Structure and Function of the Pituitary Gland in Gonadal Tumor-bearing and Normal Cyprinid Fish

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SUMMARY

The F₁ generation hybrids of carp and goldfish exhibited a marked hyperplasia of pars distalis basophil (gonadotrophic) cells, which first became evident in 3- to 4-year-old fish, the age at which carp and goldfish normally become sexually mature. Many of these hybrids also developed gonadal tumors, the genesis of which also coincided with the onset of gonadotroph proliferation. In these fish the basophil hyperplasia was more marked than in nontumorous hybrids. Tumor-bearing carp also exhibited gonadotroph hyperplasia similar to that in tumorous hybrids. Both tumor-bearing and nontumorous hybrids showed a marked reduction in gonadotroph cell number when fed a methallibure diet, although there was no apparent change in either the gross pathology or the histopathology of the tumors. These findings suggest that the hyperplasia of the gonadotrophs in tumorous fish is not, ipso facto, responsible for the maintenance of the tumors. On the contrary, since gonadotroph hyperplasia was evident in both tumorous and nontumorous hybrids (all of which were sterile), it may be symptomatic of the sterile condition of the fish rather than of the tumor per se. Evidence for the presence of a second gonadotrophic cell type in cyprinids is presented and discussed. Other pituitary cell types were similar in structure and ultrastructure in carp, goldfish, and hybrids and were unaffected by methallibure treatment.

INTRODUCTION

Our aquatic environments are known to be fouled with chemicals and classes of chemicals that have carcinogenic potential (6, 24, 25, 35, 41). This area has been excellently reviewed by Kraybill (18). Although there is little evidence that waterborne carcinogens have produced widespread cancer problems in man, it is not difficult to envisage such a possibility. At present, because of ground water depletion, many cities are processing for drinking purposes water that may be in excess of 30% recycled (9). Many inorganic and organic compounds (some known carcinogens) are not removed in current water treatment processes (5, 30). In fact, treatment may be producing carcinogens; i.e., chlorination may produce chloroform and carbon tetrachloride, both known carcinogens (3, 15, 33).

Of particular concern with waterborne carcinogens are the reports of Harris and coworkers (12, 31), which present epidemiological evidence suggesting a significant relationship between cancer mortality in white males and drinking water that was obtained from the Mississippi River. Although the results of the New Orleans study cannot be considered conclusive evidence that cancer in man is caused by the consumption of contaminated water, these findings must be taken into consideration.

Recently, the technology for the quantitation and qualification of dilute organic and metallic agents in water has been rapidly improved (34). However, the problems are complex, and the need for surveillance is acute. It is increasingly apparent that investigations must be addressed to waterborne factor(s), the existence and effects of which on animal health are unknown. A sentinel system for the early detection and identification of waterborne environmental carcinogens is urgently needed. As an approach to this situation, we have made a survey monitoring the frequency of occurrence of fish tumors in polluted and nonpolluted waters in the Great Lakes to explore the potential utility of neoplasia in feral fish as an indicator of environmental carcinogens (36-38, 40). Epizootics of gonadal tumors in carp (Cyprinus carpio), goldfish (Carassius auratus), and goldfish-carp hybrids were found, and they appear to have environmental relevance (36-38, 40). The tumors appear to be of a Sertoli cell origin, although some may also contain Leydig cell elements (40). The tumor incidence is as high as 100% in some age-sex groups (40).

One of the characteristics of the gonadal tumor-bearing fish and also of nontumor-bearing (sterile) hybrids is a massive proliferation of pituitary basophils (40). In this paper we report investigations undertaken to elucidate the pituitary-gonadal feedback axis in these fish. In addition, the effect of methallibure on the number and apparent activity of the gonadotrophs was examined. In teleosts methallibure appears to act as a gonadal inhibitor by blocking gonadotropin synthesis or release (13, 19, 32), possibly by inhibiting the synthesis or release of hypothalamic releasing factors (26). Methallibure was given to tumor-bearing hybrids to determine whether the proliferation of gonadotrophs evident in these fish can be repressed in the same way that it is in other methallibure-treated teleosts. It was hoped that these experiments might provide an insight into the cause(s) of the gonadotroph hypertrophy.

