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Circadian Changes in Prolactin Cell Activity in the Pituitary of the Teleost *Poecilia latipinna* in Freshwater

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Summary. Quantitative morphometric studies with the electron microscope were made on the prolactin cells of wild freshwater sailfin mollies taken in Florida in August at four different times of day. The results indicate a circadian rhythm in the prolactin cell, the period of highest synthetic activity being from midday to evening, as indicated by the condition of nucleus, Golgi apparatus and rough endoplasmic reticulum, and by the incidence of granule release profiles. No circadian changes were detected in the ACTH cells.

Several distinct sites of prolactin granule release were recognised. However, there was no conclusive evidence of granule release by any mechanism other than classical exocytosis.

Key words: Prolactin cell – *Poecilia latipinna* – Circadian changes – Morphometry.

Introduction

Recent evidence indicates that both plasma and pituitary levels of various adenohypophysial hormones vary with circadian periodicity. Circadian variations in the circulating levels of prolactin (PRL) have been demonstrated, for example, in rats (Koch *et al.*, 1971; Butcher *et al.*, 1972), man (Sassin *et al.*, 1973), goldfish (Leatherland and McKeown, 1973) and salmon (Leatherland *et al.*, 1974). The physiological significance of circadian changes in plasma PRL levels is perhaps still conjectural in the mammal (Tindal, 1974) but seems clearer in other vertebrates. Thus, the response of PRL target organs shows evidence of circadian rhythmicity in birds, reptiles and amphibians (see Meier, 1972), and the fattening

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response to PRL shows circadian periodicity in species of the cyprinodontid teleost genus *Fundulus* (Lee and Meier, 1967; Joseph and Meier, 1971; Mehrle and Fleming, 1970; de Vlaming and Sage, 1972) and *Cyprinodon variegatus* (de Vlaming and Sage, 1972). Circadian periodicity in pituitary PRL content has been found in the rat (Clark and Baker, 1964), hamster (Kent *et al.*, 1964) and the white-throated sparrow, *Zonotrichia albicollis* (Meier *et al.*, 1969). A circadian cycle in pituitary PRL content has been demonstrated by bioassay in the cyprinodont *Fundulus similis*, the content being high during the dark phase and low in the light phase (Sage and de Vlaming, 1973).

The ultrastructure of the PRL cell in the cyprinodont *Poecilia latipinna* was described recently (Batten *et al.*, 1975), and PRL cell secretory activity was found to be greater in freshwater (FW) than in one-third seawater (1/3SW), in agreement with earlier light microscope studies (Ball and Ingleton, 1973), and with demonstrations that pituitary PRL content in this species is higher in FW than $^1/_3$ SW (Ensor and Ball, 1968; Ball and Ingleton, 1973). In the present investigation FW fish were used, and the activity of their PRL cells was quantitatively analysed at different times during the 24 h cycle using the morphometric method of Weibl (1969); this method has previously been used in endocrinological studies to characterize cell-types and to detect ultrastructural changes in the pituitary of *Xenopus laevis* (Weatherhead and Whur, 1972) and *Gasterosteus aculeatus* form *leiurus* (Benjamin, 1974a, b).

Materials and Methods

Gravid female *P. latipinna* were seined from freshwater in the Hillsboro River just north of Tampa, Florida, at the University of South Florida Student Park, on 14 August 1974. All fish were collected before 10.00 h Eastern Standard Time (E.S.T.). In order to minimize stress and injury the fish were seined in short hauls and were rapidly removed from the net. They were placed in well-aerated river water in a styrofoam collecting box which contained *Elodea densa* and/or *Hydrilla verticillata*, the two commonest plants in the fish's natural habitat; these were added to provide cover and reduce stress. After a 10-minute drive the fish were transferred to a large outdoor 120 l aquarium containing a vigorously aerated mixture of river water and aged well water. They were maintained under natural photoperiod (sunrise c. 06.30 and sunset c. 17.40 E.S.T.) and temperature (c. 29° C) during the next 24 h, in which fish were sampled at the following times:

morning (06.00–08.00); *midday* (12.00–14.00); *evening* (18.00–20.00); and *midnight* (23.00–01.00). Examination of the fish revealed no detectable injury from collection and transport. For the purposes of this study, only females which had ovaries in stages 5 and 6 (Ball and Baker, 1969) were selected. Besides developing embryos, the ovary contained only primary oocytes; no vitellogenesis was in progress.

Fixation and Embedding. The brain with pituitary attached was removed from the neurocranium by an operation that lasted approximately 20 seconds following decapitation of the fish. Fixation was in 3% glutaraldehyde (GTA) in 0.1 M sodium cacodylate at pH 7.4. During the rinse (0.1 M sodium cacodylate with 5% sucrose) following GTA fixation, the pituitary was removed from the brain and cut into thirds to separate the three main regions of the adenohypophysis (Rostral pars distalis, proximal pars distalis and pars intermedia). Osmium postfixation (1% OsO_4) was limited to 30 minutes. After dehydration in graded alcohols the material was embedded in Spurr's

Fig. 1. Prolactin cells at midnight (23.00–01.00 h). Dense granulation evenly distributed throughout the cytoplasm. Rough endoplasmic reticulum in the form of short isolated pieces and small parallel arrays. Granule release profiles are very rare at this time. *s*, stellate cell. × 10,000

