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The Goldfish Pituitary

I. Cytology*

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Summary. In the goldfish, *Carassius auratus*, morphological and functional aspects of the pituitary gland were studied at the ultrastructural level and six cell types could be distinguished in the pars distalis. Acidophilic cells of the rostral pars distalis were identified as prolactin cells, the chromophobic cells of the rostral pars distalis as ACTH cells, the non-globular basophilic cells of the rostral and the proximal pars distalis as TSH cells, the globular basophils of the proximal pars distalis as gonadotropic cells and the acidophils of the proximal pars distalis as somatotrophs.

Besides some of the well established criteria of morphological and functional identification of different cell types, two new approaches have been used in the present study. One was to express the electron density of secretory granules objectively by means of a photometric method. It was found that both types of acidophilic cells which produce the proteohormones prolactin and somatotropin respectively, had granules with the highest electron densities. The basophilic cells producing the glycoproteins gonadotropin and TSH respectively, possessed granules of intermediate electron density whereas the chromophobic cells storing the peptide hormone ACTH had granules of lowest densities. The second new approach was the administration of the synthetic mammalian releasing hormones LH-RF and TRF, which helped in identifying gonadotropic and thyrotropic cells respectively. In the goldfish there is evidence for the presence of only one type of gonadotropic cell.

Key words: Pituitary — Goldfish — Cell types — Ultrastructure.

Introduction

The innervation of the intrinsic endocrine cells of the teleost pituitary, though extensively studied in recent years, still poses some fundamental problems (see Kaul and Vollrath, 1974) and it was for this reason that the present series of investigations was undertaken. In teleosts, hypothalamic control of pars distalis function is perhaps best understood in the goldfish, *Carassius auratus*, for which there is strong evidence for the presence of CRF (Sage and Purrott, 1969), GTH-RF (Peter, 1970), TIF (Peter, 1970, 1971, 1972) and PIF (Peter and McKeown, 1974). The pituitary gland of this species therefore appeared to be a most suitable object for studying the morphological and functional interrelations between the nervous and the endocrine tissues.

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The aim of the present investigation was to lay the foundation for such a study by providing the basic morphological and functional information on the endocrine target cells. Besides using some of the well established criteria of morphological and functional identification two new approaches have been used in the present study. One was to express the electron density of secretory granules objectively by means of a photometric method and to assess whether or not this method represents a useful means of distinguishing different cell types. The second approach was to administer the synthetic mammalian releasing hormones LH-RF and TRF respectively, hoping that they might be effective in teleosts and might help to clarify whether in *Carassius* there is just one type of gonadotropic cells, as suggested by Nagahama and Yamamoto (1969), or two types, as reported by Olivereau (1962) and Leatherland (1972).

Material and Methods

In the present study a total of 99 mature goldfish, *Carassius auratus*, 18–20 cm in length and collected over a period of one and a half years were used. The animals were kept in 70 l aquaria containing well aerated tap water of 17–19°C. They received fish food and Tubifex twice daily. Both control and experimental animals were allowed to acclimatize to laboratory conditions for one week before they were killed or before experimentation started.

Light Microscopy. For light microscopic investigations ten animals were used. They were killed, by decapitation, one animal per month, from January to May and July to November. The pituitaries were rapidly dissected out and fixed in Bouin's fluid for twelve hrs. They were embedded in paraffin wax. Serial sections of 5 μm thickness were stained with Alcian blue-PAS-Orange G after oxidation with potassium permanganate, Aldehyde fuchsin after oxidation with potassium permanganate, Herlant's tetrachrome stain, Azan, Lead Haematoxylin or van Gieson.

Electron Microscopy. The pituitaries used for electron microscopy were fixed in 1% buffered (phosphate buffer, 0.2 M, pH 7.4–7.6) osmium tetroxide for 2–3 hrs. at 4°C and embedded in Araldite. Some pituitaries were fixed in 3% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4–7.6) for 3 hrs. and postfixed in 1% osmium tetroxide in 0.2 M phosphate buffer for $1^1/_2$ hrs. Sections were cut on an Ultratome III using glass or diamond knives. Sections mounted on carbon coated copper grids were stained with Uranyl acetate and examined under an AEI 6 B electron microscope. A total of 32 apparently normal animals were killed at weekly intervals from April to June and at two-weekly intervals from July to March. These animals were used to study the ultrastructure of the pituitary gland at different seasons. Moreover, they served as controls for the following experimental studies.

TSH Function of the pituitary was studied as follows. Twelve animals were immersed in 0.1% thiourea (British Drug House) solution, which was changed weekly, and killed after respectively 3 weeks (3 animals), 5 weeks (3 animals), 7 weeks (2 animals), 9 weeks (2 animals) and 12 weeks (2 animals). Six animals received daily intraperitoneal thyroxine (L-Thyroxine sodium salt–$5H_2O$, Sigma) injections of 0.25 mg (dissolved in 0.5 ml of fish saline). Two of these six fishes received 4 injections and were killed 24 hrs. after the last injection. The remaining four animals received 5 and 6 injections respectively and were killed 3 days after the last injection. One fish was given two intraperitoneal injections of 300 μg TRF (Beckman) dissolved in 1.0 ml fish saline) one hour apart and it was killed one hour later.

Gonadotropic Function. Twelve animals received 2, 3 or 4 intramuscular injections of 2.5 mg/0.5 ml Oestradiol benzoate (Benztrone–Paines and Byrne Ltd.) on either consecutive or alternate days and were killed 1 to 12 days after the last injection. Progesteron (Paines and Byrne Ltd.) (5 mg/0.5 ml) was administered intramuscularly to five animals on two or three alternate days and the animals were killed 1 or 3 days after the last injection. LH-RF (Beckman) at a dose of 100 ng/0.1 ml fish saline was given intraperitoneally to four fishes. Two of these were killed 30 min after one injection; the remainder received 2 injections, one hour apart, and were killed one hour later. Four animals were kept in an aquarium with the water temperature raised from 17 to 25°C for 3 days.

ACTH Function. Ten animals received 2 to 9 intraperitoneal injections of 25 mg metopiron (Ciba)/0.5 ml fish saline on consecutive or alternate days. Eight of these animals were killed 24 hrs. after the last injection. One animal died during the experiment and one spawned after the second injection and was killed immediately after spawning. Three fishes received three intramuscular injections of 25 mg hydrocortisone (Hydrocortisyl, Roussel)/0.5 ml on alternate days and were killed 24 hrs. after the last injection.

Particle Size Analysis. Particle size analysis (Zeiss Particle Size Analyzer TGZ3) was carried out on electron micrographs with final magnifications of between 12500 and 25000. In each case all the secretory granules of individual cell profiles were measured and distribution curves plotted. In a next step the particle sizes of a number of cells of the same type were pooled and the mean formed to obtain representative values for the various cell types. In the case of elongate particles the long diameter was measured.

Assessment of Electron Density. For the objective assessment of the electron density of secretory granules a Zeiss IPM-2 Integrating Photometer was used. The measuring principle of this photometer is based on the observation by Zeitler and Bahr (1962, 1967) that under certain conditions there is an almost linear relationship between the mass thickness of an object detail and the transmission measured photometrically at the corresponding point in the electron micrograph *negative*. Originally the IPM-2 was designed to determine the total mass or dry weight of an object area or an isolated particle. In the present study the photometric method is applied to particles contained in sections rather than isolated structures. To measure dry mass of particles contained in sections has been little used, mainly because of problems of contamination, sublimation and variation in section thickness (Casley-Smith, 1972; Silverman *et al.*, 1969). It is apparent that these problems matter less if only relative values are to be obtained and it was assumed in the present investigation that all the cells of a relatively small tissue area were equally affected by contamination and sublimation. Since the present approach represents a pilot study, the problem of variation in section thickness was overcome by taking photographs of adjacent cells only, though bearing in mind that even in those small areas variation in section thickness might occur.

