*g*). Some intercellular spaces in this region are dilated (asterisk). ×15000

The GtH cells occupied the whole of the ventral and lateral parts of the PPD (Fig. 1). Large cells, more-or-less rounded though often elongated at one pole, they displayed a central round or oval indented nucleus containing a usually indistinct nucleolus. The secretory granules were less electron-dense than those of

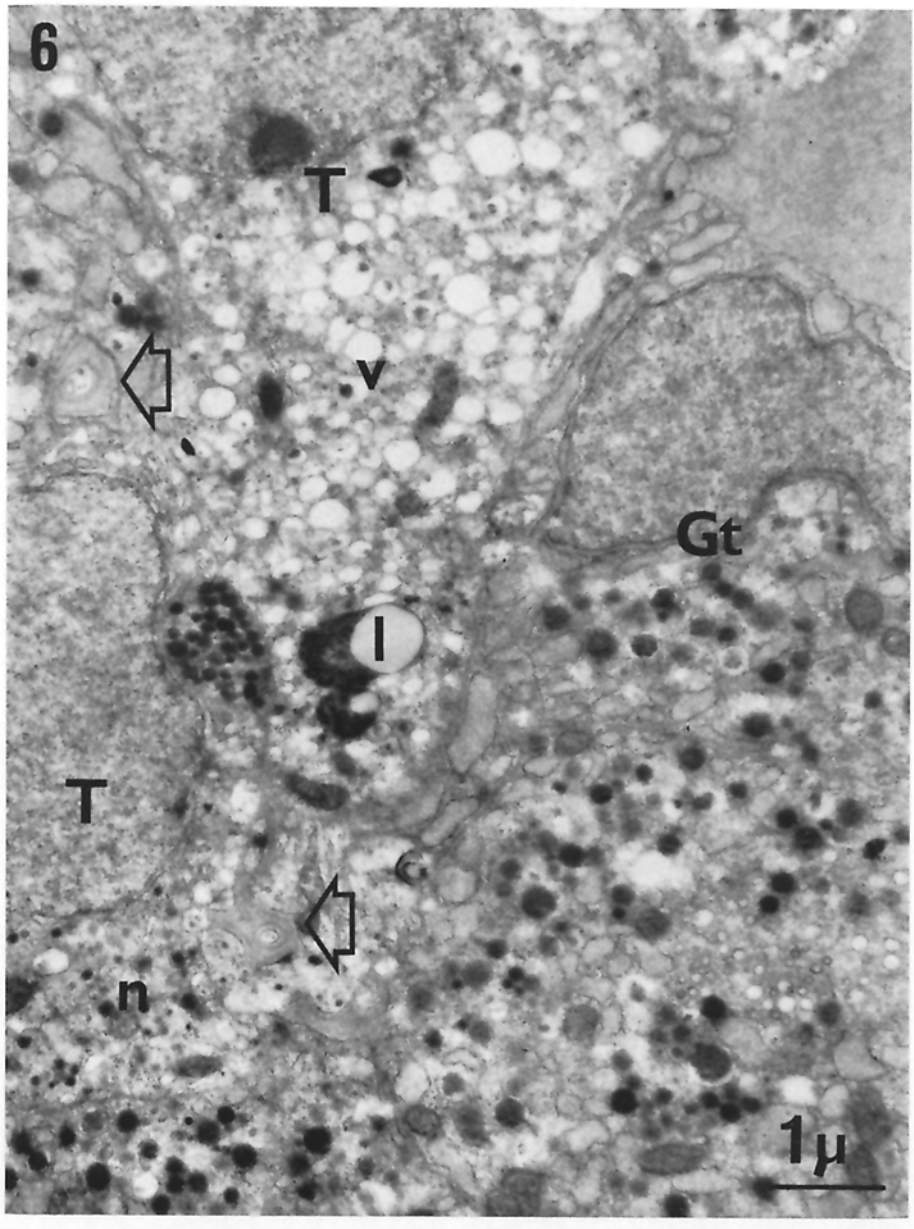
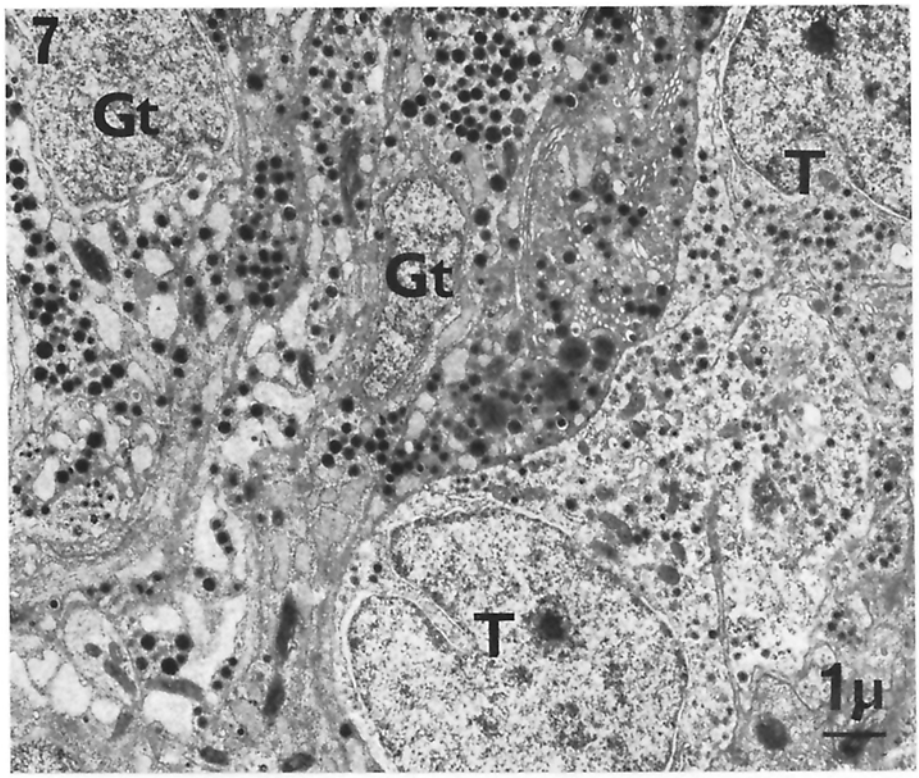
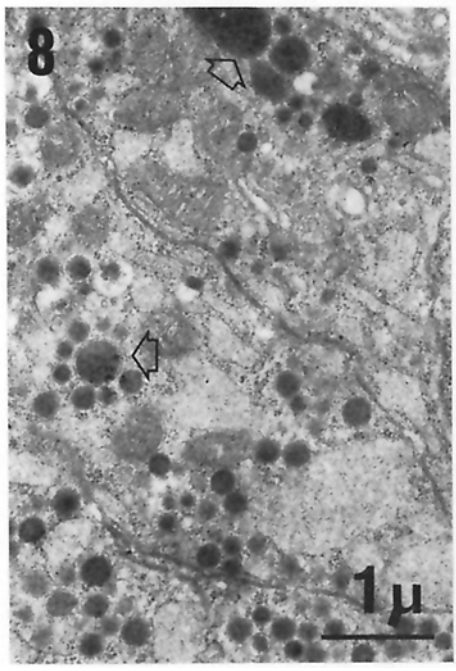
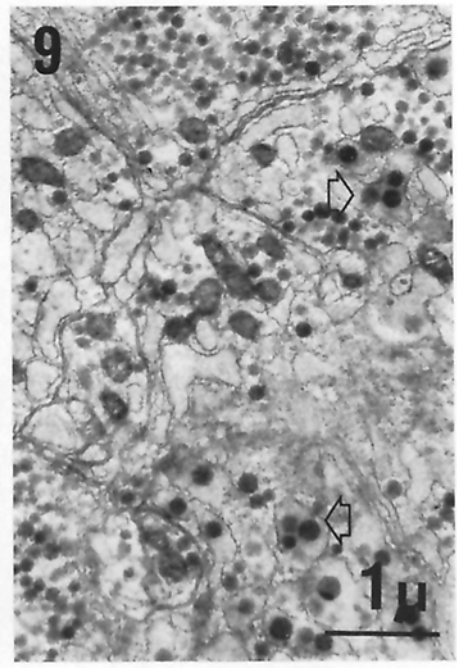