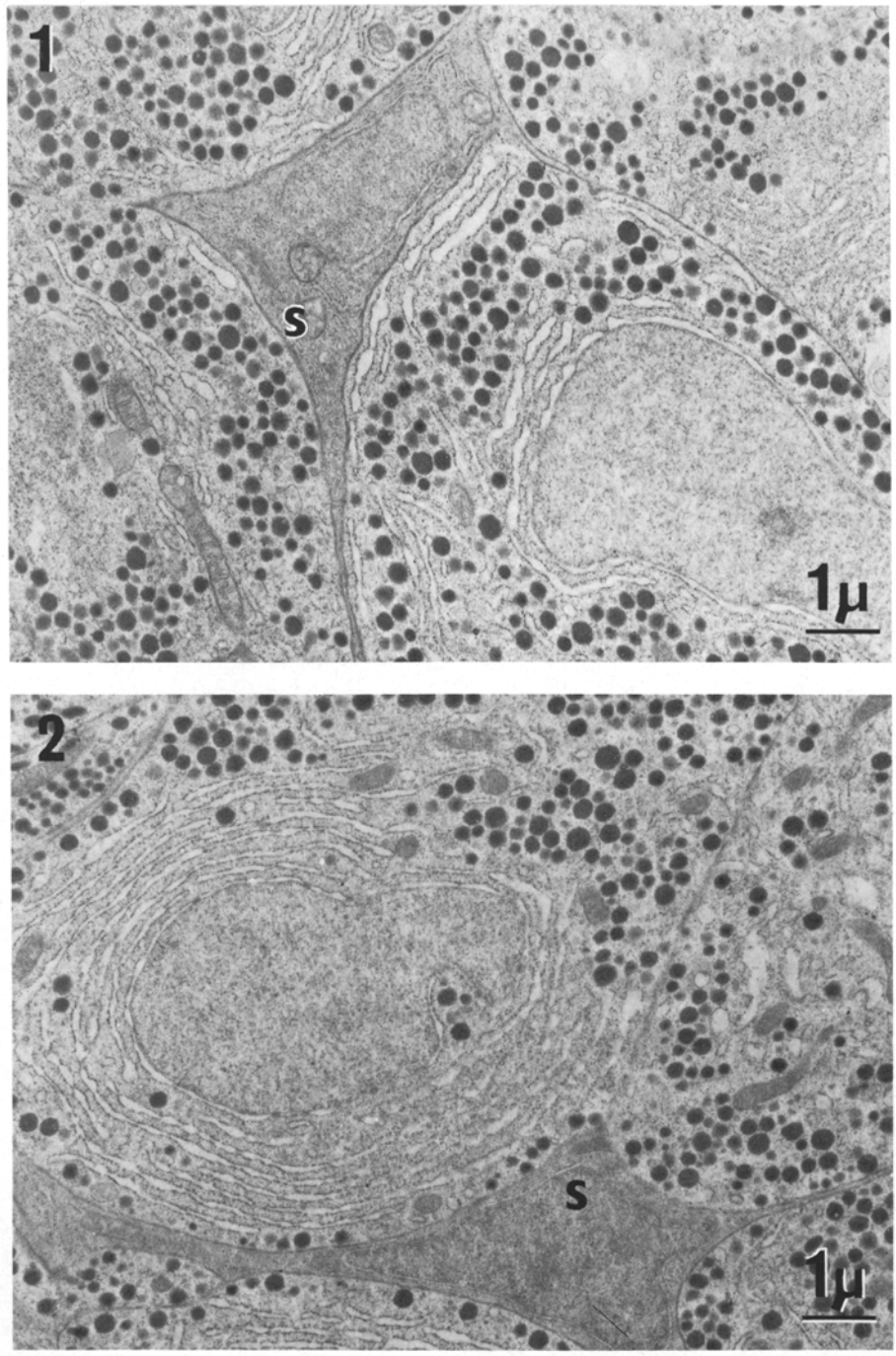

Fig. 2. Prolactin cells in the morning (06.00–08.00 h). Fairly heavy granulation, evenly distributed. Rough endoplasmic reticulum in the form of short isolated pieces, and perinuclear arrays which may be quite extensive in some cells. Granule release profiles are rare. *s*, stellate cell. × 10,000

resin. For each time period six rostral pars distalis (RPD) fragments were sectioned with a Reichert OM U3 ultramicrotome, and the silver to grey thin sections were stained with 5% uranyl acetate and Reynold's lead citrate.

Electron Microscopy and Morphometry. Six RPD fragments in each time group were sectioned at random. They were examined with a JEOL JEM T7 electron microscope, and 3 electron micrographs were taken at random for each specimen, with a final print magnification of ×10,000. The relative areas of the prolactin cells occupied by various organelles was determined by Weibel's (1969) point counting method. The test system consisted of two superimposed quadratic lattices, the cross points of which served as markers for point counting volumetry. The relative areas were first determined using a thick line system (15 mm spacing), and then organelles which occupied less than 1% of the total PRL cell volume were re-estimated using a thin line system (1.5 mm spacing). The organelles were those characterized by Benjamin (1974a), with the addition of 4 others: droplets; membrane whorls; sectory granule (SG) release profiles; and SGs in intercellular spaces.

Statistics. For each specimen the point counting data allowed the calculation of the percentage of the total volume of the PRL cell occupied by each organelle. The mean percentage volumes were then calculated for each time-group. Because of non-normality of distribution, arc-sine angular transformations of the percentages were made before assessing the statistical significance of differences between time-groups, using one-way analysis of variance and Duncan's (1955) New Multiple Range Test. Calculations were made using an Olivetti-Underwood Programma 101.

Results

The RPD fragments consisted mainly of the mass of PRL cells together with some ACTH cells and neurohypophysial tissue (Batten *et al.*, 1975).

Typical examples of PRL cells at the different times are illustrated in Figs. 1–4. Table 1 gives the relative volumes of the PRL cell occupied by the various organelles at the different times. It can be seen that significant changes occurred in the main parameters of PRL cell activity during the 24 h period. *Nucleoli* were only rarely encountered in the morning, but they were significantly more common at midday and evening. The volume occupied by the *Golgi apparatus* was significantly greater at midday than in the morning, the difference being largely attributable to an increase in the small Golgi vesicles. *Immature secretory granules* (immature SGs) were more numerous at midday and evening, none being detected in the morning. *Oblong and irregularly-shaped SGs* were more numerous in the evening, less so at midnight. The *total number of SGs* was less at midday and evening than at midnight. *SG release profiles* were frequent at midday and evening, but were not seen at other times. The total amount of *rough endoplasmic reticulum* (RER) was significantly greater at midday and evening than at other times, due mainly to increase in parallel arrays near the cell membrane. The number of *free ribosomes* was lower in the evening than at midnight and in the morning. Other notable features were the presence of large numbers of *dense bodies* and *droplets* at midnight and in the morning (Fig. 5), and the presence of *large curvilinear whorls of RER* in some cells at midday (Fig. 3).