In the present study some 4000 secretory granules contained in 47 cell profiles were measured. In addition the electron density of the cytoplasm of each cell investigated was assessed by measuring the transmission at ten different sites devoid of cell organelles.

The electron microscopic material and the sections used for assessment were processed in exactly the same way, (material was fixed in osmium tetroxide only, see above). Since the electron microscope used was not equipped with an automatic exposure timer, an exposure time of 1 sec was chosen. The illumination was adjusted accordingly and left unchanged until all the photographs of a series were taken. Photographs taken from different sections and with (probably) a different illumination were assigned to different groups. Altogether four different groups of photographs were taken. The processing of the photographic plates was carried out under identical conditions and the photometric measurement of the transmission of the secretory granules and the cytoplasm according to the instructions of the manufacturer of the IPM-2.

The measurements obtained from the granules were first used to plot frequency histograms for individual cell profiles. Moreover, the mean of the measurements of all the granules of an individual cell profile was formed to obtain representative values for individual cells which were then compared.

Results

Light Microscopy: The pituitary gland of *Carassius auratus* consists of three lobes (Fig. 1). The *rostral pars distalis*, the smallest of the three lobes, occupies the dorsal area of the posterior part of the gland and is intimately related to the posterior aspect of the short and delicate pituitary stalk. It contains acidophilic, basophilic and chromophobic cells. *Acidophilic cells*, the predominant cell type, were found to be evenly distributed throughout the lobe. They were oval or irregular in shape. Their nuclei were spherical and the cytoplasm exhibited a

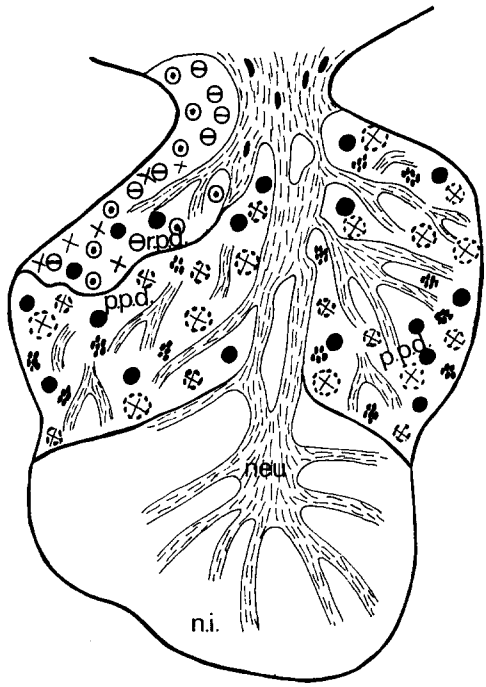

Fig. 1. A semidiagrammatic representation of a medial sagittal section through the pituitary gland of *Carassius*. *r.p.d.* rostral pars distalis; *p.p.d.* proximal pars distalis; *n.i.* neurointermediate lobe; *neu.* neurohypophysis. ⊙ Prolactin cells, ⊖ ACTH cells, × TSH cells, ● Gonadotropes, ·:· STH cells

discrete acidophilic granulation. *Basophilic cells*, relatively few in number, lay mainly near the free surface of the lobe. They were small and mostly elongate with round nuclei. Their cytoplasm was PAS-, alcian blue- and aldehyde fuchsin-positive and enclosed a fine granulation with similar staining properties. Near the proximal pars distalis a few globular basophils were observed (see below). *Chromophobic cells* were scattered all over the rostral pars distalis and additionally formed a distinct layer of two to three cells in thickness near the neurohypophysis which comes into more or less close contact with all three pituitary lobes. Chromophobic cells were usually small and polymorphous with spherical nuclei.

The *proximal pars distalis*, the second pituitary lobe, was found beneath both the rostral pars distalis and the pituitary stalk. It extended to a varying degree into the anterior part of the gland and contained two types of basophilic and one type of acidophilic cell. The most abundant cell type of this lobe was the *globular basophils*. Their cytoplasm was characterized by the presence of large globular inclusions and small secretory granules, both of which stained intensely with PAS, alcian blue and aldehyde fuchsin. Moreover, it contained a varying number of vacuoles. The nuclei were round and eccentrically positioned. The *second type of basophilic cells* were light microscopically indistinguishable from the basophilic cells of the rostral pars distalis. These cells were usually arranged in clusters and

surrounded by cords of acidophilic cells. *Acidophilic cells*, arranged in cords or clusters, had relatively large, oval, centrally placed nuclei and a homogenous cytoplasm.

In the *neuro-intermediate lobe*, the largest pituitary lobe, the intrinsic cells did not exhibit conspicuous staining properties. Particularly striking was the absence of PAS-positive cells. Nevertheless, two cell types could be distinguished. One type was relatively large and possessed a pale cytoplasm with a faint granulation. These cells, arranged in cords, were the predominant cell type. The second type, considerably smaller than the first one, showed a slight affinity for lead haematoxylin and lay scattered in the periphery of the cell cords.

Seasonal Changes: Although a relatively small number of specimens were investigated by light microscopy, seasonal changes were conspicuous. In the rostral pars distalis the basophilic cells were particularly abundant from October to December. They gradually decreased in number from January to August. The proximal pars distalis was particularly large in April and May and the globular basophils were more abundant than in the other months and exhibited a prominent cytoplasmic vacuolation.

Electron Microscopy: The six cell types of the pars distalis distinguished by light microscopy could be easily identified by electron microscopy.

Size of Secretory Granules: The size histograms of the secretory granules of the six cell types are shown in Fig. 2. It can be seen that in most cases the localization of the histogram peaks differed strikingly from cell type to cell type. The exceptions were the peaks of the acidophilic cells of the rostral pars distalis and the non-globular basophils of the proximal pars distalis. In this instance, however, there were clear differences in the shapes of the histograms and also in the mean diameter of the secretory granules (see below).

Electron Density of Secretory Granules: The results of the electron density assessment are shown in Fig. 3(a, b) and Table 1. Fig. 3 depicts the histograms of the electron densities of the secretory granules of all the cells investigated. It can be seen that the shape of the histograms is fairly characteristic for each cell type, though it is apparent that the position of the histogram peaks differs. The histograms of the acidophilic cells of the rostral pars distalis are relatively wide with a number of peaks, whereas those of the acidophilic cells of the proximal pars distalis are relatively narrow with one distinct peak only. Inspection of corresponding electron micrographs reveals that the electron density of the secretory granules of the former is much more heterogenous than that of the latter. Very variable in electron density are also the granules of the chromophobic cells. The relatively large widths of the histograms of the globular basophils are mainly due to the fact that the electron densities of the globular inclusions are distinctly different from those of the small secretory granules.

Table 1 shows the mean transmission values of both secretory granules and cytoplasm of all the cell profiles investigated. It can be seen that, within each group, the granules of the acidophilic cells possessed higher transmission values than those of the non-globular basophils and the chromophobic cells.

Comparing the transmission values of the different cell types assigned to different groups reveals fairly striking differences which seem to preclude a direct comparison of the values obtained. Nevertheless, such a comparison is possible.