MATERIALS AND METHODS

Collection and Maintenance of Fish. One hundred and
two fish were used in the study of pituitary histology, including 32 carp (19 males and 13 females), 83 hybrids (38 males, 23 females, and 2 hermaphrodites), and 7 goldfish (4 males, 2 females, a 1 hermaphrodite). A further 41 fish (19 males, 19 females, and 3 of indeterminate sex) were used to determine the effect of methallibure on pituitary histology, and 39 5- to 6-year-old fish were used for measurements of pituitary weight. Fish were collected by gill nets from Hamilton Harbour, Lake Ontario. Some were biopsied as they were removed from the nets, some were returned to Guelph for biopsy, and others (to be treated with methallibure) were returned to Guelph and acclimated for 4 to 10 weeks in 150-gallon aquarium of running well water. The age of each fish was determined at biopsy by scale ring counts.

Methallibure Treatment. Methallibure was administered to hybrids in 2 separate experiments. In the 1st experiment hybrids that displayed a distinct tumorous condition, evidenced by a distended abdomen, were chosen. They were maintained in the laboratory in continuously running and aerated well water. They were fed daily with a pelleted fish diet to which had been added methallibure (I.C.I. 33,838). A known amount of methallibure-treated diet was given so that, assuming equal food consumption, each fish received 1 mg of methallibure per kg of body weight each day. In the 1st experiment a group of 12 fish was administered the diet for 6 weeks, and the pituitaries were fixed for light microscopy. Pituitaries from 5 control fish, which had been fed a diet identical with that of the methallibure-treated fish except that methallibure had been omitted, were also fixed for light microscopy.

In the 2nd experiment sterile and tumor-bearing hybrids were used. A group of 5 fish was randomly selected as the initial control group, and the remainder of the fish were administered the methallibure diet. Random selections of 3 to 4 fish were made 3, 5, 10, 15, 20, and 35 days after first giving the methallibure diet. The pituitary from each fish was preserved for light microscopy.

Histology. Each fish was killed by transection of the spinal cord, and the pituitary was rapidly dissected from the skull, cut into halves in the sagittal plane, and placed into Bouin's Hollande sublimate. The tissues were dehydrated in alcohol, embedded in Paraplast, and sectioned at 5 μm. The sections were stained with 1 of the following procedures: AB-PAS-OG, Herlant's tetrachrome, Cleveland-Wolf trichrome, Mallory's trichrome, or Gabe's aldehyde fuchsin.

Pieces of partes intermedia and distalis were taken from 5 nontumorous carp, 5 nontumorous goldfish, 5 tumorous hybrids, and 2 tumorous carp for examination by means of an electron microscope. The pieces were fixed overnight in ice-cold 5% cacodylate-buffered (pH 7.4) glutaraldehyde. They were then postfixed in cacodylate-buffered (pH 7.4) 1% osmium tetroxide, dehydrated in acetone, and embedded in Epon. Sections were mounted on uncoated copper grids, stained with uranyl acetate and lead citrate, and examined on a Philips 200 electron microscope.

Pituitary Weights. Pituitary glands were dissected from 39 freshly killed carp and hybrids, fixed for 48 hr in Bouin's fluid, transferred to 70% alcohol for 48 hr, and weighed. The data were compared by 1-way analysis of variance. Individual means were compared by least significant difference.