Fig. 3. Prolactin cells at midday (12.00–14.00 h). Light granulation, largely restricted to cell periphery, and with a high proportion of irregularly-shaped granules. Numerous granule release profiles. All forms of rough endoplasmic reticulum are well-developed, and some cells contain extensive curvilinear whorls (*W*). ×10,000

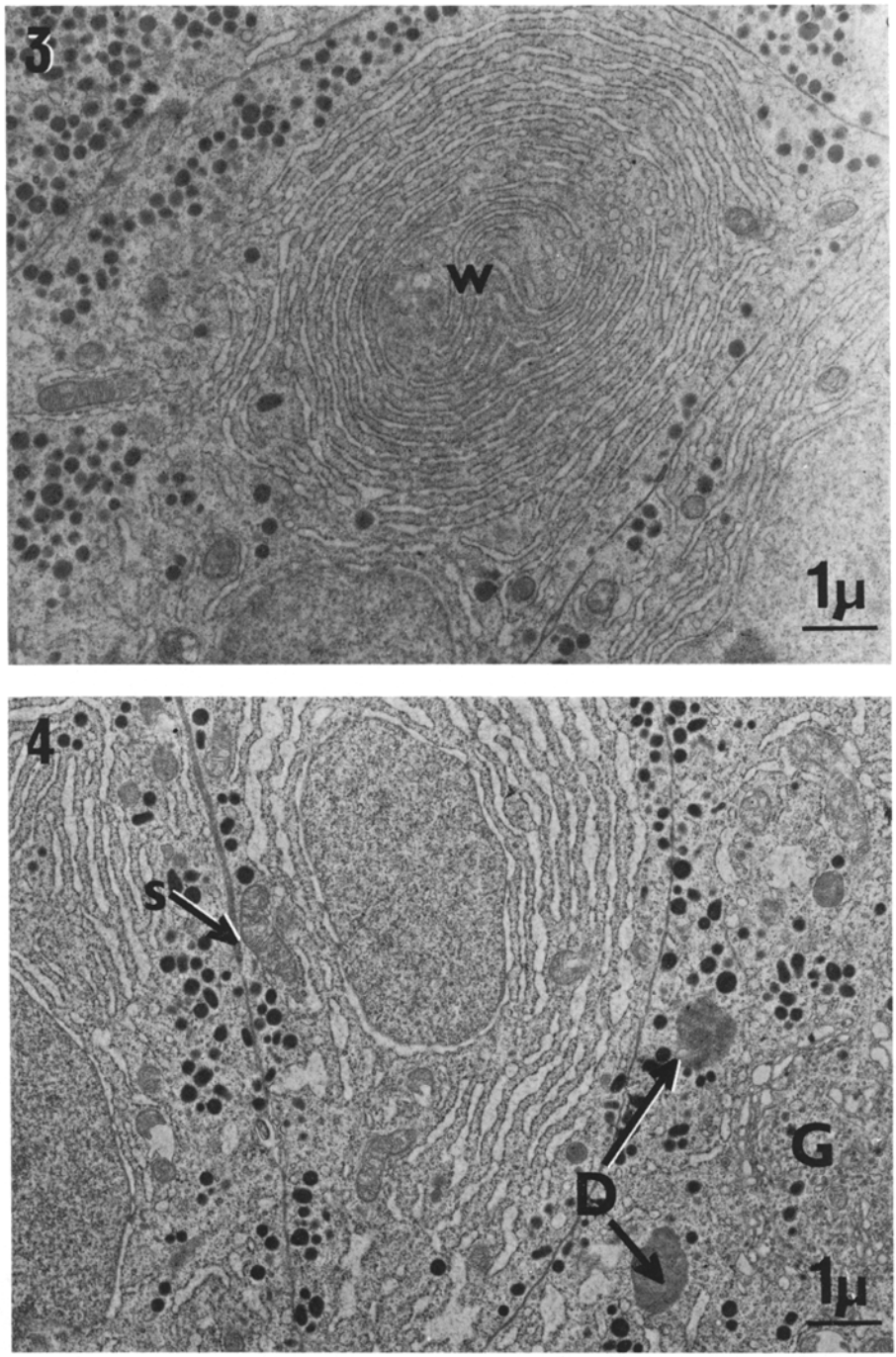

Fig. 4. Prolactin cells in the evening (18.00–20.00 h). Sparse granulation, granules irregularly-shaped and clustered at cell periphery, where granule release profiles are common, especially adjacent to long processes of stellate cells (*s*). All forms of rough endoplasmic reticulum are well-developed, and the Golgi apparatus (*G*) is extensive and contains numerous secretory granules. Dense bodies (*D*) are uncommon at this time. × 10,000

Table 1. Percentage of total PRL cell volume occupied by the various cell organelles at different times