Fig. 6. Adjacent TSH cells (*T*) and gonadotrophs (*Gt*). The vesicular cytoplasm (*v*) is typical of some TSH cells, and contrasts with the more normal cytoplasm (*n*). Note two lytic bodies (*l*) with droplets, and short sections of basement membrane (arrowed). ×15000

the PRL and GH cells. A frequent but variable feature was that the granules were often haloed, with the dense core being distinctly separated from the limiting membrane (Figs. 5–7). Granule diameter varied a great deal (Fig. 2), with the


7
Gt
Gt
T
T
1μ


8
1μ


9
1μ

smaller granules lying in the Golgi region. Some fish had GtH cells containing larger granule-like bodies, usually more electron-lucent than the secretory granules, their electron density increasing with size. These probably correspond to the R granules seen with the light microscope (Ball and Baker, 1969), and are probably lytic. They reached up to 700 nm in diameter, and were usually rounded, though many had an irregular shape (e.g. triangular), and they were often surrounded by a ring of secretory granules (Fig. 8).

The cytoplasm varied in its general appearance. In some fish it was packed with clear vesicles of smooth membrane, resembling secretory granules without the dense core, though some vesicles contained a small amount of dense material. In other fish, the cytoplasm contained variable amounts of dilated RER enclosing electron-lucent flocculent material, and sometimes including intracisternal granules (Fig. 9). In some cells the clear vesicles and distended RER occurred together. Classical parallel arrays of non-distended RER were only occasionally seen, adjacent to the cell membrane or the nucleus, but free ribosomes were distributed throughout the cytoplasm, often in clusters. The Golgi apparatus was usually very extensive, generally positioned near the side of the nucleus facing the elongated pole of the cell (Fig. 5), and comprised flattened parallel cisternae with terminal sacs, numerous small vesicles, and small (immature) secretory granules. Within each cell the abundant mitochondria displayed a wide variety of shapes and sizes, and had a dense matrix with distinct cristae arranged perpendicular to the long axis. Multivesicular bodies and acanthosomes were common in the vicinity of the Golgi apparatus. In some specimens, usually those which displayed GtH cells with an extensive system of dilated RER filled with electron-lucent material, the intercellular spaces along the ventral surface of the pituitary were filled with a similar flocculent substance (Fig. 5).

Thyrotrophin (TSH) Cells, Thyrotrophs. The TSH cells varied greatly in appearance between two extremes. Most often they were large cells, irregularly shaped and containing a large nucleus that was often so deeply indented as to appear divided into two or three lobes. The nucleus usually lay towards one corner of the cell, and occasionally displayed a small rounded nucleolus. The RER did not form parallel arrays, but was scattered in short pieces throughout the cell. It was sometimes expanded into vacuoles containing an electron-dense flocculent material, but these RER dilations never attained the size of those in the GtH cells. The abundant mitochondria were round or rod-shaped, and contained a dense matrix with irregularly organised cristae. Golgi elements, never very conspicuous, appeared in many parts of the cell and comprised stacks of straight flat and dilated cisternae. The secretory granules in these large TSH cells varied greatly in size (Fig. 2), shape, and electron density. Typically they appeared as haloed granules, 70–170 nm in diameter and round or sometimes rod-

Fig. 7. TSH cells (*T*) bordering an area of gonadotrophs (*Gt*). × 8000

Fig. 8. Gonadotroph cytoplasm showing large dense bodies (arrowed), often surrounded by a ring of smaller secretory granules. × 15000

Fig. 9. Gonadotroph cytoplasm showing secretory granules within the dilated RER cisternae (arrowed). × 15000

shaped in section; the core was less electron-dense in the smaller granules (Fig. 10). In some cells much of the cytoplasm was filled with empty membrane-bound vesicles, the same size as the haloed secretory granules, together with larger vesicles which were empty or contained only a little electron-dense material (Fig. 6). Presumptive lytic inclusions took two forms: commonly there were non-haloed semi-dense bodies, larger than the secretory granules (c. 200 nm diameter) and sometimes containing a clear droplet; and some very active cells there were numerous large multivesicular bodies. Frequently the larger TSH cells contained many acanthosomes, often arranged in groups.

At the other extreme of the range of thyrotroph variation was the small rounded cell, with a round or oval nucleus almost filling the cell. The nucleus was generally dark and granular, and the nucleolus was only rarely visible. In the sparse cytoplasm were large mitochondria, rod-shaped or crescentic, and linear arrays of Golgi vacuoles. The scarce secretory granules took the form of typical haloed structures, smaller than in the large TSH cell, and ordered RER was only rarely encountered. In addition the cytoplasm contained a few larger semi-dense granule-like bodies together with some larger oval dense inclusions (Fig. 10).

Growth Hormone (GH) Cells, Somatotrophs. The GH cells lay intimately mixed with TSH cells in the inner regions of the PPD, forming the bulk of the adeno-hypophysial "fingers" interpenetrating the neurohypophysis (Fig. 1). The nucleus was small, usually irregularly oval and occasionally indented, and generally lay in one corner of the cell. The compact cytoplasm had a notably dense ground substance and contained many secretory granules, round and usually non-haloed (Fig. 10). Also present were a few larger membrane-bound bodies (presumably lytic), less electron-dense than the secretory granules and often containing an irregular "woolly" inclusion. RER was usually limited to one or two rows around the nucleus, a few parallel arrays at the edge of the cell, and some scattered isolated short pieces. The centrally-placed Golgi apparatus consisted of small vesicles and stacks of straight flattened cisternae which were sometimes dilated. The small mitochondria had a dense matrix, and were round, oval or rod-shaped. In general, the GH cell exhibited less variation than the other cell-types in the PPD.