RESULTS

Adult (>4 Years Old) Nontumorous Carp and Goldfish. The structure of the pituitary gland was essentially similar in nontumorous carp and goldfish (Figs. 1 and 4). The organization and cellular characteristics of the cell types closely resembled the descriptions of goldfish pituitary given by other authors (2, 7, 16, 20, 21, 23, 28, 35). The rostral pars distalis (Figs. 1, 2, and 11) formed a thin anterior cap, which extended dorsally from the dorsoanterior region of the pituitary stalk and ventrally to meet the most anterior regions of the pars intermedia. The rostral pars distalis region was composed of 4 cell types, an acidophil, an amphiphil (possibly a chromophobe), and 2 basophils. The acidophils [prolactin cells (11)] were spherical or elongated cells with spherical nuclei and prominent nucleoli. The cytoplasm was poorly granulated; the granules stained deep red with erythrogin but only pale orange with OG (Fig. 2). The amphiphilic cell type [probably ACTH (16, 29)] was interspersed with the acidophils and, together with the acidophils, composed the major component of this region of the gland. The amphiphils were conversely elongated cells with oval or spherical nuclei but with no prominent nucleoli. Their cytoplasm contained little stainable material and appeared chromophobic with the stains used here.

In electron micrographs the prolactin (Figs. 14 and 16) and ACTH cells (Figs. 15 and 16) were similar in appearance. Both contained numerous membrane-bound electron-dense cytoplasmic granules, and both showed signs of moderate secretory activity, as evidenced by their content of mitochondria, endoplasmic reticulum, and Golgi bodies. The cells differed in the diameter of the cytoplasmic granules, those in the prolactin cells being larger than those in the ACTH cells (Figs. 14 to 16) (16, 20). Interspersed among the prolactin and ACTH cells were nongranulated cells with moderately electron-dense cytoplasm (Fig. 17).

The 2 basophil cell types were present in small numbers. One type (Fig. 2), the presumptive thyroid-stimulating hormone cells (type 2 basophils) (16, 20), were small angular...
Pituitary Gland Structure and Function in Cyprinid Fish

Fig. 2. a, part of the rostral pars distalis in a hybrid, showing a group of type 2 basophils (thyrotrophs) (arrows) among the pale prolactin and ACTH cells; these 2 cell types cannot be distinguished in the preparation. Paraffin section, AB-PAS-OG, x 270. b, cyst, probably granuloma reaction to parasitic or bacterial infection, in the proximal pars distalis of a hybrid. Paraffin section, Cleveland-Wolfe, x 270.

The proximal pars distalis (Figs. 1 and 6) formed the largest component of the pars distalis. The region was composed of 4 cell types, an acidophil and 3 basophils. The acidophils of the proximal pars distalis (Fig. 6), probably somatotrophs (16, 20), were also found as small groups of cells. They were large cells with irregularly shaped cytoplasm. The cytoplasmic granules were only weakly erythrosinophilic but tended to be more orangeophilic than the prolactin cells. The nuclei were round or oval, with moderately prominent nucleoli. In electron micrographs the somatotrophs (Fig. 20) were seen to contain numerous rod-shaped, membrane-bound, electron-dense cytoplasmic granules, together with moderate numbers of mitochondria and Golgi bodies. Certain regions of cytoplasm were composed almost wholly of whorls of endoplasmic reticulum (Fig. 19).