Organelle	Midnight (23.00–01.00 h)	Morning (06.00–08.00 h)	Midday (12.00–14.00 h)	Evening (18.00–20.00 h)
Nucleus	16.78 ± 1.126	19.57 ± 1.283	15.00 ± 2.447	19.41 ± 2.066
Nucleolus	0.43 ± 0.152	0.16 ± 0.115^{ab}	0.71 ± 0.171^{a}	0.67 ± 0.124^{b}
Golgi apparatus:				
small vesicles	1.32 ± 0.287	0.67 ± 0.178^{ab}	2.15 ± 0.635^{a}	1.52 ± 0.156^{b}
large vacuoles	0.23 ± 0.225	0.51 ± 0.169	0.73 ± 0.237	0.76 ± 0.180
flat cisternae	0.51 ± 0.268	0.21 ± 0.105	0.62 ± 0.319	0.59 ± 0.150
total Golgi app.	2.63 ± 0.754	1.42 ± 0.317^{a}	3.50 ± 1.078^{a}	2.90 ± 0.282
Multivesicular bodies	0.07 ± 0.042	0.17 ± 0.062	0.05 ± 0.039	0.08 ± 0.052
Dense bodies	0.66 ± 0.212	0.83 ± 0.418^{ab}	0.24 ± 0.142^{a}	0.26 ± 0.115^{b}
Droplets	0.13 ± 0.081	0.31 ± 0.280^{ab}	0.00 ± 0.000^{a}	0.00 ± 0.000^{b}
Membrane whorls	0.50 ± 0.332	0.27 ± 0.177	0.27 ± 0.130	0.12 ± 0.041
Acanthosomes	0.04 ± 0.024	0.02 ± 0.017	0.00 ± 0.000	0.02 ± 0.017
Secretory granules:				
immature	0.13 ± 0.069^{abc}	0.00 ± 0.000^{abc}	0.50 ± 0.130^{b}	0.30 ± 0.091^{c}
mature, round	20.49 ± 0.834^{ab}	19.62 ± 1.366	13.20 ± 3.982^{a}	11.45 ± 2.067^{b}
mature, oblong	0.11 ± 0.055^{a}	0.17 ± 0.082	0.24 ± 0.112	0.51 ± 0.181^{a}
release profiles	0.00 ± 0.000^{ab}	0.00 ± 0.000^{cd}	0.31 ± 0.104^{ac}	0.16 ± 0.041^{bd}
intercellular	0.00 ± 0.000	0.07 ± 0.071	0.07 ± 0.045	0.15 ± 0.129
Translucent vesicles	0.10 ± 0.048	0.21 ± 0.100	0.23 ± 0.084	0.08 ± 0.047
Mitochondria	1.91 ± 0.306	2.50 ± 0.416	2.77 ± 0.509	2.96 ± 0.377
Rough Endoplasmic Reticulum (RER): isolated pieces, not dilated	5.33 ± 1.046	3.88 ± 0.362	5.75 ± 0.986	5.17 ± 0.977
isolated pieces, dilated	0.28 ± 0.106	0.30 ± 0.246	0.52 ± 0.172	0.45 ± 0.158
perinuclear	4.09 ± 0.869	6.20 ± 1.128	4.43 ± 1.459	7.27 ± 1.641
curvilinear	0.00 ± 0.000	0.00 ± 0.000	2.29 ± 1.682	0.33 ± 0.332
parallel, near cell membrane	1.26 ± 0.367^{ac}	2.20 ± 0.482^{b}	7.31 ± 2.301^{ab}	3.87 ± 0.950^{c}
parallel, in main cell body	2.03 ± 0.793	1.81 ± 0.624	4.09 ± 1.687	5.99 ± 1.613
total RER	12.99 ± 1.246^{ab}	14.37 ± 1.247^{cd}	24.27 ± 3.987^{ac}	23.09 ± 2.992^{bd}
Cytoplasmic ground substance	25.00 ± 0.501	24.87 ± 1.352	21.63 ± 1.334	22.23 ± 1.553
Free ribosomes	18.30 ± 0.485^{a}	18.88 ± 0.797^{b}	16.92 ± 1.162	15.17 ± 0.855^{ab}

Values paired by superscripts (a – a, b – b, etc.) differ significantly ($P < 0.05$).

Inspection of Table 1 indicates that the overall increase in PRL cell secretory activity occurred between morning and midday when synthetic activity increased (nucleolus, total RER, parallel arrays of RER, Golgi, immature SGs), PRL release increased (decline in stored mature SGs, appearance of SG release profiles) and lytic activity decreased (dense bodies, droplets). Subsequently, PRL cell activity, by the same criteria, declined between evening and midnight.

Evidence from the PRL cells in the midday and evening groups suggests several possible methods of SG release: (i) exocytosis into the intercellular

Fig. 5. Prolactin cells at 06.00–08.00 h, showing large lytic bodies present in many cells at this time. × 7,500