Stellate Cells. These were less prominent than in the RPD. They were found mostly between GH cells close to the neurohypophysis, and were never encountered between GtH cells. They contained a very electron-dense nucleus, usually flask-shaped, and a sparse dense cytoplasm containing free ribosomes and small mitochondria. Cytoplasmic projections between GH and TSH cells certainly existed, but were more difficult to trace than the stellate cell processes in the RPD.

Z-Cells. Another cell-type could be distinguished in the PPD, and for non-committal convenience it was called the Z-cell. These were found mainly between GH, TSH and stellate cells close to the neurohypophysial border. They occurred in groups, and were characterized by the presence of very small haloed granules 80–90 nm in diameter. The round or oval nuclei were very granular and though little RER was present polyribosomes occurred throughout the cytoplasm. The extensive Golgi apparatus consisted of concentric arrays of flattened and dilatated cisternae. The numerous mitochondria, often grouped, were rounded or rod-shaped, and had a very dense matrix. Some large dense structures, very like mito-

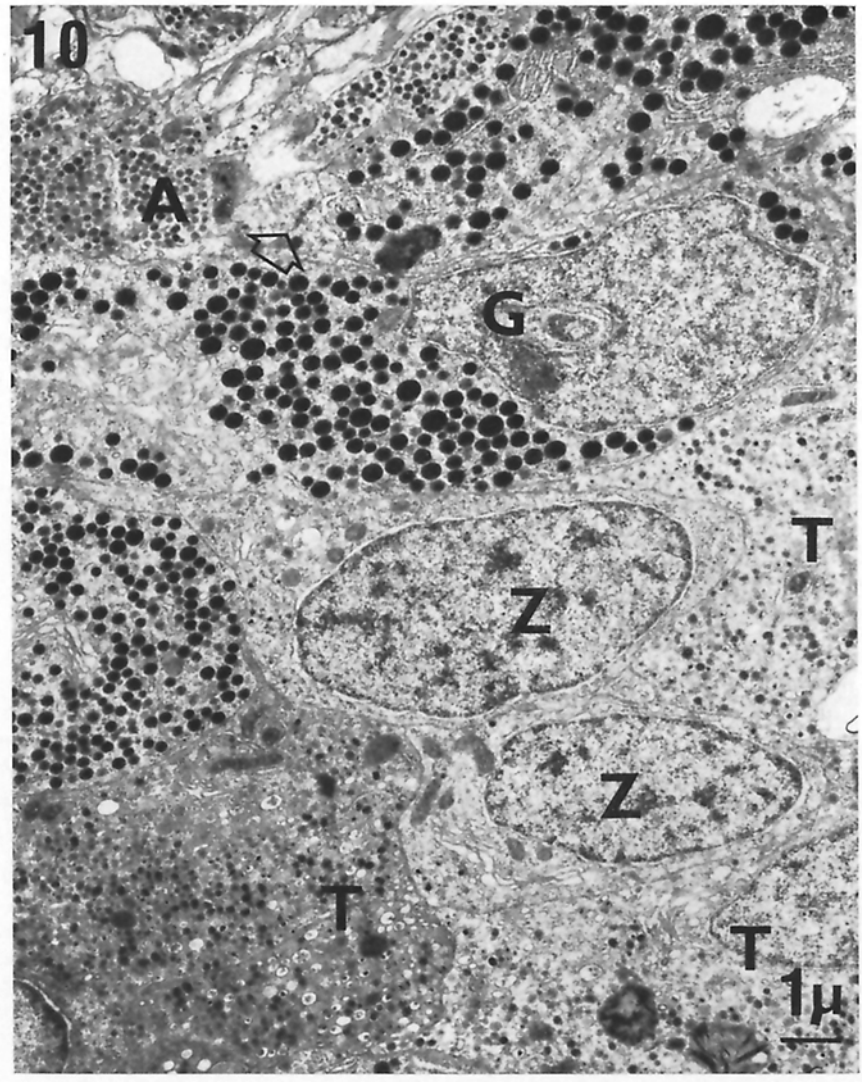
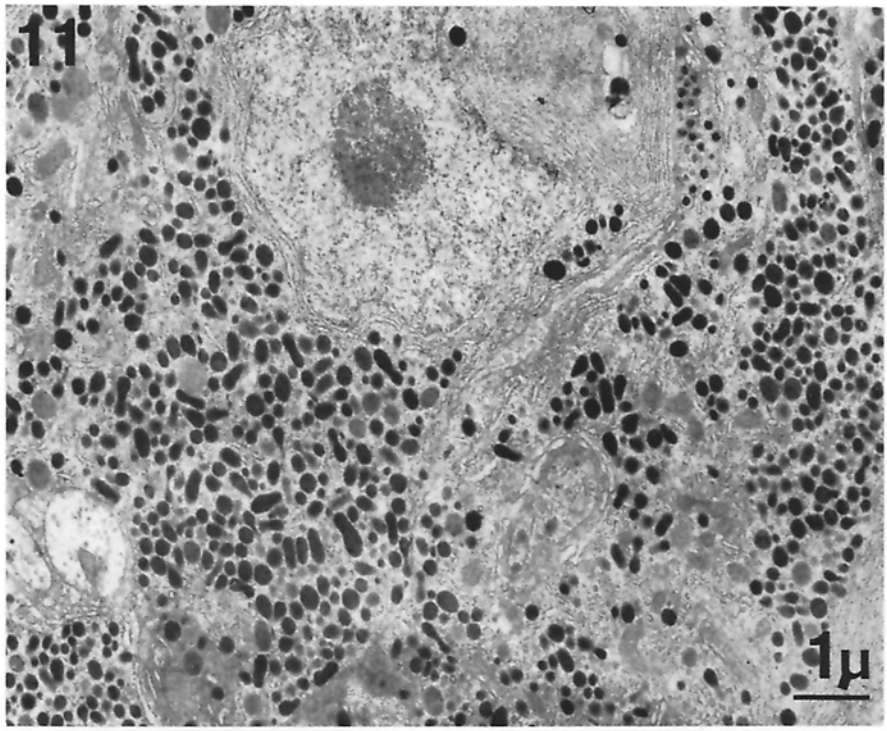