Basophil cell types 1 and 3 (Figs. 6 and 7) were found in the form of lobes, with the type 3 cells in the center of a type 1 cell group. Type 1 cells were large spherical or oval cells, which in many fish contained a prominent, large vacuole occupying approximately 50% of the cell cytoplasm. The remainder of the cytoplasm contained coarse granules, which stained intensely with AB, aniline blue, and alizarin blue. There was a marked variability in the vacuolation of these cells in different fish, which could not be correlated with age, sex, or season of capture. The type 1 basophils are very similar in appearance to the cells in young goldfish that were previously termed gonadotrophic (16, 20, 22, 27). In electron micrographs these cells contained a number of types of cytoplasmic inclusions (Figs. 21 to 25). The most numerous were spherical electron-dense, membrane-bound granules (diameter, 3000 to 5000 Å), which were scattered throughout the cytoplasm. Some of these granules were less electron dense than others and may be undergoing structural changes (Fig. 24). The cells also contained larger (diameter, 1 to 2 μm) membrane-bound spherical droplets of varying electron density, from very electron dense to virtually electron translucent (Fig. 22). The "paler" droplets commonly contained fibrillar inclusions (Figs. 22 and 23). The presence of droplets containing an electron-dense core with peripheral fibrillar organelles suggests that 1 form of the droplet is derived from the other. In addition to the granules and droplets, these cells also contained cytoplasmic vacuoles, which formed the large organelles evident in light micrographs; they contained an amorphous, granular matrix (Figs. 21, 22, 24, and 25). Some of these type 1 basophils had a cytoplasmic matrix that was more electron dense than other cells; these may represent degenerating cells (Fig. 25).

Type 3 basophils were smaller cells than the type 1 cells, usually grouped together in small clusters (Figs. 6 and 7) or, less commonly, scattered as single cells throughout the proximal pars distalis. Their cytoplasm was more finely granulated and stained less intensely than that of type 1 cells; the cytoplasm commonly appeared flocculated due to its content of small vacuoles. In some fish the vacuoles contained intensely PAS- or aniline blue-stainable rod-shaped bodies, but these were not present in all fish. In electron micrographs these cells were similar in general appearance to the type 1 basophils but differed in a number of ways. Firstly, the electron-dense granules were smaller (diameter, 1500 to 2000 Å, compared with 3000 to 5000 Å in cells, mostly situated toward the periphery of the gland or in the median part of the rostral pars distalis. The heavily granulated cytoplasm stained moderately with AB, aniline blue, and alizarin blue. In electron micrographs (Fig. 18), the cells were identical in appearance with the thyrotrophs described in goldfish previously (16, 20). The 2nd basophil type found in the rostral pars distalis was identical with the type 1 basophil cells of the proximal pars distalis (see below).

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the type 1 cells) and tended to be grouped together into clumps of granules rather than scattered, as they were in the type 1 cells (Fig. 24). Secondly, the droplets were fewer in number in the type 3 cells. Thirdly, the type 3 cells did not have the large vacuoles evident in the type 1 components; in the former the vacuoles took the form of scattered, smaller, multiple vacuoles (Fig. 24). The rod-shaped organelles present in light micrographs were not identified in electron micrographs. In some fish several of the globules (and possibly the granules also) had fused to form large electron-dense masses (Fig. 26), which may account for the light microscope observations.

The 3rd basophil cell type was similar in every way to the putative thyroid-stimulating hormone cells also found in the rostral pars distalis. In electron micrographs a further cell type, a nongranulated cell, was evident in all regions of the pars intermedia of some older (10 + years) carp (Fig. 20). A smaller number of type 3 gonadotrophs were present in the pars intermedia of some younger specimens.

Nontumorous Hybrids. In hybrid fish between 1 and 4 years old that showed no evidence of gonadal tumors, the pituitary and gonad were identical in morphology with those in carp and goldfish of similar ages [see above; also see Leatherland (20) for literature review].

In nontumorous hybrids older than 4 years (these fish were sterile and the gonads were small), there was a hyperplasia of the basophil population of the proximal pars distalis that was similar to, but less extensive than, that found in tumor-bearing hybrids (see below). The pituitary weight of nontumorous hybrids was considerably smaller than in the older fish. The large vacuoles, which were characteristic of type 1 basophils in larger animals, were only rarely found in the younger specimens.