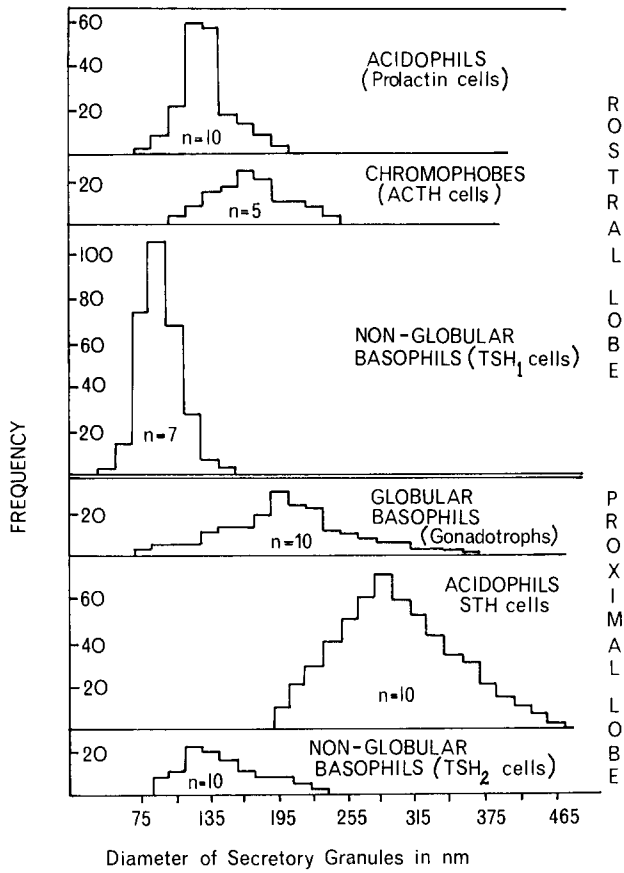

Fig. 2. Histograms showing diameter of secretory granules of different cell types in the pars distalis. *n* number of cells investigated. The vertical axis represents the number of secretory granules, the horizontal axis the diameter of secretory granules in nm

Taking into consideration that variations in illumination and section thickness affect the transmission of secretory granules and cytoplasm correspondingly it was thought that it might be possible to characterize different cell types by the difference in electron density between secretory granules and cytoplasm. Table 1 shows that the values obtained by subtracting the mean transmission of the cytoplasm from the mean transmission of the secretory granules were fairly similar for the cells belonging to the same type but strikingly dissimilar for the different cell types. The difference in transmission or electron density was high in the acidophilic cells, intermediate in the basophils and small in the chromophobic cells. The only cell type in which the differences were inconsistent was the globular basophils. There were statistically significant differences (Wilcoxon's rank test, 95% probability) between the values obtained by subtracting the mean transmission of the cytoplasm from the mean transmission of the secretory granules of different cell types, except between the prolactin and STH cells.

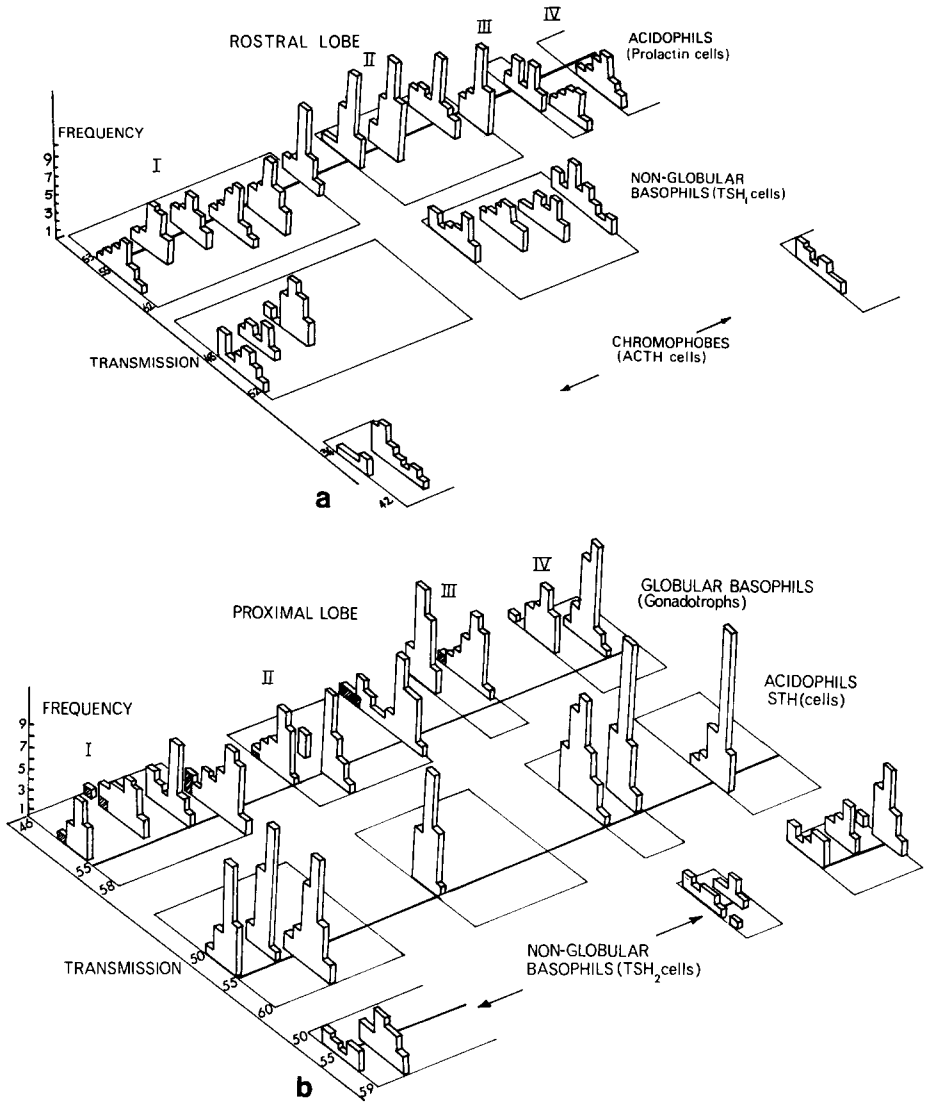

Fig. 3. Three-dimensional histograms showing the transmission of the secretory granules of the different cell types in the rostral (a) and proximal (b) pars distalis. Each histogram represents the transmission values of the granules of one cell profile. On the abscissa, the transmission values are shown in working units; for ease of comparison, a line of reference is drawn at the transmission value 55. On the ordinate, the frequency is shown. *I*, *II*, *III* and *IV* denote different series of photographs. The cross-hatched columns in Fig. 3b represent the transmission of the globular inclusions of the globular basophils

Other Ultrastructural Features of the Various Cell Types (Figs. 4 and 5). The acidophilic cells of the rostral pars distalis were characterized by a large number of mostly oval or elongate secretory granules, the long diameter of which measured