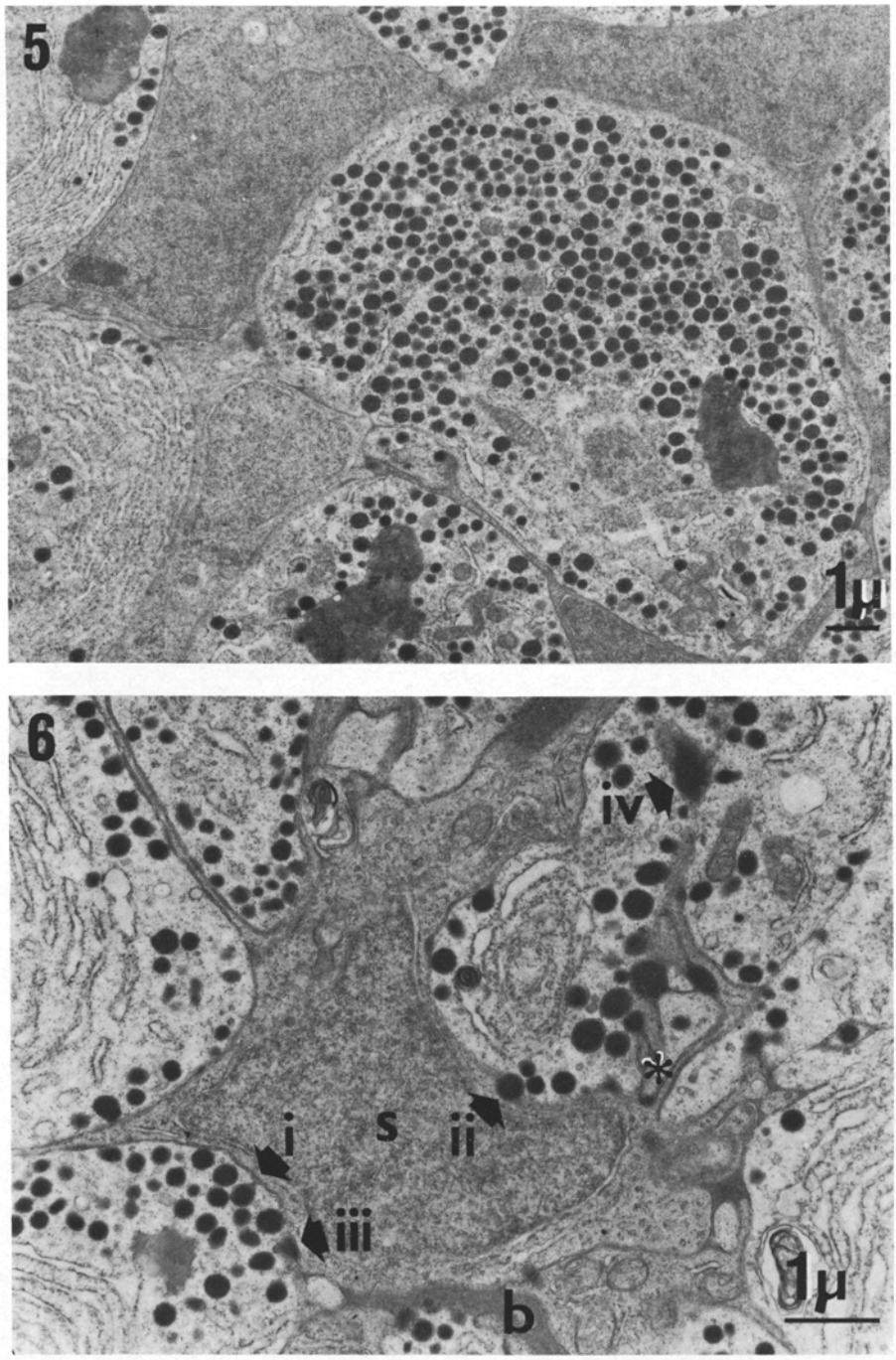

Fig. 6. Prolactin cells at 12.00–14.00 h, showing stages in granule release into the intercellular space adjacent to stellate cells (*s*) and into the basement membrane (*b*): (i) granule membrane contacts and becomes continuous with plasmalemma; (ii) granule without its membrane, free in intercellular space; (iii) granule moulded by enclosing structures before either dissolving, or (iv) apparently increasing in size, by fusing with other granules released at the same place. Note also apparent cell buds (asterisk). ×12,500

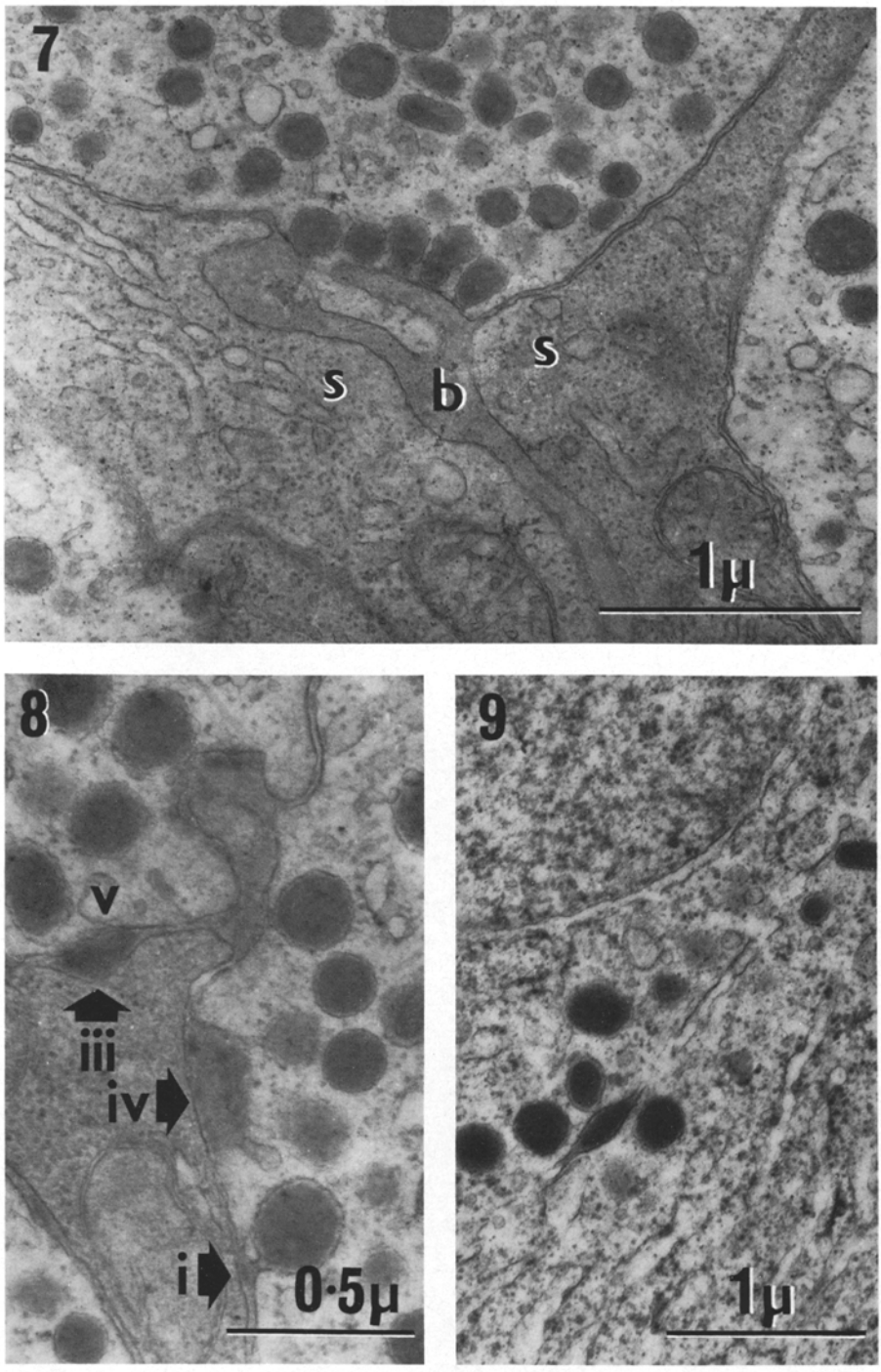

Fig. 7. Prolactin granules about to be released into basement membrane (*b*) between two stellate cells (*s*). 18.00–20.00 h, × 35,000