Tumor-bearing Hybrids, Goldfish, and Carp. The pituitary in tumor-bearing fish was commonly 2 or 3 times larger than that in nontumorous goldfish and carp (compare Fig. 5 with Fig. 4). Pituitary weights in tumor-bearing hybrids and hybrids were significantly greater than in nontumorous carp (p < 0.01) (Table 1). The increase in volume was so marked that, in nontumorous hybrids older than 4 years (these fish were sterile and the gonads were small), there was a hyperplasia of the basophil population of the proximal pars distalis that was similar to, but less extensive than, that found in tumor-bearing hybrids (see below). The pituitary weight of nontumorous hybrids was considerably smaller than in the older fish.

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### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Pituitary wt (mg)</th>
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<tbody>
<tr>
<td>Nontumorous carp</td>
<td>8</td>
<td>11.6 ± 3.4*</td>
</tr>
<tr>
<td>Tumorous carp</td>
<td>6</td>
<td>28.1 ± 3.1</td>
</tr>
<tr>
<td>Nontumorous carp</td>
<td>6</td>
<td>19.4 ± 3.0</td>
</tr>
<tr>
<td>Tumorous hybrids</td>
<td>19</td>
<td>27.9 ± 2.1</td>
</tr>
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* The gland was dissected from each fish and fixed in Bouin's fluid for 48 hr, transferred to 70% alcohol for 48 hr, blotted dry, and weighed.
* Mean ± S.E.
body weight. However, in tumorous fish, the rostral pars distalis cells formed a narrow zone of cells bordering the periphery of the proximal pars distalis (Fig. 5). The structural and ultrastructural characteristics of the various cell types were indistinguishable from those in nontumorous carp and goldfish. The proximal pars distalis was markedly increased in volume, apparently due to an increase in the number of type 1 and type 3 basophils (Figs. 5 and 8). There appeared to be no difference in the cytological or ultrastructural characteristics of the somatotrophs and thyrotrophs, nor of the type 1 or type 3 basophils, but both basophil cell types were vastly increased in number. Whereas, in the nontumorous carp and goldfish, the type 1 cells surrounded the type 3 cells, in the tumorous fish there were areas of the proximal pars distalis that contained only either type 1 or type 3 basophilic cells. In addition central areas of the pituitaries in tumorous fish were necrotic.

Methallibure-treated Hybrids. In the hybrids in both Experiments 1 and 2, fed a methallibure diet for periods greater than 25 days, type 1 and 3 basophils were both greatly reduced in number (Fig. 9). In light and electron micrographs, the basophil type 1 and 3 cells were similar in general appearance in methallibure- and nonmethallibure-treated fish, except for the extent of vacuolation of the type 1 basophils, which was less in the former group. At the end of Experiment 2, 1 fish was found to be a carp; the reduction in number of basophils was more marked in this fish (Fig. 10) than it was in the hybrids. Other pituitary cell types did not seem to differ, either in number or appearance, from comparable cells in untreated animals. The pituitary gland in treated hybrids was generally smaller and firmer to the touch than in untreated animals. In hybrids given the methallibure diet for periods less than 25 days, the response of type 1 and 3 basophils was variable. Some fish demonstrated a reduction in cell number, whereas others did not. In Experiment 1, in which only markedly tumorous fish were used, there was no evidence of gross or histopathological changes in the gonads of methallibure-treated fish compared with control hybrids.

DISCUSSION

This study shows a marked hypertrophy of proximal pars distalis basophils in tumor-bearing cyprinids and also in nontumorous goldfish hybrids, resulting in a marked increase in pituitary weight and volume. Since, in teleosts, gonadotrophic cells appear to be under stimulatory hypothalamic control (1), the proliferation of the gonadotrophs in tumorous fish is probably due to continued hypothalamic stimulation as a result of the low sex steroid levels in these fish (J. Sumpter, J. F. Leatherland, and R. A. Sonstegard, unpublished data). However, paradoxically, the gonadotroph hyperplasia does not result in an increase in plasma GTH levels (E. Tan, J. F. Leatherland, and R. A. Sonstegard, unpublished data). Thus, in hybrids older than 4 years (both tumorous and nontumorous) and in tumorous carp and goldfish, the negative feedback system, which is thought to regulate the pituitary-gonad axis in teleosts, is either impaired or inoperative.