Table 1. Transmission of secretory granules of pituitary cell types

	Group I			Group II			Group III			Group IV		
	T_{Gr}	T_{Cy}	$T_{Gr}-T_{Cy}$	T_{Gr}	T_{Cy}	$T_{Gr}-T_{Cy}$	T_{Gr}	T_{Cy}	$T_{Gr}-T_{Cy}$	T_{Gr}	T_{Cy}	$T_{Gr}-T_{Cy}$
Rostral lobe												
Prolactin cells (acidophilic)	48.0	30.5	17.5	56.3	39.7	16.6	49.6	33.2	16.4	48.1	29.4	18.7
	51.6	34.4	17.2	57.2	40.2	17.0	45.2	26.5	18.7			
	50.9	35.5	15.4	57.0	38.4	18.6						
	57.6	38.2	19.4	52.6	34.4	18.2						
	54.4	34.6	19.8									
	47.9	30.6	17.3									
Mean ±S.E.M.	51.7	33.9	17.7	55.7	38.2	17.6	47.4	29.8	17.5	48.1	29.4	18.7
	±1.5	±1.2	±0.6	±1.0	±1.3	±0.5	±2.2	±3.3	±1.5			
ACTH cells (chromophobic)	38.8	30.9	7.9							34.0	27.5	6.5
	30.0	22.7	7.3									
Mean ±S.E.M.	34.4	26.8	7.6							34.0	27.5	6.5
	±4.4	±4.1	±0.3									
TSH_1 cells (basophilic)	33.1	22.5	10.6	47.4	34.8	12.6						
	46.2	34.3	11.9	45.2	35.0	10.2						
	48.6	35.8	12.8	46.9	37.6	9.3						
				50.3	39.1	11.2						
Mean ±S.E.M.	42.6	30.9	11.7	47.4	36.6	10.8						
	±4.8	±4.2	±0.6	±1.0	±1.0	±0.7						
Proximal lobe												
Gonadotrophs (basophilic)	56.5	42.9	13.6	41.6	27.4	14.2	37.1	28.0	9.1	40.0	28.9	11.1
	55.1	39.6	15.5	42.0	28.4	13.6	44.0	34.3	9.7	43.3	32.3	11.0
	54.5	38.9	15.6	48.1	33.1	15.0						
	39.5	25.7	13.8									
Mean ±S.E.M.	51.4	36.8	14.6	43.9	29.6	14.3	40.5	31.1	9.4	41.6	30.6	11.0
	±3.9	±3.7	±0.5	±2.1	±1.7	±0.4	±3.4	±3.1	±0.3	±1.6	±1.7	±0.7
STH cells (acidophilic)	37.9	21.9	16.0	53.2	34.1	19.1	55.3	38.5	16.8	55.9	38.9	17.0
	52.9	35.0	17.9				54.8	36.9	17.9			
	55.7	36.2	19.5									
Mean ±S.E.M.	48.8	31.0	17.8	53.2	34.1	19.1	55.0	37.7	17.3	55.9	38.9	17.0
	±5.5	±4.5	±1.0				±0.2	±0.8	±0.5			
TSH_2 cells (basophilic)	47.1	36.0	11.1				53.4	42.4	11.0	34.1	26.4	7.7
	38.6	27.4	11.2				55.5	44.4	11.1	53.7	43.9	9.8
										41.7	34.9	6.8
Mean ±S.E.M.	42.8	31.7	11.1				54.4	43.4	11.0	43.1	35.0	8.1
	±4.2	±4.3	±0.7				±1.0	±1.0	±0.7	±5.7	±5.0	±1.2

Abbreviations denote: T_{Gr} Transmission of secretory granules, T_{Cy} Transmission of cytoplasm, $T_{Gr}-T_{Cy}$. T_{Gr} minus T_{Cy}. For details see Text.

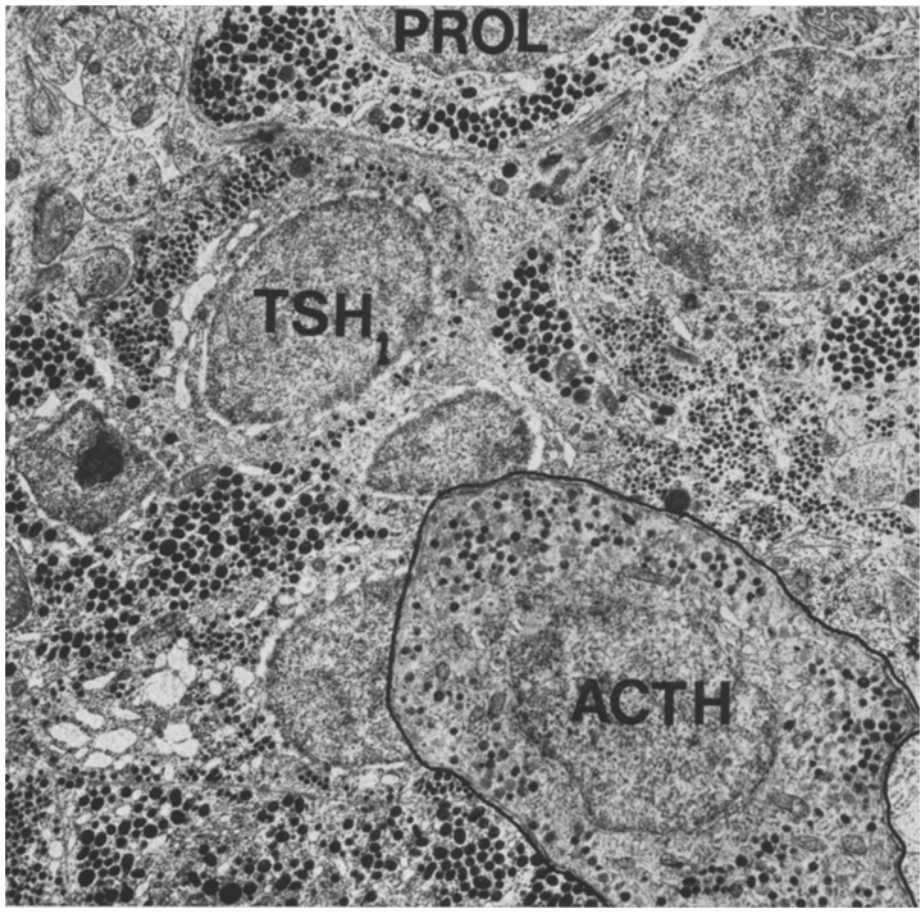

Fig. 4. An electron micrograph showing three different cell types of the rostral pars distalis. *Prol* Prolactin cell; TSH_1 TSH cell; *ACTH* ACTH cell (inset). ×7900

127 ± 14 nm (mean). The granules were evenly distributed throughout the cytoplasm. Mitochondria were present in moderate number. Rough endoplasmic reticulum, slightly dilated, was scarce.

The chromophobic cells of the rostral pars distalis contained only a few round secretory granules with a mean diameter of 172 ± 14 nm. Rough endoplasmic reticulum was present in small amount and the Golgi apparatus lying closely related to the nucleus consisted of but a few narrow cisternae. Membrane-bound inclusions resembling lysosomes were fairly frequent.

The basophilic cells of the rostral pars distalis contained a large number of mainly spherical secretory granules with a mean diameter of 105 ± 12 nm. Rough endoplasmic reticulum with moderately dilated cisternae was particularly prominent around the nucleus. The Golgi apparatus was small and lysosome-like inclusions were moderately numerous.