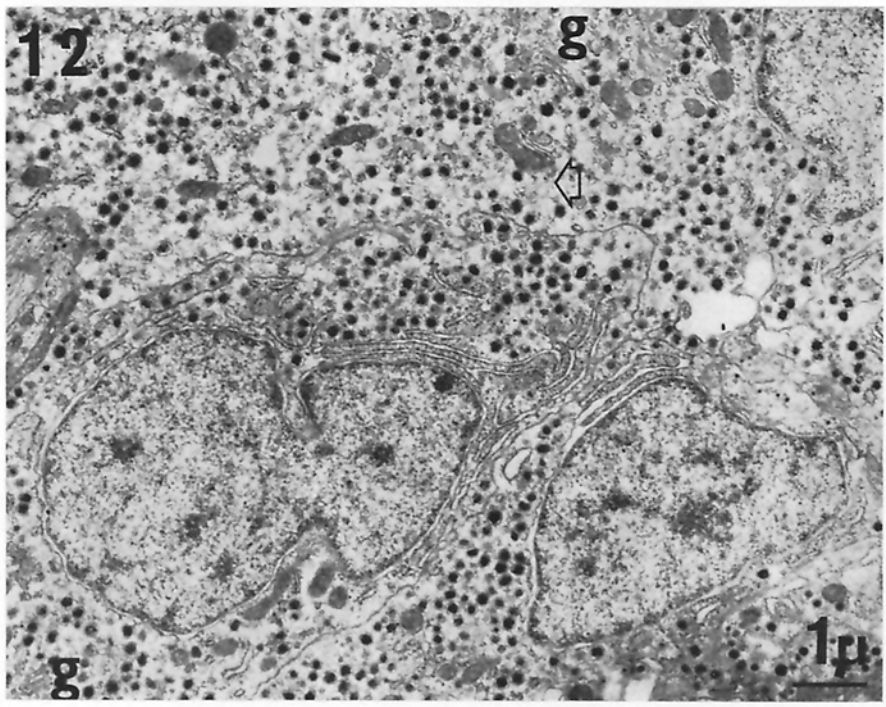
Fig. 10. Inner part of the PPD showing GH cells (*G*), TSH cells (*T*) and Z-cells (*Z*), which contain fewer and smaller granules than the TSH cells. Note the two types of nerve fibres in the neurohypophysis, Type A (*A*) and (arrowed) Type B, synapsing with a GH cell. × 8000

chondria, may in fact belong to a different structural category. Microfibrils occurred throughout the cytoplasm (Fig. 10).

Pars intermedia

Forming a rather loosely-packed region at the posterior edge of the gland (Fig. 1), the pars intermedia (PI) contained two endocrine cell-types: one predominated (PI2 cell), and the other (PI1 cell) occurred locally in clumps or


11
1μ


12
g
g
1μ

columns. Present also were a few non-secretory cells, structurally similar to stellate cells but lacking the extensive intercellular processes, and these were found mainly between the PI1 cells. The fine structure of both endocrine cell-types was essentially the same in two groups of male fish living in 1/3SW and adapted to either a black (9 fish) or a white (9 fish) background for 4 weeks, and the following account is based on these 18 males. However, in the 18 females maintained on an indifferent background in 1/3SW the PI cells presented the same features, and this account applies equally to them.

PI1 Cells (Fig. 11). Corresponding to the periodic acid-Schiff-positive cell (PAS + ve cell) of LM studies, the rounded PI1 cell had a nucleus which was often extensively indented and usually flattened against the cell membrane. The nucleolus was sometimes large, but was often scarcely visible against the dark granular nucleoplasm. The sparse RER, usually arranged in linear arrays around the nucleus or against the cell membrane, was not heavily coated with ribosomes, but numerous free ribosomes were scattered uniformly throughout the dark cytoplasmic ground substance. In the more active state the usual short isolated pieces of RER in the cell body were occasionally organised into modest parallel arrays. All these cells were heavily granulated, the secretory granules being oval rather than round, with a very thin translucent rim. The granule core varied in electron-density, so that there were dark and light granules in each cell (Fig. 11). The size range of the granules was notably great (Fig. 2) and many granules were elongated, sometimes dumbell-shaped, perhaps indicative of fusion of small round granules (Fig. 11). Lysosome-like structures, less electron-dense and larger than secretory granules (up to 500 nm diameter) were common, especially in the rather less heavily granulated cells; however, irregular dense lytic bodies with lipid droplets were rare. In active cells the Golgi apparatus was very extensive and centrally placed. It comprised small vesicles, parallel stacks of flattened cisternae, and larger dilated cisternae in concentric arrays. The mitochondria, round or elongated, contained a very dense matrix with indistinct cristae arranged haphazardly. As in the earlier LM studies, groups of PI1 cells were commonly found in the RPD and occasionally in the PPD (Ball and Baker, 1969).

PI2 Cells (Fig. 12). The PI2 cell corresponds to the light-microscope category of the lead haematoxylin-positive cell (PbH + ve cell). The irregularly-shaped nuclei were usually centrally placed, and in active cells they each contained a distinct central nucleolus. The cytoplasm was often very extensive, with numerous attenuated projections or processes folded against the cell periphery, and although the cell membrane was very distinct its continuity was obscured by these processes (Fig. 12). The RER was usually very abundant, sometimes taking the form of concentric perinuclear rings but more often forming parallel arcs along one edge of the nucleus (Fig. 12). This perinuclear RER was often continuous with the RER in the body of the cell, frequently resulting in a complex network

Fig. 11. PI1 cells showing variations in density and shape of the secretory granules. × 10000

Fig. 12. PI2 cells showing small regular "haloed" granules. Note microtubules (arrowed) and small compact Golgi apparatus (*g*). × 10000

of RER extending from the edge of the nucleus to the nearest portion of the cell membrane. In addition the cytoplasm often contained many scattered small RER pieces.

The secretory granules of the PI2 cells took the form of a moderately electron-dense core surrounded by a clear halo of variable width (Fig. 12). A few granules in each cell were rod-shaped. The Golgi apparatus consisted mainly of numerous small vesicles and small flattened cisternae. The numerous mitochondria varied a great deal in shape (Fig. 12) and usually had a fairly lucent matrix with cristae arranged at various angles; they sometimes contained intramitochondrial granules. Polyribosomes and small vesicles were scattered throughout the cytoplasm, and acanthosomes were common. Lytic structures, often numerous, took two forms: either multivesicular bodies, or rounded dense bodies which were often associated with a clear droplet. Microtubules occurred throughout the cytoplasm.

Discussion

In the present investigation five secretory cell-types have been identified by electron microscopy in the pars distalis of *Poecilia latipinna*. From their location within the gland, and by comparing the features of each cell-type in adjacent LM sections with earlier LM accounts of the pituitary in this species, each EM cell-type could be allocated to the secretion of one of the five hormones known to be secreted by the pars distalis (Ball and Baker, 1969; Chester Jones *et al.*, 1974; Holmes and Ball, 1974). In addition, two secretory cell-types were found in the pars intermedia, and again their architecture and distribution allowed them to be assimilated to the two pars intermedia cell-types of LM studies (Ball and Baker, 1969). Non-secretory stellate cells were observed between the endocrine cells in all regions, and a cell-type not hitherto found in this species with the LM was identified in the PPD, the Z-cell.

Secretory granule size was not a major feature used to identify the cell-types. There undoubtedly are some significant differences in mean granule size between different cell-types (Fig. 2), but mean granule size cannot necessarily be used as an immutable "finger print" of a cell-type, since the size of secretory granules can vary with physiological condition, location of the cell within the gland, and different methods of fixation (Hopkins, 1969; Costoff, 1973; Kaul and Vollrath, 1974; Thornton and Howe, 1974).