The commencement of gonadotroph proliferation in the carp-goldfish hybrids is concomitant with the normal onset of maturity in carp and goldfish. This also coincides with the age at which the tumor frequency begins to increase markedly, which suggests that the gonadal tumors may be induced by hypersecretion of the hypothalamus-pituitary axis. However, since the proliferation of gonadotrophs does not result in an elevation of plasma GTH (see above), the effect of hypothalamic stimulation in the hybrids may be to induce the differentiation of gonadotroph cells rather than of GTH release. Conversely, if the hypothalamic factor stimulates both gonadotroph cell differentiation and GTH release in carp and goldfish, the gonadotrophs in hybrids may be unable to release GTH. This may well give rise to a continued production of the hypothalamic factor(s), since there is no negative feedback inhibition.

Methallibure caused a marked reduction of pituitary basophils in hybrid fish. The drug inhibits gonadal development in cyprinids (13), probably by inhibiting gonadotrophin secretion at the pituitary (19) or hypothalamic level (26). That methallibure caused such a marked regression in the gonadotrophs without apparently affecting the histopathology of the tumor suggests that the onset of tumor proliferation, although coincident with the onset of gonadotroph proliferation, is not brought about by the increased gonadotroph activity. Further evidence for this comes from the nontumorous hybrids. The latter have a gonadotroph population similar to that of the tumorous fish but are, in fact, sterile. It is probable that the gonadotroph proliferation in both tumor-bearing and nontumorous hybrids is a response to the sterile condition of the fish rather than to the tumor, per se.

The structure of the rostral pars distalis, pars intermedia, and pars nervosa in carp, goldfish, and hybrids was essentially similar to the descriptions given for goldfish previously (see “Results” for references). There was a marked variation in the appearance of the pars intermedia cells between groups, which could not be correlated with species or tumors but which may well be due to the variation in pigmentation of the specimens used, since this was found to affect pituitary cytology in goldfish (21). The significance of the basophilic pars intermedia (PAS-negative) cells in young hybrids is not known.

The formation of follicles of pars intermedia cells surrounding a colloid-filled lumen, which is found in older cyprinids, has not, to our knowledge, been reported previously in fish, although these structures are commonly found in mammals [e.g., humans (14) and seals (22)]. Their significance and function is not known.

In this study of carp, goldfish, and their hybrids, as in the earlier study of the pituitary in goldfish (20), 3 basophil cell types were found in the proximal pars distalis. One cell type probably represents a thyrotrophic cell (basophil type 2), whereas the other 2 are probably gonadotrophic (types 1 and 3). Evidence for gonadotrophic roles for both these cells is provided by methallibure-treated fish in which both cell types were partly diminished in a manner similar to that found in other species treated with methallibure (19, 32). Kaul and Vollrath (16) were able to identify only 1 putative gonadotrophic cell type, the description of which corresponds to the type 1 basophil reported here. The differences in observations are difficult to explain, since in both
this study and that of Kaul and Vollrath (16) mature fish were used. It is possible that the discrepancies between the observations described here and those of Kaul and Vollrath (16) were due to the different sizes of fish used in the 2 studies. Kaul and Vollrath used specimens of unknown age, 18 to 20 cm in total length, whereas specimens up to 12 years old and 60 to 70 cm in total length were used in this study. Type 3 basophils differ in size, position within the gland, and several cytoplasmic characteristics, including granule diameter, vacuolation, and the presence of the PAS- or aniline blue-stained rod-shaped bodies. These differences provide strong evidence for separate cell types. This hypothesis may be supported by findings in salmonoid species, in which only 1 gonadotroph has been described during the major portion of the life cycle, but where a 2nd gonadotroph type becomes evident in the sexually mature fish (4, 8, 10, 29, 39).