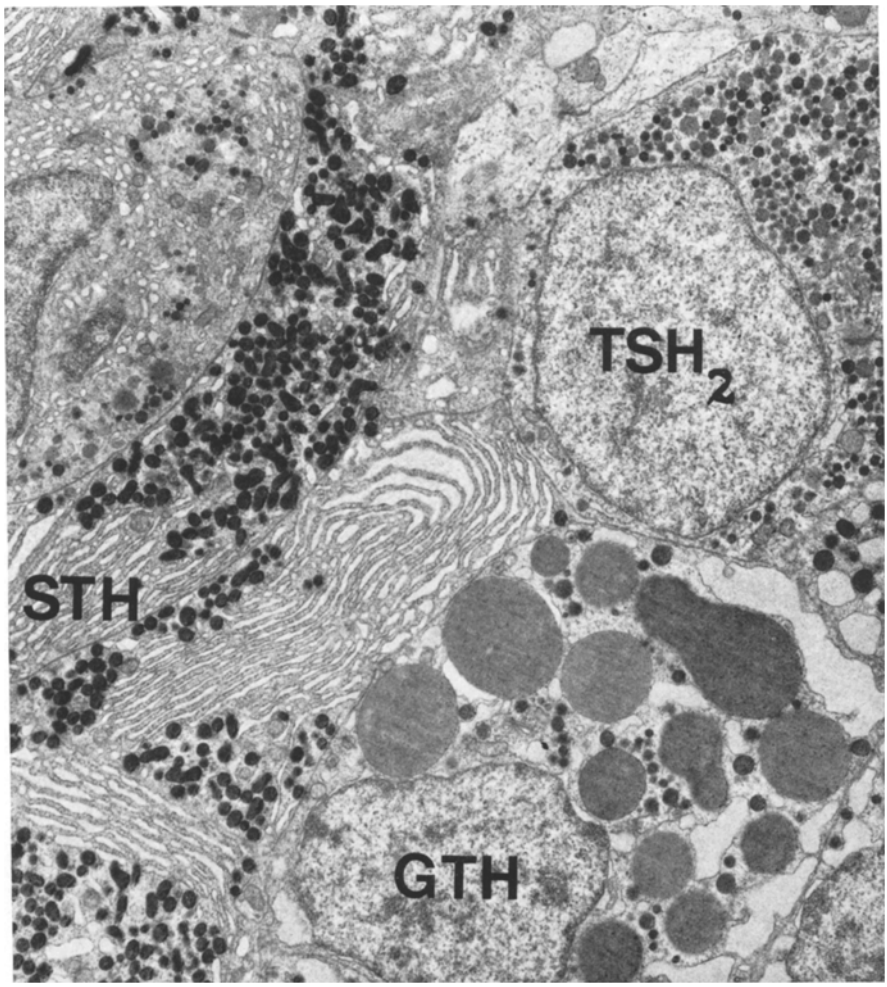

Fig. 5. An electron micrograph showing three different cell types in the proximal pars distalis. *STH* somatotropic cell; *TSH_2* TSH_2 cell; *GTH* gonadotropic cell. ×8250

The globular basophils of the proximal pars distalis contained a large number of mostly spherical secretory granules with a mean diameter of 216 ± 20 nm. The globular inclusions seen under the light microscope corresponded to round, membrane-bound structures measuring between 1.3 and 3.0 μm which consisted of a fine-granular, homogenous material. Another conspicuous feature was the prominent rough endoplasmic reticulum with extremely wide cisternae, particularly at certain times of the year, which comprised a fine-granular material of low electron density.

The acidophilic cells of the proximal pars distalis were characterized by the presence of a large number of mostly kidney-, rod-shaped or oval secretory granules. Rough endoplasmic reticulum, slightly dilated and showing a parallel arrangement, was abundant, particularly in the immediate vicinity of the nucleus.

The non-globular basophilic cells of the proximal pars distalis were electron microscopically similar in appearance to the basophilic cells of the rostral pars distalis. The spherical secretory granules were however distinctly larger (164 ± 15 nm mean diameter). Some of the cells contained a few spherical globular inclusions similar to those found in the globular basophils.

The most frequently occurring cell type (Type 1) of the *neurointermediate lobe* was characterized by a large number of electron-dense membrane-bound secretory granules, round or oval in shape, the long axis measuring 214 ± 10 nm. Rough endoplasmic reticulum was scarce and the Golgi apparatus was well developed.

Type 2 cells contained only a few round secretory granules with a mean diameter of 225 ± 8 nm and with a very low electron density. Electron lucent vesicles measuring 200–280 nm in diameter were abundant.

Seasonal Changes: Seasonal changes, at the ultrastructural level, were observed only in the cell types of the proximal pars distalis. The globular basophils showed a striking increase in both the number and size of the globular inclusions from February to April. The cisternae of the endoplasmic reticulum became wider and the electron density of the secretory granules increased, as compared to the previous months. Some fairly large secretory granules measuring up to 500 nm in length and 100 nm in width were present. In May and June, just before spawning, the secretory granules were smaller in number and the globules larger and less electron dense than in the preceding months. Moreover, the dilatations of the endoplasmic reticulum had increased in width. Immediately after spawning the globular basophils were almost devoid of secretory granules and globules and the few remaining inclusions showed a strong decrease in electron density. The dilatations of the endoplasmic reticulum were maximal (Fig. 6). New was the occurrence of a varying number of rod-like electron dense particles (Fig. 6, inset) similar to those observed in presumed FSH cells of migrating European eels approaching sexual maturity (Knowles and Vollrath, 1966).

In the post-spawning season (June and July) the globular basophils were characterized by only a few, moderately electron dense secretory granules and little endoplasmic reticulum with narrow cisternae. In August and September, a slight increase in the number of both globules and secretory granules and a striking increase in the amount of rough endoplasmic reticulum were observed. From October to January, the globular basophils showed no changes.

The acidophilic cells of the proximal pars distalis possessed a small amount of endoplasmic reticulum and a relatively large number of secretory granules from October to January. In February and March, both the secretory granules and the endoplasmic reticulum increased in amount. In the summer months, the amount of endoplasmic reticulum increased even further, while the secretory granules decreased in number.

The non-globular basophils contained, from October to March, a considerably larger amount of secretory granules than in the summer months. From March to August, an increase in the amount of both endoplasmic reticulum and lysosome-like inclusions was noted.

Administration of Oestradiol. After administration of oestradiol benzoate the globular inclusions of the globular basophils had coalesced to form large irregular masses exhibiting a fine striation either near the edges or in the centre (Fig. 7). In

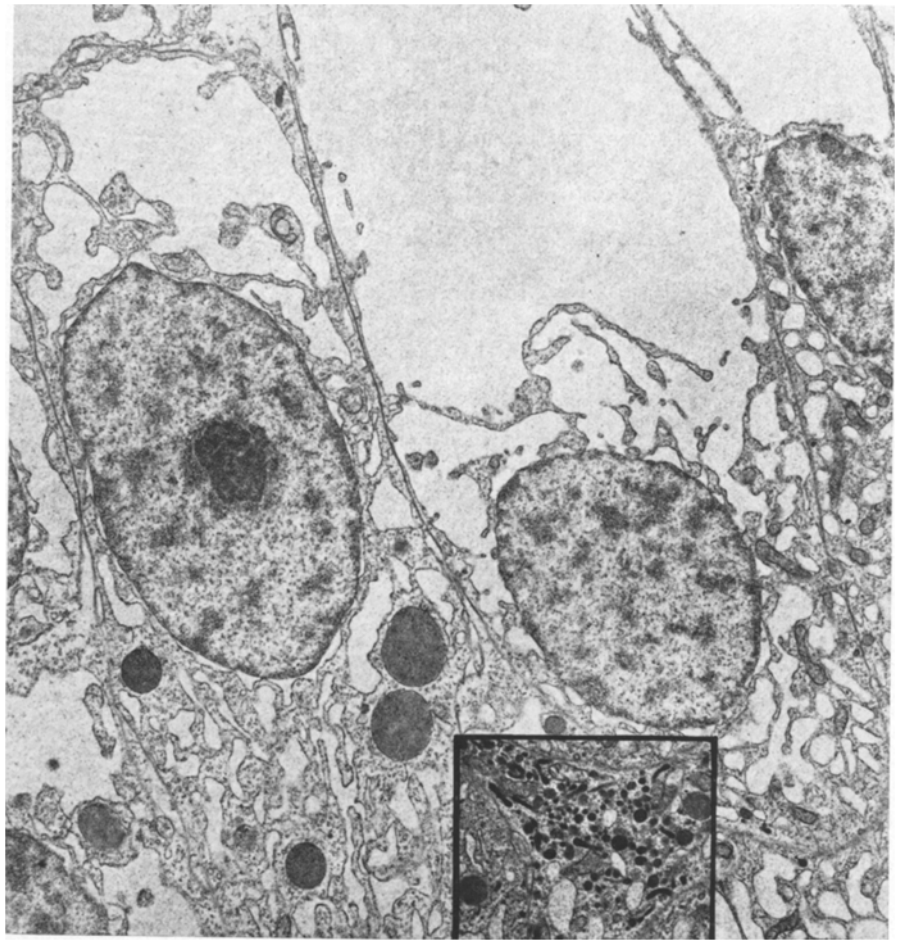

Fig. 6. An electron micrograph showing gonadotropic cells after spawning. Note the loss of secretory granules and the extremely wide dilatations of the endoplasmic reticulum. Inset: After spawning, in some gonadotropic cells the occurrence of rod-like electron dense particles is typical. ×8000