There is no difficulty in identifying the *ACTH cells* in *P. latipinna*, given their restricted location on the neurohypophysial-RPD border (Fig. 1). Their ultrastructure in general corresponds closely to that described in other teleosts, in the lack of extensive RER and in the wide separation of the secretory granule core from its limiting membrane; these features have been described in *Tilapia* (Dharmamba and Nishioka, 1968; Leatherland *et al.*, 1974), *Oncorhynchus* (Nagahama and Yamamoto, 1969a; Cook and van Overbeeke, 1972), *Gasterosteus* (Benjamin, 1974) and *Mugil* (Abraham, 1971). However, the ACTH secretory granules are smaller in *P. latipinna* than in other teleosts (Table 1). Possibly their unusually small size explains why these granules in *P. latipinna* stain only very faintly with the appropriate techniques used in light microscopy (Ball and Olivereau, 1966). Again the cells resemble the corticotrophs of *Perca fluviatilis*

Table 1. Range of diameters of ACTH granules in various teleosts

Species	Granule diameter (nm)	Reference
P. latipinna	70–170	present work
Anguilla anguilla	200–250	Knowles and Vollrath (1966)
Perca fluviatilis	120–160	Follénius and Porte (1961)
Gasterosteus aculeatus form *trachurus*	150–220	Follénius (1968); Leatherland (1970a)
form *leiurus*	50–250	Benjamin (1974)
Oncorhynchus nerka and *O. keta*	150–250	Nagahama and Yamamoto (1969a, 1970)
Mugil cephalus	110–250	Abraham (1971)
Tilapia mossambica	100–400	Dharmamba and Nishioka (1968)
Tilapia spp.	120–240	Leatherland *et al.* (1974)
Leuciscus rutilus	150–200	Båge *et al.* (1974a)
Carassius auratus	160–210	Leatherland (1972)
	ca. 100–245	Kaul and Vollrath (1974)
Misgurnus fossilis	150–250	Jasiński (1973)

(Follénius and Porte, 1961), *Anguilla anguilla* and *Conger conger* (Knowles and Vollrath, 1966) in having numerous small vesicles and microtubules dispersed throughout the cytoplasm, but they differ from the corticotrophs of *Perca* (Follénius and Porte, 1961) and *Gasterosteus aculeatus* (Benjamin, 1974) in lacking dilated RER.

The *PRL cells* are also easily recognised, since as in other teleosts they constitute the bulk of the RPD (Fig. 1). The size range of the PRL granules, measured in 1/3SW adapted fish, is similar (100–270 nm) to that in other teleosts, the extension of the lower end of the range in *P. latipinna* being in part at least due to our including smaller (immature?) granules in the Golgi region in our measurements. In closely related cyprinodonts adapted to FW, PRL granule size was 200–250 nm in *Poecilia* (*Lebistes*) *reticulata*, *P.* (*Mollienisia*) *sphenops* and *Xiphophorus helleri* (Follénius and Porte, 1960), and 200–300 nm in *X. maculatus* (Weiss, 1965). Changes in PRL granule size with salinity changes have been described for several teleosts, including *P. latipinna*, in which Hopkins (1969) found that fish adapted to full-strength seawater had many mature secretory granules, c. 300 nm in diameter, and smaller, probably immature, granules close to the Golgi region, 150–250 nm in diameter; 72 hours after transfer to FW, the granules were fewer and smaller, and like the immature granules in SW fish they had a translucent rim between the dense core and the membrane. Hopkins interpreted his observations as evidence that in FW PRL was being released more rapidly than in SW without attaining the mature storage phase represented by the larger granules in SW.

In contrast to Hopkins' study on the PRL cells *during adaptation* to FW, other workers on other species have examined the PRL granules in specimens

fully adapted to their medium, and have consistently reported that PRL granule size is *greater* in FW than in high salinities (see Holmes and Ball, 1974; also Nagahama *et al.*, 1973; Leatherland *et al.*, 1974). *Xiphophorus helleri* showed a progressive reduction in mean PRL granule size during the 30 days following transfer from FW to 1/3SW (Holtzman and Schreibman, 1972), and a study is now in progress on the PRL cells of *P. latipinna* in relation to external salinity.

Båge *et al.* (1974a) have described two types of PRL cells in *Leuciscus rutilus*, one with granules 160–220 nm diameter, the other with granules 110–160 nm diameter; they concluded that both types were indeed PRL cells, partly because in the fluorescent antibody study on this species by Aler (1970) both types gave evidence of containing PRL. It may be suggested in the light of Hopkins' work discussed above, that the population of PRL cells in *Leuciscus* is heterogeneous in terms of the rate of PRL turnover in the individual cell. In *P. latipinna* and *Fundulus heteroclitus*, the PRL cells display at the LM level some size and tinctorial heterogeneity, the larger central cells staining more intensely than those at the periphery (Emmart *et al.*, 1966; Ball, unpublished); for *P. latipinna*, the present EM observations indicate that this tinctorial diversity is based on differences in PRL granule size, and suggest, as in the case of *Leuciscus*, the possibility of non-homogeneity of PRL turnover.

The extensive perinuclear RER and well-developed Golgi apparatus seen in the PRL cells of FW *P. latipinna* are also found in *Leuciscus* (Båge *et al.*, 1974a), *Gasterosteus* (Benjamin, 1974) and *Carassius* (Leatherland, 1972) in FW. In contrast, these organelles were poorly-developed in 1/3SW *P. latipinna*, as in the marine *Zoarces* (Öztan, 1966). Ultrastructural studies on euryhaline teleosts have demonstrated that the degree of development of RER and Golgi elements is inversely related to environmental salinity (*Mugil*, Abraham, 1971; *Tilapia*, Dharmamba and Nishioka, 1968; Leatherland *et al.*, 1974; *P. latipinna*, Hopkins, 1969; *Carassius*, Leatherland, 1972; *Oryzias* and *Anguilla*, Nagahama, 1973). The purely FW *Misgurnus fossilis* seems to be anomalous in that its PRL cells contain only rudimentary RER cisternae (Jasiński, 1973). Our present observations confirm extensive earlier LM and physiological work on *P. latipinna* (Ball and Ingleton, 1973) in demonstrating greater PRL cell activity in FW than in 1/3SW.

Hopkins (1969) noted the presence of multivesicular bodies and large lytic bodies in the PRL cells of *P. latipinna*, corresponding to those we have described, and he demonstrated that these structures, which increase in frequency when PRL secretion is suddenly reduced by raising external salinity, contain acid phosphatase and therefore are true lysosomes. Hopkins concluded that the multivesicular bodies digest material from the secretory granules and give rise to the large irregular lytic bodies containing lipid droplets, together with whorls of lamellae derived from the secretory granules. He also found that acanthosomes became numerous in the Golgi region during the 24–48 h period after *P. latipinna* was transferred from SW to FW, in association with signs of increased synthetic activity; as Hopkins points out, these observations are in agreement with the suggestion that acanthosomes (junctional vesicles) may be responsible for the transport of newly-synthesized protein from the RER to the Golgi complex. However, it is difficult in these terms to interpret our observations (on *adapted* fish) that acanthosomes were more numerous in 1/3SW than in FW.