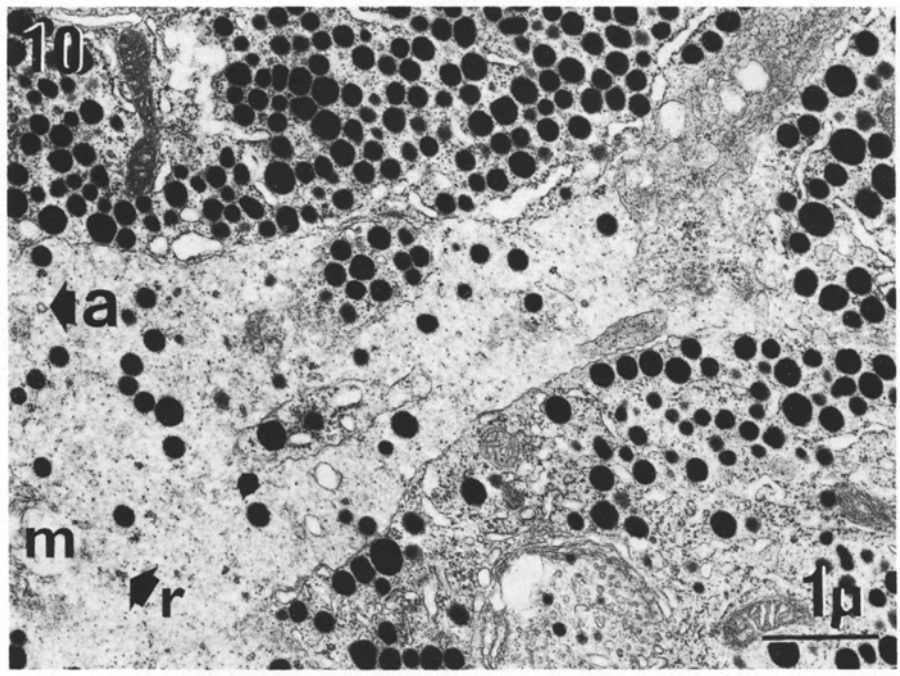

Fig. 10. Dilated intercellular space among prolactin cells, containing flocculent material, prolactin granules within their membranes, apparent prolactin cell buds, and presumed fragments of cell organelles (polyribosomes, *r*; membrane whorls, *m*; acanthosomes, *a*. This could represent cell degradation rather than normal granule release. × 15,000

spaces between PRL cells and stellate cells, or between two PRL cells (Figs. 6, 8, 9), the space in some cases being greatly distended and filled with a finely-granular material similar to that in the pericapillary spaces; (ii) exocytosis into the basement membrane (BM) adjacent to the neurohypophysis or around pericapillary spaces, or into extensions of the BM among the PRL cells (Figs. 6, 7). In all cases of exocytosis, certain characteristic features were observed such as loosening of the SG membrane before fusion with the plasmalemma, fusion of several SGs exocytosed in the same place to form irregular extracellular SGs, and the moulding of the extruded SGs by contiguous membranes before the SG apparently dissolved (Fig. 8). Other appearances of SG release occurred: (iii) release into certain distended spaces between PRL cells which contained what appeared to be fragments of cell organelles together with a flocculent material; the SGs retained their membrane and their shape in these spaces

Fig. 8. Prolactin cells at 18.00–20.00 h, showing stages in granule release into intercellular space adjacent to stellate cells (*s*). Stages numbered as in Fig. 6. Note empty vesicles (*v*) which appear to be pinched-off from the plasmalemma after granule extrusion. × 50,000

Fig. 9. Prolactin cells at 12.00–14.00 h, showing granule release into intercellular space between two prolactin cells, apparently not associated with presence of stellate cells or basement membrane. × 27,000

(Fig. 10). No "classical" SG exocytosis into these spaces was encountered, and it is possible that we are here dealing with cell degeneration rather than with normal processes of SG release. Finally, (iv), we also encountered possible instances of budding of the PRL cells into stellate cell processes or BM (Fig. 6).

No differences could be detected in the ACTH cells in the different time-groups.

Discussion

The differences found in the incidence of various organelles at different times indicates that there is a circadian rhythm of PRL cell activity in *P. latipinna*. On the basis of our observations, it seems that the time of major nuclear, Golgi and RER synthetic activity and of most PRL release is between midday and evening, i.e. in the second half of the photoperiod. The increase in dense bodies and droplets in the period midnight-morning is consistent with reduced PRL release during the night, since these structures are known to be lytic bodies (Farquhar *et al.*, 1975), active in the digestion of SGs: for example, when PRL secretion in *P. latipinna* is suddenly reduced by raising the external salinity (Hopkins, 1969). The activity of these lytic organelles would also explain the greater proportion of large irregular SGs during midday–evening, because in the midnight–morning period newly-formed SGs would tend to succumb to lytic digestion before they could fuse to form larger irregular granules.