what appeared to be cross sections through the striation a fingerprint-like or porous appearance was typical, probably indicating a tubular nature of the striation. Moreover, a decrease in electron density, as assessed visually, was observed in both the globular inclusions and the secretory granules, the latter of which showed also a decrease in number and size (mean diameter of 194 ± 17 nm). The endoplasmic reticulum was less in amount and showed narrower cisternae than in the controls. Lysosomes were more frequent. In animals killed twelve days after the last injection the electron density of the globular inclusions was very pronounced.

The acidophilic cells of the proximal pars distalis showed a decrease in the amount of secretory granules.

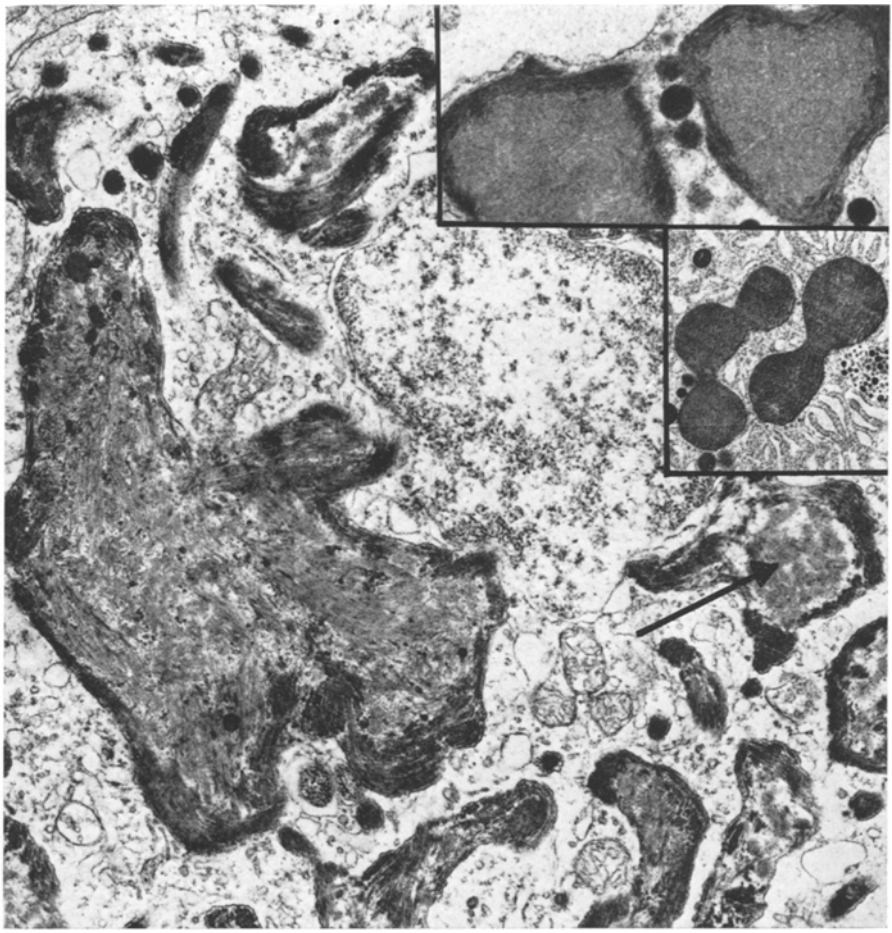

Fig. 7. The effect of oestradiol administration on gonadotropic cells. Small inset: In a first stage, the globular inclusions appear to fuse. ×7900. Large inset: In the periphery of the coalesced inclusions a dark striation occurs, which probably represents a second developmental stage. ×15800. Main figure: In the third stage, the whole of the inclusion exhibits a striated appearance. The arrow points to a fingerprint-like arrangement of the striation. ×15800

Administration of Progesteron. After injection of progesteron the secretory granules of the globular basophils decreased in number and size (mean diameter 194 ± 17 nm). Characteristically, the globular inclusions coalesced and showed, near their edges, the same striation as observed after administration of oestradiol. Endoplasmic reticulum, only slightly dilated, increased in amount.

In the non-globular basophils of the proximal pars distalis the secretory granules appeared less electron dense and the cytoplasm and the nucleus more electron dense than in the controls. The nuclei were highly indented.

Administration of LH-RF. Administration of LH-RF led to striking changes in the globular basophils. Half an hour after a single injection, most of the globular baso-

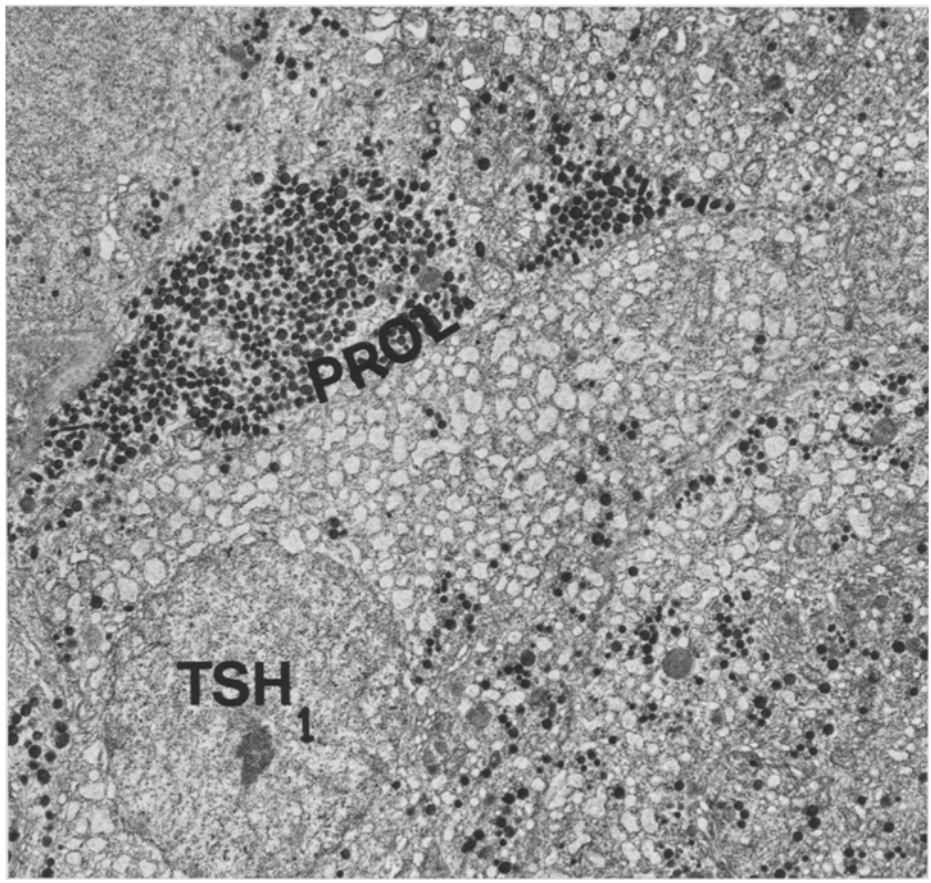

Fig. 8. Thyrotropic cell (TSH_1) of the rostral pars distalis after 12 weeks of thiourea treatment. Note the reduction in number of secretory granules and the increase in the amount of endoplasmic reticulum. *Prol* Prolactin cell. ×8000