The *GH cells* can readily be identified with the large, round, densely granulated somatotrophs of LM studies, with their characteristic distribution in the inner part of the PPD (Ball and Baker, 1969). Ultrastructurally they resemble the GH cells of *Anguilla* (Knowles and Vollrath, 1966), *Gasterosteus* (Leatherland, 1970b; Benjamin, 1974) and *Mugil* (Abraham, 1974) in lacking well-developed RER and Golgi elements, and in granule size, rather than the GH cells of *Leuciscus* (Båge *et al.*, 1974b) and *Carassius* (Kaul and Vollrath, 1974), which contain extensive parallel RER and active Golgi apparatus. Although we often observed large bodies of variable electron density in GH cells, they were never as frequent or as large as those in *Misgurnus fossilis* (Jasiński, 1973) and unlike Jasiński, we interpret them as lytic bodies rather than as the ultimate stages in maturation of the secretory granules.

The *TSH cells* are more difficult to identify than other cell-types. Those of the inner regions of the PPD present no great difficulties, since they occur as islands of finely-granulated cells amongst the coarsely-granulated GH cells. However, in some specimens TSH cells mostly occur ventral and ventro-lateral to the somatotroph region, and lie amongst or adjacent to the GtH cells. GtH cells and TSH cells have many structural features in common, which sometimes hinders the allocation of particular cells in this region to either type. Nevertheless, careful examination usually resolves the difficulty, since TSH cells contain smaller granules with a more prominent halo, and have much less dilated RER than the gonadotrophs.

TSH cells are found in the PPD in many teleosts, but in others, such as *Anguilla* (Knowles and Vollrath, 1966), *Leuciscus* (Båge *et al.*, 1974a) and salmonids (Nagahama and Yamamoto, 1969a, 1970), they lie amongst the PRL cells in the RPD (see Ball and Baker, 1969). In *Carassius auratus*, Kaul and Vollrath (1974) recognised two types of TSH cells, one in the RPD and one in the PPD. These are virtually indistinguishable by the LM (cf. Nagahama and Yamamoto, 1969b; Leatherland, 1972), but according to Kaul and Vollrath (1974) those in the PPD have larger granules (mean diameter 164 ± 15 nm) than those in the RPD (105 ± 12 nm). This distinction was not noticed by other workers on *Carassius* (Nagahama and Yamamoto, 1969b; Leatherland, 1972), and Nagahama (1973) does not appear to differentiate the PPD thyrotrophs from gonadotrophs. The wide variation we found in the structure of these cells in *P. latipinna* seems to be general in teleosts. Most of the TSH cells that we observed resemble closely those in *Misgurnus fossilis* (Jasiński, 1973), in having large heterogeneous nuclei, small numbers of secretory granules of variable electron density and characteristically haloed, and mostly in the size range 70–150 nm; in having the few larger lytic dense bodies; and in having variably abundant RER with its oval or round vacuoles. However, mitochondria, acanthosomes and Golgi elements appear to be better developed or more numerous in *Poecilia* than in *Misgurnus*. The large nucleus, abundant mitochondria and small haloed granules are also seen in the TSH cells of *Gasterosteus* (Benjamin, 1974), but this teleost differs from *Poecilia* in having fairly extensive parallel arrays and curvilinear whorls of RER, and more extensive RER vacuoles. TSH cells similar in structure and granule size to those of *Poecilia* occur in *Anguilla* and *Conger* (Knowles and Vollrath, 1966), *Carassius* (Nagahama and Yamamoto, 1969b; Kaul and Vollrath, 1974); *Oryzias* (Nagahama, 1973), *Tilapia* (Leatherland *et al.*, 1974), *Oncorhynchus* (Nagahama

and Yamamoto, 1969a) and *Zoarces* (Öztan, 1966). In the last two teleosts, granules occurred within the dilated RER cisternae, but this feature was not found in *P. latipinna*, nor did we observe the large, dense rod-shaped bodies characteristic of the TSH cells of *Tilapia* (Leatherland *et al.*, 1974), although the TSH cells of *Poecilia* and *Tilapia* are similar in other respects. In some *P. latipinna* TSH cells, a large proportion of the cytoplasm was occupied by membrane-bound vesicles similar to secretory granules but with the dense core reduced or absent. This condition has not been described for other species, and it may possibly be a fixation artifact rather than a reflection of physiological state.

Although two types of *GtH cells* have been described in several teleosts, e.g. *Anguilla*, *Oncorhynchus*, *Zoarces* (see Ball and Baker, 1969; Holmes and Ball, 1974), we found no evidence of more than one type in *P. latipinna*. Nevertheless, the variable structure of *Poecilia* GtH cells, correlated with gonad state (Ball and Baker, 1969), means that it is difficult to discount the possibility that this teleost, too, possesses two kinds of gonadotrophs. The dilated RER cavities containing finely-granular material seem characteristic of these cells in many teleosts e.g. *Carassius* (Nagahama and Yamamoto, 1969b; Leatherland, 1972; Kaul and Vollrath, 1974), *Perca* (Follénius and Porte, 1961), *Mugil* (Abraham, 1974), *Gasterosteus* (Follénius, 1968; Leatherland, 1970b; Benjamin, 1974), *Leuciscus* (Båge *et al.*, 1974b), *Misgurnus* (Jasiński, 1973) and *Tilapia* (Leatherland *et al.*, 1974). The function of these dilated RER cavities is uncertain; they may reach fantastic dimensions in fully-mature and spawning fish, as in the illustrations of Nagahama and Yamamoto (1969b), Jasiński (1973) and Kaul and Vollrath (1974). They could result from the inability of the cell to transport newly-synthesized protein quickly enough away from the RER to the Golgi region when synthesis is very rapid, in which case their contents would presumably include newly-synthesized (non-granular) gonadotrophin. The secretory granules of the GtH cells in *P. latipinna* are in the size range reported for other teleosts (Ball and Baker, 1969; Holmes and Ball, 1974; Båge *et al.*, 1974b; Benjamin, 1974; Leatherland, 1972; Kaul and Vollrath, 1974; Jasiński, 1973). The presence of larger lytic inclusions, frequently irregularly-shaped and corresponding to the acidophilic R granules seen with the LM, is a striking feature of GtH cells in teleosts, amphibians and reptiles (Holmes and Ball, 1974). Examples are illustrated by Nagahama and Yamamoto (1969a, b), Leatherland *et al.* (1974), Kaul and Vollrath (1974), and Båge *et al.* (1974b).