We do not know the biological half-life of fish PRL. However, it is not likely to be grossly different from the short half-life of human PRL (less than 15 minutes, Perez-Lopez and Robyn, 1975). On this assumption, it would appear that the times of the active and non-active phases of the PRL cell in *P. latipinna* differ from those in two other teleosts, *Oncorhynchus nerka*, which showed a peak in plasma PRL between 03.00 and 06.00 h (Leatherland *et al.*, 1974), and *Carassius auratus*, which showed peaks in plasma PRL at 09.00 h and at 24.00 h when in FW and at 06.00 h and 24.00 h when in $^1/_3$ SW (Leatherland and McKeown, 1973). Both these studies on fish plasma used a heterologous radioimmunoassay to measure PRL and caution is undoubtedly needed in comparing them with our ultrastructural observations on the PRL cells. It is also necessary to be careful in deducing changes in plasma PRL levels from our observation of SG release profiles in the adenohypophysis. Nevertheless, any inferences about plasma PRL drawn from the prevalence of SG release profiles during the period 12.00–20.00 h seem to be substantiated by the associated reduction in the number of storage SGs (stored PRL), coupled with the simultaneous increase in the morphological indices of synthetic activity (nucleolus, Golgi apparatus, RER). It is also important to note that earlier studies on *P. latipinna* demonstrated that PRL cell granulation (visible by light microscopy) closely parallels pituitary PRL content, measured by bioassay (Ensor and Ball, 1968) or by disc electrophoresis-densitometry (Ball and Ingleton, 1973). It thus seems reasonable to infer that, on natural summer photoperiod, plasma PRL levels in *P. latipinna* would be higher during the period 12.00–20.00 h than at other times.

A different circadian rhythm in PRL exists in another cyprinodont *Fundulus similis*, in which pituitary PRL, measured by bioassay, is stored during the dark phase and released shortly after the onset of the light (Sage and de Vlaming, 1973). In this species, presumably plasma PRL would be low during the second half of the light phase, in contrast to *P. latipinna*.

Other evidence of circadian endocrine rhythms in teleosts has come from the circadian variations in the fattening response to exogenous PRL in *Fundulus chryotus* (Lee and Meier, 1967), *F. grandis* (Joseph and Meier, 1971), *F. kansae* (Mehrle and Fleming, 1970), *F. similis* and *Cyprinodon variegata* (de Vlaming and Sage, 1972). The indications are that the circadian changes in the fattening response to injected PRL depend on a circadian rhythm in plasma cortisol levels, which has been demonstrated in *F. grandis* (Garcia and Meier, 1973). Interestingly this cortisol rhythm perists in *F. grandis* even after hypophysectomy (Srivastava and Meier, 1972) and therefore seems not to depend on parallel changes in pituitary ACTH secretion. This recalls our failure to detect morphological changes in the ACTH cells of *P. latipinna* at different times, although we should note that in this species, in contrast to *F. grandis*, detectable levels of plasma cortisol disappear within two days of hypophysectomy (Hawkins and Ball, 1973).

Exocytosis of SGs from PRL cells into intercellular spaces adjacent to stellate cells has been described in several teleosts, including *Leuciscus rutilus* (Bage *et al.*, 1974), *Gillichthys mirabilis* (Nagahama *et al.*, 1972), *Gasterosteus aculeatus* (Leatherland, 1972; Benjamin, 1974b) and the cyprinodont (poeciliid) *Xiphophorus maculatus* (Weiss, 1965). Weiss and Leatherland also described PRL cell budding as a method of SG release. Some indications of cell budding were found in the present work, but verification of this interpretation of these images would require extensive serial sectioning to determine whether or not apparent buds were connected to the main cell body. We should note that studies have shown that where thoroughly investigated, in mammalian protein-secreting cells "exocytosis is the *only* release mechanism so far demonstrated. *No* alternatives have been found, although several... have been proposed" (Farquhar *et al.*, 1975). Our material furnished many instances of PRL granule release into the BM, and whole SGs were sometimes seen in the BM. These observations implicate the BM and its ramifications as a transport system connecting the PRL cells with pericapillary spaces. The disposition of the BM and its extensions, interconnecting PRL cells, stellate cells, pericapillary spaces and neurohypophysis (Batten *et al.*, 1975) lends support to this concept. Elaborate extensions of the BM of primary portal capillaries in the median eminence of bufonid amphibians seemingly play an analogous transport role, in this case conveying neurosecretory material from hypothalamic nerve endings to the median eminence capillaries (see Holmes and Ball, 1974). In *P. latipinna* as in other teleosts (Leatherland *et al.*, 1974) and other vertebrates (Holmes and Ball, 1974), the frequent release of PRL granules into the intercellular space adjacent to processes of stellate cells also suggests that the stellate cells may play a role in hormonal transport. SG release is occasionally seen into a space between two PRL cells, with no stellate cell in the vicinity; however stellate cell processes may in fact connect with such a space, but out of the

section plane. This may not be the only, or even the main, function of these elements; evidence points to the stellate (follicular) cell in mammals as playing a scavenger phagocytic role (Farquhar *et al.*, 1975).

Although exocytosis of PRL granules in *P. latipinna* follows the usual pattern, two "classical" features were only rarely observed, viz. the presence of numerous small vesicles in contact with the SG before release (Weiss, 1965), and the appearance of a "fuzz coat" on the plasmalemma at the site of exocytosis (Benjamin, 1974b).

Finally, it must be said that it is unclear how *P. latipinna*, which has an absolute requirement for PRL to maintain ionic balance in FW (Ensor and Ball, 1972), is able to cope with circadian changes in the rate of PRL secretion. In the normal circadian cycle the fish will presumably never experience anything like the sharp decline in circulating PRL that is fatal within 24 h of hypophysectomy in FW (Ball and Ensor, 1967, 1969). Nevertheless, since the sodium-retaining action of PRL in *P. latipinna* is dose-dependent (Ensor and Ball, 1968), circadian changes in PRL secretion at least raise the possibility of circadian changes in ionic balance. Alternatively, the fish may avoid these ionic disturbances by biological features we can only guess at. *P. latipinna* is a markedly diurnal animal, very active in the light and resting during hours of darkness. Possibly it requires higher circulating levels of PRL for electrolyte regulation when it is active during the day than when quiescent at night. Or the biological half-life of endogeneous PRL may change with the day/night shift, so as to compensate for changes in the rate of PRL release by the pituitary. Measurements of circulating levels of PRL are obviously needed to clarify these and many other questions.