phils showed a distinct decrease in the amount of secretory granules. The endoplasmic reticulum was dilated and the large globular inclusions were less electron dense than in the controls. One hour after two injections of LH-RF, given one hour apart, the globular basophils had the same appearance as immediately after spawning. The cells were almost devoid of secretory granules. Globules and both types of inclusion showed a decrease in electron density. The cisternae of the endoplasmic reticulum were extremely wide and the nuclei shrunken and indented.

Administration of Thiourea. After three weeks of thiourea treatment no changes were noted in both the rostral and the proximal pars distalis. After five weeks, a decrease in the amount of secretory granules and an increase in the amount of endoplasmic reticulum was observed in both the basophils of the rostral pars distalis and the non-globular basophils of the proximal pars distalis. Seven and nine weeks exposure to thiourea led to a further reduction in the number of secretory granules and to a further increase in the amount of endoplasmic reticulum (Fig. 8). In addition, mitochondria increased in number. After twelve weeks,

both cell types showed an increase in nuclear size and nucleoli were prominent. The endoplasmic reticulum appeared foam-like and the secretory granules decreased in number. The size of the secretory granules increased in the basophils of the rostral pars distalis to 149 ± 13 nm, as compared to 105 ± 12 nm in the controls, and remained unchanged in the non-globular basophils of the proximal pars distalis.

Administration of Thyroxine. Administration of thyroxine was followed by an increase in the amount of lysosomes and a decrease in the amount of secretory granules in both the basophils of the rostral pars distalis and the non-globular basophils of the proximal pars distalis. The secretory granules of the basophils of the rostral pars distalis increased in size to 157 ± 15 nm, as compared to 105 ± 12 nm in the controls, while those of the non-globular basophils of the proximal pars distalis remained unchanged.

TRF Administration. Administration of TRF affected the basophils of the rostral pars distalis, the non-globular basophils of the proximal pars distalis and to a lesser extent the globular basophils of the proximal pars distalis. The two former cell types showed a striking decrease in the amount of secretory granules and in some cells only a few granules remained. Occasionally secretory granules were encountered in the intercellular spaces indicating exocytosis. The cytoplasm of the basophils of the rostral pars distalis was vacuolated. The endoplasmic reticulum was dilated in both the globular and non-globular basophils of the proximal pars distalis.

Administration of Metopiron. After administration of metopiron the chromophobic cells of the rostral pars distalis increased in size and the nuclei were elongated and highly indented. Both lysosomes and secretory granules increased in amount, the latter measuring 141 ± 12 nm in diameter, as compared to 172 ± 14 nm in the controls. Furthermore, the endoplasmic reticulum increased in amount.

The globular basophils of the proximal pars distalis were characterized by a striking decrease in the amount of secretory granules and an increase in the amount of endoplasmic reticulum with very wide cisterns. The overall picture of the globular basophils was similar to that immediately after spawning. One of the mature fishes actually spawned after metopiron injections.

Administration of Hydrocortisone. Administration of hydrocortisone led, in the chromophobic cells of the rostral pars distalis, to a slight increase in the amount of lysosomes, some of which were particularly large (600–1000 nm). Secretory granules, whose size was similar to that after administration of metopiron, decreased slightly in amount.

Discussion

In the teleost pars distalis the identification of different cell types by means of light and electron microscopy is greatly helped by the fact that the cells of a single type are frequently grouped together (Sage and Bern, 1971). The results of the present study show that the pituitary gland of the goldfish, *Carassius auratus*, is no exception in this respect. In the present investigation six cell types have been distinguished in this species and it is assumed that the acidophilic cells of the rostral pars distalis produce prolactin, the chromophobic cells of the rostral pars distalis ACTH, the basophilic cells of the rostral pars distalis and the non-globular

basophils of the proximal pars distalis TSH, the globular basophils of the proximal pars distalis gonadotropic hormone and the acidophilic cells of the proximal pars distalis growth hormone. That all these hormones are indeed present in teleosts has recently been reviewed (Sage and Bern, 1971).

Prolactin Cells. There can be no doubt that the acidophilic cells of the rostral pars distalis represent the prolactin cells. This conclusion is partly based on the fact that in *Carassius* there is only one type of acidophilic cell present in the rostral pars distalis and that in teleosts the acidophilic prolactin cells are restricted to this lobe (Ball and Baker, 1969). Direct evidence for their prolactin nature was obtained by immunohistochemistry (Emmart, 1969).

ACTH Cells. To ascribe ACTH function to the chromophobic cells of the rostral pars distalis also presents no difficulties. To begin with, most of the reputed ACTH cells of *Carassius* have precisely the same localization that is typical of ACTH cells in teleosts generally, namely in the rostral pars distalis adjacent to the neurohypophysis (Olivereau, 1964, 1970; Ball and Baker, 1969; Oguri, 1971). Furthermore, ACTH cells of teleosts stain characteristically with lead haematoxylin (Olivereau, 1964; Oguri, 1971). In *Carassius* a positive staining reaction of the chromophobic cells with this dye was described by Oguri (1971), though it was not observed in the present study. The lack of stainability in the present study is perhaps not too surprising because, as Oguri (1971) has pointed out, the cells stained only moderately in *Carassius*. Moreover, the observation by Nagahama (1973) that the stainability with lead haematoxylin decreased in the corticotropic cells after metopiron injections which inhibit the 11-hydroxylation of adrenal steroids indicates that the stainability might depend on the functional state of the cells. Direct evidence for the ACTH nature of the chromophobic cells was obtained in the present study by the administration of metopiron and hydrocortisone which were followed by cellular activation and inactivation respectively. Similar results were obtained by Leatherland (1972) and Nagahama (1973).

The fact that metopiron also affected the globular basophils of the proximal pars distalis does not, in our view, speak against the ACTH nature of the chromophobic cells. The globular basophils are gonadotropic beyond doubt (see below) and side effects of metopiron seem to be common. In *Anguilla anguilla* (Ball and Olivereau, 1966) and *Clarias* (Dixit, 1970) the TSH cells were affected by this drug.

TSH Cells. Despite a number of recent experimental studies on goldfish TSH cells there is still some controversy as to which cells are involved in TSH production. The most likely candidates are the basophilic cells of the rostral pars distalis and the non-globular basophils of the proximal pars distalis which are light microscopically indistinguishable. Originally TSH function was only attributed to the basophils of the rostral pars distalis because this appeared to be the only cell type that responded to radiothyroidectomy (Olivereau, 1962; Chavin *et al.*, 1962; Cukrowski and Chavin, 1964). Later these cells were also shown to be affected by goitrogens (Nagahama and Yamamoto, 1969; Nagahama, 1973). Leatherland (1972), on the other hand, did not mention a response of these cells to thiourea. The non-globular basophils of the proximal pars distalis were regarded as TSH cells because they were degranulated and hypertrophied after thiourea treatment (Nagahama and Yamamoto, 1969). According to Leatherland (1972) only some of these basophils were larger and apparently more active in thiourea-treated fish and

smaller and less active in thyroxine-treated goldfish when compared with controls. The extreme degranulation of the cells, as reported by Nagahama and Yamamoto (1969), was not confirmed by Leatherland and it is perhaps relevant to note that in a recent study Nagahama (1973) regarded only the basophils of the rostral pars distalis as TSH cells.