Non-secretory *stellate cells* have been described in all parts of the adenohypophysis of many teleosts, sometimes as "neck cells", interstitial cells or chromophobes (e.g. Leatherland, 1969, 1970a; Abraham, 1971, 1974; Båge *et al.*, 1974a, b). Knowles and Vollrath (1966) suggested that the "neck cells" in *Anguilla* RPD might regulate the passage of substances along intercellular channels between the PRL cells, by alterations in their size and shape. The frequent presence of microfibrils and microtubules in the stellate cells certainly suggests that they might possess some degree of motility. In the stellate form, as in *P. latipinna*, the network of fine processes between the endocrine cells, which forms close associations with basement membrane and with pericapillary spaces, suggests that these cells could be involved in transport of hormones from endocrine cells to capillaries (Weiss, 1965; Hopkins, 1969). The increased prominence and elaborated ramifications of the RPD stellate cells in FW-adapted *P. latipinna* certainly indicates

that they are linked functionally with the neighbouring PRL cells, as does the frequent release of PRL granules into their vicinity. Together with findings from other teleosts, the evidence indicates that the stellate cells act as transfer or "secretory aid" elements (Leatherland, 1970a; Leatherland *et al.*, 1974). Similar cells, with indications of similar functions, occur in the adenohypophysis of all vertebrates (Holmes and Ball, 1974). In *Misgurnus fossilis* two types of non-secretory cell have been described, one resembling the stellate cell of *P. latipinna* and the other containing numerous ribosomes and occasional haloed granules (Jasiński, 1973). The *Z-cells*, found in *P. latipinna* near the PPD-neurohypophysis interface, correspond in ultrastructure, granule size, and position, to the catecholaminergic cells demonstrated in *Leuciscus* by Båge *et al.* (1974b). Their function is unknown.

Two types of secretory cells have been distinguished in the *pars intermedia* of most teleosts that have been examined, corresponding to the PAS + ve and PbH + ve cells identified with the LM (see Ball and Baker, 1969). The appearance of each cell-type at the EM level varies from species to species. In *Anguilla* (Knowles and Vollrath, 1966), the PAS + ve (Type I) cell contains diffuse RER and secretory granules less than 180 nm diameter, while the PbH + ve (Type II) cell contains extensive RER with granules 270–360 nm in diameter, together with larger (c. 1 μm) inclusions. In *Perca* (Follénius and Porte, 1961) the PAS + ve cell contains 250–300 nm diameter granules, and granules were found only rarely in the PbH + ve cell. In *Gasterosteus* (Follénius, 1968; Leatherland, 1970b; Benjamin, 1974) the PAS + ve cell contains the larger granules, variable in shape and 100–400 nm in diameter; these cells also have parallel arrays of RER around the nucleus and next to the cell membrane. The PbH + ve cell of *Gasterosteus* contains only a few electron-dense granules, 100–300 nm in diameter, together with numerous membrane-bound translucent vesicles, about the same size as the granules; these cells have extensive parallel arrays of RER, often as perinuclear whorls, and abundant mitochondria with intramitochondrial granules. The two PI cell-types in *Misgurnus fossilis* (Jasiński and Kilarski, 1970) are very like those in *Gasterosteus*, as are the cell-types of *P. latipinna* apart from the ultrastructural details of the PbH + ve cell: in *P. latipinna* this cell strikingly differs from its homologue in *Gasterosteus* in having numerous small dense secretory granules and only very few translucent vesicles. However, recent work on *Anguilla* indicates that the preservation of the dense core of these PbH + ve secretory granules is exceptionally dependent on the pH of the fixative (Thornton and Howe, 1974). Thus it seems quite possible that the empty vesicles in these cells may be derived from dense secretory granules as a fixation artifact.

The functional roles of the two PI cell-types have long been debated. The cytophysiological evidence now available indicates that the PbH + ve (Type II) cell is the source of MSH (Olivereau, 1971; Baker, 1972; Thornton and Howe, 1974). Furthermore, antibody to alpha-MSH locates specifically in the PbH + ve cell in *Gasterosteus* (Follénius and Dubois, 1974), and in *Anguilla* and *P. latipinna* (B. I. Baker, unpublished). Non-pigmentary functions, osmoregulatory (*Anguilla*, *Mugil*, *Oryzias*) or reproductive (*Zoarces*), have been suggested for the PAS + ve cell, but the evidence is not conclusive (see Holmes and Ball, 1974). Confusingly, LM studies have shown that in *P. latipinna* adapted to a black background the PAS + ve cells become larger and more numerous than on a white background,

while the PbH + ve cells show no detectable change; neither cell-type seems to respond to salinity changes in this teleost (Baker and Ball, 1970; Ball, unpublished).

References

Abraham, M.: The ultrastructure of the cell types and of the neurosecretory innervation in the pituitary of *Mugil cephalus* L. from fresh water, the sea, and a hypersaline lagoon. I. The rostral pars distalis. Gen. comp. Endocr. **17**, 334–350 (1971)

Abraham, M.: The ultrastructure of the cell types and of the neurosecretory innervation in the pituitary of *Mugil cephalus* L. from freshwater, the sea, and a hypersaline lagoon. II. The proximal pars distalis. Gen. comp. Endocr. **24**, 121–132 (1974)

Aler, G. M.: Prolactin cells: study of the pituitary gland of the roach (*Leuciscus rutilus*) by immuno-histochemical methods. Acta zool. **51**, 149–157 (1970)

Båge, G., Ekengren, B., Fernholm, B., Fridberg, G.: The pituitary gland of the roach *Leuciscus rutilus*. I. The rostral pars distalis and its innervation. Acta zool. **55**, 25–45 (1974a)

Båge, G., Ekengren, B., Fernholm, B., Fridberg, G.: The pituitary gland of the roach *Leuciscus rutilus*. II. The proximal pars distalis and its innervation. Acta zool. **55**, 191–204 (1974b)

Baker, B. I.: The cellular source of melanocyte stimulating hormone in *Anguilla* pituitary. Gen. comp. Endocr. **19**, 515–521 (1972)

Baker, B. I., Ball, J. N.: Background adaptation and the pituitary in teleosts. J. Endocr. **48**, xxvi (1970)

Ball, J. N.: Partial hypophysectomy in the teleost *Poecilia*: separate identities of teleostean growth hormone and teleostean prolactin-like hormone. Gen. comp. Endocr. **5**, 654–661 (1965)

Ball, J. N., Baker, B. I.: The pituitary gland: anatomy and histophysiology. In: Fish physiology (Hoar, W. S. and Randall, D. J., eds.), vol. II, p. 1–110. New York: Academic Press 1969

Ball, J. N., Ingleton, P. M.: Adaptive variations in prolactin secretion in relation to external salinity in the teleost *Poecilia latipinna*. Gen. comp. Endocr. **20**, 312–325 (1973)

Ball, J. N., Olivereau, M.: Experimental identification of the ACTH cells in the pituitary of two teleosts, *Poecilia latipinna* and *Anguilla anguilla*: correlated changes in the interrenal and in the pars distalis resulting from the administration of metopirone (SU 4885). Gen. comp. Endocr. **6**, 5–18 (1966)

Benjamin, M.: A morphometric study of the pituitary cell types in the freshwater stickleback, *Gasterosteus aculeatus*, form *leiurus*. Cell Tiss. Res. **152**, 69–92 (1974)