References

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 (1974)

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., Ensor, D.M.: Specific action of prolactin on plasma sodium levels in hypophysectomized *Poecilia latipinna* (Teleostei). Gen. Comp. Endocr. **8**, 432–440 (1967)

Ball, J.N., Ensor, D.M.: Aspects of the action of prolactin on sodium metabolism in cyprinodont fishes. Coll. internat. C.N.R.S. (Paris) **177**, 215–224 (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)

Batten, T., Ball, J.N., Benjamin, M.: Ultrastructure of the adenohypophysis in the teleost *Poecilia latipinna*. Cell Tiss. Res. **161**, 239–261 (1975)

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

Benjamin, M.: Seasonal changes in the prolactin cell of the pituitary gland of the freshwater stickleback, *Gasterosteus aculeatus*, form *leiurus*. Cell Tiss. Res. **152**, 93–102 (1974b)

Butcher, R.L., Fugo, N.W., Collins, W.E.: Semicircadian rhythm in plasma levels of prolactin during early gestation in the rat. Endocrinology **90**, 1125–1127 (1972)

Clark, R.H., Baker, B.L.: Circadian periodicity in the concentration of prolactin in the rat hypophysis. Science **143**, 375–376 (1964)

Duncan, D.B.: Multiple range and multiple F. tests. Biometrics **11**, 1–42 (1955)

Ensor, D.M., Ball, J.N.: A bioassay for fish prolactin (paralactin). Gen. comp. Endocr. **11**, 104–110 (1968)

Ensor, D.M., Ball, J.N.: Prolactin and osmoregulation in fishes. Fed. Proc. **31**, 1615–1623 (1972)

Farquhar, M.G., Skutelsky, E.H., Hopkins, C.R.: Structure and function of the anterior pituitary and dispersed pituitary cells. *In vitro* studies. In: The anterior pituitary (Tixier-Vidal, A. and Farquhar, M.G., eds.), p. 83–135. New York: Academic Press 1975

Garcia, L.E., Meier, A.H.: Daily rhythms in concentration of plasma cortisol in male and female Gulf killifish *Fundulus grandis*. Biol. Bull **144**, 471–479 (1973)

Hawkins, E.F., Ball, J.N.: Current knowledge of the mechanisms involved in the control of ACTH secretion in teleost fishes. In: Brain-pituitary-adrenal interrelationships (Brodish, A. and Redgate, E.S., eds.), p. 293–315. Basel: S. Karger 1973

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

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)

Joseph, M.M., Meier, A.H.: Daily variations in the fattening response to prolactin in *Fundulus grandis* held on different photoperiods. J. exp. Zool. **178**, 59–62 (1971)

Kent, G.C., Turnbull, J.G., Kirby, A.C.: A daily rhythm in prolactin secretion or release in hamsters. Ass. Southeastern Biol. Bull. **11**, 48 (abstract) (1964)

Koch, Y., Chow, Y.F., Meites, J.: Metabolic clearance and secretion rates of prolactin in the rat. Endocrinology **89**, 1301–1308 (1971)

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

Leatherland, J.F., McKeown, B.A.: Circadian rhythm in the plasma levels of prolactin in the goldfish, *Carassius auratus* L. J. interdiscipl. Cycle Res. **4**, 137–143 (1973)

Leatherland, J.F., McKeown, B.A., John, T.M.: Circadian rhythm of plasma prolactin, growth hormone, glucose and free fatty acid in juvenile kokanee salmon, *Oncorhynchus nerka*. Comp. Biochem. Physiol. **47A**, 821–828 (1974)

Lee, R.W., Meier, A.H.: Diurnal variations of the fattening response to prolactin in the golden topminnow, *Fundulus chrysotus*. J. exp. Zool. **166**, 307–316 (1967)

Mehrle, P.M., Fleming, W.R.: The effect of early and midday prolactin injection on the lipid content of *Fundulus kansae* held on a constant photoperiod. Comp. Biochem. Physiol. **36**, 597–603 (1970)

Meier, A.H.: Diurnal variations of metabolic responses to prolactin in lower vertebrates. Gen. comp. Endocr., Suppl. **2**, 55–62 (1969)

Meier, A.H.: Temporal synergism of prolactin and adrenal steroids. Gen. comp. Endocr., Suppl. **3**, 499–508 (1972)

Meier, A.H., Burns, J.T., Dusseau, J.W.: Seasonal variations in the diurnal rhythm of pituitary prolactin content in the white-throated sparrow, *Zonotrichia albicollis*. Gen. comp. Endocr. **12**, 282–289 (2969)

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)

Perez-Lopez, F.R., Robyn, C.: Studies on human prolactin physiology. Life Sci. **15**, 599–616 (1975)

Sage, M., de Vlaming, V.L.: A diurnal rhythm in hormone effectiveness and in pituitary content of prolactin in *Fundulus similis*. Tex. Rep. Biol. Med. **31**, 101–102 (1973)

Sassin, J.F., Frantz, A.G., Kapen, S., Weitzman, E.D.: The nocturnal rise of human prolactin is dependent on sleep. J. clin. Endocr. **37**, 436–440 (1973)

Srivastava, A., Meier, A.H.: Daily variations in concentrations of plasma cortisol in intact and hypophysectomized Gulf killifish, *Fundulus grandis*. Science **177**, 185–187 (1972)

Tindal, J.S.: Hypothalamic control of secretion and release of prolactin. J. Reprod. Fertil. **39**, 437–461 (1974)

Vlaming, V.L., de, Sage, M.: Diurnal variation in fattening response to prolactin treatment in two cyprinodont fishes, *Cyprinodon variegatus* and *Fundulus similis*. Contrib. in Marine Sci. **16**, 59–63 (1972)

Weatherhead, B., Whur, P.: Quantification of ultrastructural changes in the melanocyte-stimulating hormone cells of the pars intermedia of the pituitary of *Xenopus laevis*, produced by changes of background colour. J. Endocr. **53**, 303–310

Weibel, E.R.: Stereological principles for morphometry in electron microscopic cytology. Int. Rev. Cytol. **26**, 235–302 (1969)

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

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