The results obtained in the present study indicate that indeed both cell types are involved in TSH production. Both cell types responded to thiourea, thyroxine and the synthetic mammalian releasing hormone TRF. That the non-globular basophils of the rostral and the proximal pars distalis are functionally very similar is also suggested by the fact that their granules have the same electron density. The differences in size of the secretory granules are perhaps not too surprising: TSH cells are known to be variable in structure (Schreibman *et al.*, 1973) and granule size has been shown to depend on the functional state of the cell (Hemme, 1972).

It has to be noted, however, that the two types of TSH cells exhibited clear differences in their response to experimentation. Some of the differences could be due to the fact that the drugs were administered differently and over different lengths of time. Thiourea was given for 15 days (Nagahama and Yamamoto, 1969), 4 weeks (Leatherland, 1972) or 5 to 30 days (Nagahama, 1973) whereas in the present study the animals were exposed to the drug for 3 to 12 weeks. Thyroxine was given intra-peritoneally in the present study but added to the aquarium water in Leatherland's (1972) investigation. But it seems that the TSH cells of the different regions of the pituitary gland do indeed respond differently. In the present study it was found that progesteron affected the TSH cells of the proximal pars distalis but not those of the rostral lobe. Seasonal changes, at the ultrastructural level, were only observed in the TSH cells of the proximal pars distalis and thiourea and thyroxine treatments increased the size of the secretory granules only in the TSH cells of the rostral pars distalis. These differences may be due to a different innervation pattern: the TSH cells of the rostral pars distalis are innervated by Type B fibres only whereas those of the proximal pars distalis are innervated by both Type A and Type B fibres (Kaul and Vollrath, 1974).

The finding that administration of TRF was followed by a striking degranulation of the TSH cells is most interesting from a comparative point of view, though its physiological significance is unclear. Since in the goldfish TSH function appears to be under inhibitory hypothalamic control, by means of a TIF (Peter, 1970, 1971, 1973), the above finding could be interpreted to mean that the TSH cells of teleosts, despite the absence of a hypothalamic TRF, are equipped with the same receptors for TRF as the TSH cells of mammals. The particular advantage of using a substance which is unphysiological for a teleost is that it makes possible a much quicker identification of the TSH cells than by using goitrogens which have to be administered over several weeks before clear-cut results can be obtained.

Gonadotropic Cells. Gonadotropic cells, usually localized in the proximal pars distalis, can be easily identified by their changes during the reproductive cycle (Ball and Baker, 1969; Sage and Bern, 1971) and it was these changes that led to their identification also in *Carassius* (Scruggs, 1951). Additional experimental evidence was obtained by heat induced spawning (Yamamoto and Yamazaki, 1967; Nagahama and Yamamoto, 1969), castration (Nagahama and Yamamoto,

1969; Nagahama, 1973), administration of oestradiol (Nagahama, 1973; and the present study) and, in the present study, by administration of oestradiol, progesteron and the synthetic mammalian hypothalamic releasing hormone LH-RF. The cell type unequivocally regarded as gonadotropic is the globular basophil of the proximal pars distalis. There is, however, still some controversy as to whether there is just one type of gonadotropic cell or two. Although two types have been described in a number of teleosts (Ball and Baker, 1969; Sage and Bern, 1971; Holmes and Ball, 1974) only one gonadotropic principle seems to be present in *Carassius* (Yamazaki and Donaldson, 1968; Sage and Bern, 1971). The results of the present study support the assumption of just one type of gonadotropic cell. The globular basophils were the only cell type that responded to LH-RF administration.

Additional evidence was obtained by oestradiol and progesteron administration, though it has to be noted that also the somatotropic cells were affected, which is in accordance with Nagahama's (1973) observations.

The conclusion that the small non-globular basophils of the proximal pars distalis, which are in fact TSH cells, represent a second type of gonadotropic cell is understandable if one considers that in teleosts a close functional relationship appears to exist between the gonads and the thyroid gland (Sage and Bromage, 1970). In the present study this point is illustrated by the observation that the TSH cells of the proximal pars distalis were affected by progesteron administration and the gonadotropic cells by administration of TRF. We have the suspicion that Leatherland's (1971) second type of gonadotropic cell, which was characterized by markedly fewer and smaller secretory granules and globular inclusions and narrower lacunae of the endoplasmic reticulum than the globular basophils, might represent a globular basophil gonadotrope in a different functional state.

The observation that administration of LH-RF led to a release of secretory granules from the gonadotropic cells is in accordance with the postulate that in the goldfish a releasing factor for gonadotropic hormone is present (Peter, 1970). It is also in agreement with the finding that in *Cyprinus carpio* administration of LH-RF was followed by a distinct increase in plasma gonadotropin (Breton and Weil, 1973).

STH Cells. In teleost fishes, STH cells are acidophilic and lie in the proximal pars distalis (Ball and Baker, 1969; Sage and Bern, 1971). Since there is just one type of acidophilic cell present in the proximal pars distalis of the goldfish, there can be no doubt that it is this cell which is involved in the production of growth hormone, in particular as the only other acidophilic cell type of the pituitary gland lying in the rostral pars distalis has been unequivocally identified as prolactin cell (Emmart, 1969).

Assessment of Electron Density. In our view the objective assessment of the electron density of the secretory granules provides an excellent additional method of characterizing and distinguishing different pituitary cell types. In the present study the assessment and the interpretation of the results was unnecessarily complicated because the work had to be carried out using an electron microscope which was not equipped with an automatic exposure timer. Clearly, with the illumination kept constant for all the photographs, directly comparable results can be obtained much more easily and—particularly important in a study of that

nature—also from different sections. From the results of the present study we are under the impression that, for obtaining relative figures for the electron density of the granules, variations in section thickness do not matter a great deal. Moreover, we think that with a sufficiently large amount of tissue sections investigated differences in section thickness should level out to make direct comparisons possible.

Our optimism about the application of this technique stems from the fact that the assessment of the electron densities yielded strikingly different results for the various cell types present within a relatively small tissue area where variations in section thickness and illumination were likely to be negligible. Not only did we detect that different cell types had characteristic frequency histograms of the electron densities of their respective secretory granules, but also that one cell type, namely the non-globular basophils of the rostral and the proximal pars distalis which used to be regarded as different cell types but turned out to be functionally identical, had granules of different sizes but of identical electron densities. We therefore suspect that in certain cases the objective assessment of the electron densities can even be superior to that of granule sizes.

Perhaps the most important result obtained is that the electron densities of the secretory granules of the different pituitary cell types in the goldfish followed a distinct pattern. Thus it was found that both types of acidophilic cells which produce the proteohormones prolactin and somatotropin respectively had granules with the highest electron density. The basophilic cells producing the glycoproteins gonadotropin and TSH respectively possessed granules of intermediate electron density whereas the chromophobic cells storing the peptide hormone ACTH had granules of lowest electron densities. This trend is clearly a most interesting and challenging one. Further studies are planned to examine whether the differences in electron density are due to (a) different affinities of the granules to heavy metal ions introduced during tissue processing, (b) differences in dry weight of the granules or (c) differences in molecular weight of the hormones contained in the granules.

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