Chester Jones, I., Ball, J. N., Sandor, T., Henderson, I. W., Baker, B. I.: Endocrinology of fishes. In: Chemical zoology (Florkin, M. and Scheer, B. T., eds.), vol. VIII, p. 524–593. New York: Academic Press 1974

Cook, H., Overbeeke, A. P. van: Ultrastructure of the pituitary gland (pars distalis) in sockeye salmon (*Oncorhynchus nerka*) during gonad maturation. Z. Zellforsch. **130**, 338–350 (1972)

Costoff, A.: Ultrastructure of rat adenohypophysis: correlation with function. New York: Academic Press 1973

Dharmamba, M., Nishioka, R. S.: Response of "prolactin-secreting" cells of *Tilapia mossambica* to environmental salinity. Gen. comp. Endocr. **10**, 409–420 (1968)

Emmart, E. W., Pickford, G. E., Wilhelmi, A. E.: Localization of prolactin within the pituitary of a cyprinodont fish, *Fundulus heteroclitus* (Linnaeus) by specific fluorescent antiovine prolactin globulin. Gen. comp. Endocr. **7**, 571–583 (1966)

Follénius, E.: Analyse de la structure fine des différents types de cellules hypophysaires des poissons téléostéens. Path. et Biol. **16**, 619–632 (1968)

Follénius, E., Dubois, M. P.: Immunocytological localization and identification of the MSH-producing cells in the pituitary of the stickleback (*Gasterosteus aculeatus* L.). Gen. comp. Endocr. **24**, 203–207 (1974)

Follénius, E., Porte, A.: Ultrastructure de l'hypophyse des cyprinodonts vivipares. Étude des types cellulaires composant l'adénohypophyse. C.R. Soc. Biol. (Paris) **154**, 1247–1250 (1960)

Follénius, E., Porte, A.: Étude des différents lobes de l'adénohypophyse de la perche, *Perca fluviatilis*, au microscope électronique. C.R. Soc. Biol. (Paris) **155**, 128–131 (1961)

Holmes, R. L., Ball, J. N.: The pituitary gland: a comparative account. Cambridge: Cambridge University Press 1974

Holtzman, S., Schreibman, M. P.: Morphological changes in the "prolactin" cell of the freshwater teleost, *Xiphophorus helleri*, in salt water. J. exp. Zool. **180**, 187–196 (1972)

Hopkins, C. R.: The fine structural localization of acid phosphatase in the prolactin cell of the teleost pituitary following the stimulation and inhibition of secretory activity. Tissue and Cell **1**, 653–671 (1969)

Jasiński, A.: Electron microscopic study of the pars distalis of the hypophysis in the pond loach, *Misgurnus fossilis* L. Acta anat. (Basel) **86**, 228–252 (1973)

Jasiński, A., Kilarski, W.: Fine structure of pars neuro-intermedia of the loach, *Misgurnus fossilis* L. Z. Zellforsch. **105**, 259–275 (1970)

Kaul, S., Vollrath, L.: The goldfish pituitary. I. Cytology. Cell Tiss. Res. **154**, 211–230 (1974)

Karnovsky, M. J.: A glutaraldehyde fixative. J. Cell Biol. **27**, 137A (1965)

Knowles, F., Vollrath, L.: Neurosecretory innervation of the pituitary of the eels *Anguilla* and *Conger*. Phil. Trans. B **250**, 311–342 (1966)

Leatherland, J. F.: Seasonal variation in the structure and ultrastructure of the pituitary of the marine form (*trachurus*) of the threespine stickleback, *Gasterosteus aculeatus* L. I. Rostral pars distalis. Z. Zellforsch. **104**, 301–317 (1970a)

Leatherland, J. F.: Seasonal variation in the structure and ultrastructure of the pituitary gland in the marine form (*trachurus*) of the threespine stickleback, *Gasterosteus aculeatus* L. II. Proximal pars distalis and neuro-intermediate lobe. Z. Zellforsch. **104**, 318–336 (1970b)

Leatherland, J. F.: Histophysiology and innervation of the pituitary gland of the goldfish, *Carassius auratus* L.: a light and electron microscope investigation. Canad. J. Zool. **50**, 835–844 (1972)

Leatherland, J. F., Ball, J. N., Hyder, M.: Structure and fine structure of the hypophyseal pars distalis in indigenous African species of the genus *Tilapia*. Cell Tiss. Res. **149**, 245–266 (1974)

Nagahama, Y.: Histo-physiological studies on the pituitary gland of some teleost fishes, with special reference to the classification of hormone-producing cells in the adenohypophysis. Mem. Fac. Fish., Hokkaido Univ. **21**, 1–63 (1973)

Nagahama, Y., Nishioka, R. S., Bern, H. A.: Responses of prolactin cells of two euryhaline marine fishes, *Gillichthys mirabilis* and *Platichthys stellatus*, to environmental salinity. Z. Zellforsch. **136**, 153–167 (1973)

Nagahama, Y., Yamamoto, K.: Fine structure of the glandular cells in the adenohypophysis of the kokanee *Oncorhynchus nerka*. Bull. Fac. Fish., Hokkaido Univ. **20**, 159–168 (1969a)

Nagahama, Y., Yamamoto, K.: Basophils in the adenohypophysis of the goldfish (*Carassius auratus*). Gunma Symp. Endocr. **6**, 39–55 (1969b)

Nagahama, Y., Yamamoto, K.: Morphological studies on the pituitary of the chum salmon, *Oncorhynchus keta*. II. Changes of the prolactin cells during the life-cycle. Bull. Fac. Fish., Hokkaido Univ. **21**, 169–177 (1970)

Olivereau, M.: Elaboration d'intermédine par les cellules colorées avec l'haematoxyline au plomb dans la pars intermedia de l'Anguille: preuves nouvelles et contrôle hypothalamique. C.R. Acad. Sci. (Paris) D **272**, 102–105 (1971)

Olivereau, M., Ball, J. N.: Contribution à l'histophysiologie de l'hypophyse des Téléostéens, en particulier de celle de *Poecilia* species. Gen. comp. Endocr. **4**, 523–532 (1964)

Olivereau, M., Ball, J. N.: Histological study of functional ectopic pituitary transplants in a teleost fish (*Poecilia formosa*). Proc. roy. Soc. B **164**, 106–129 (1966)

Öztan, N.: The fine structure of the adenohypophysis of *Zoarces viviparus* L. Z. Zellforsch. **69**, 699–718 (1966)

Reynolds, E. S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**, 208–212 (1963)

Spurr, A. R.: A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**, 31–43 (1969)

Thornton, V. F., Howe, C.: The effect of change of background colour on the ultrastructure of the pars intermedia of the pituitary of the eel (*Anguilla anguilla*). Cell Tiss. Res. **151**, 103–115 (1974)

Weiss, M.: The release of pituitary secretion in the platyfish *Xiphophorus maculatus* (Guenther). Z. Zellforsch. **68**, 783–794 (1965)