The ultrastructural appearance of the somatotrophs in these large cyprinids gave every impression of a high secretory activity. This is surprising in light of the slow growth rate of the older fish. These observations strongly suggest that the somatotrophs are involved in processes other than growth regulation throughout the animal's whole life history; a similar idea was recently proposed by Komourdjian et al. (17).

Methallibure acts as a goitrogen in some fish (13, 32). No marked effects of methallibure were found in the thyrotrrophs. This finding agrees with that of others (13), who showed that cyprinids are less susceptible to the goitrogenic action of methallibure than other species. Certain teleost fishes may be particularly suited for monitoring levels of effects of environmental carcinogens, particularly of carcinogens that cause endocrine dysfunction. The separation of the teleost pars distalis into 2 distinct zones (each of which contains functionally different cell types) permits a relatively easy identification of adenohypophysial cell types, compared with other vertebrates. Moreover, because of the process of biological magnification, fishes are often exposed to waterborne environmental insults of an order higher than those to which other animals are exposed and to which they may be unusually responsive.

ACKNOWLEDGMENTS

We are indebted to L. Lin, B. Hicks, and M. Burke for their technical help.

REFERENCES


**Fig. 3.** a, part of the neurointermediate lobe in a hybrid, showing 2 pars intermedia cell types, 1 of which is stained with PAS (small arrow). Note the penetration of pars nervosa tissue (large arrow) among the strands of intermedia tissue. Paraffin section, AB-PAS-OG, × 270. b, colloid-filled follicles (F) in the pars intermedia tissue of a 9-year-old hybrid. Paraffin section, Cleveland-Wolfe, × 270.

**Fig. 4.** Parasagittal section (10 to 20 μ to 1 side of the midline) of a pituitary gland in a 6-year-old nontumorous carp. Note the rostral pars distalis (R) and neurointermediate lobe (N) on either side of the proximal pars distalis (P). The latter is made up of more or less equal numbers of basophils (dark cells) and somatotrophic acidophils (pale cells). Paraffin section, AB-PAS-OG, × 23.

**Fig. 5.** Section in a region of the pituitary similar to that in Fig. 4, showing pituitary gland in a 6-year-old tumor-bearing hybrid. The pale rostral pars distalis cells (arrows) now form a thin layer of the periphery of the greatly enlarged proximal pars distalis (P). The latter is composed, almost in its entirety, of gonadotroph cells. N, neurointermediate lobe. Paraffin section, AB-PAS-OG, × 23.

**Fig. 6.** Part of the proximal pars distalis in a nontumorous carp, showing groups of pale acidophils (somatotrophs) interspersed with darker basophils (gonadotrophs). The clumps of basophils are divided into a central region of smaller cells (small arrows) surrounded by a ring of larger, more coarsely granulated cells (large arrows). Paraffin section, AB-PAS-OG, × 245.

**Fig. 7.** Enlarged area of a clump of proximal pars distalis basophils, showing PAS-stained rod-shaped bodies (arrows) in the small basophils. Paraffin section, AB-PAS-OG, × 290.

**Fig. 8.** Part of the proximal pars distalis in a tumorous carp. The larger gonadotrophs (which are vacuolated in this specimen) occupy much of the region: the clumps of acidophils (A) occupy only small areas compared with nontumorous fish (Fig. 6). Paraffin section, Cleveland-Wolfe, × 290.

**Fig. 9.** Part of the proximal pars distalis in a methallibure-treated hybrid. Note the marked reduction in the population of gonadotrophs compared with Fig. 8. A, acidophils (somatotrophs). Paraffin section, AB-PAS-OG, × 230.

**Fig. 10.** Region similar to Fig. 9 in a methallibure-treated carp, showing the marked reduction in gonadotroph population compared with Fig. 6. A, acidophils (somatotrophs). Paraffin section, AB-PAS-OG, × 290.

**Fig. 11.** Sagittal section of the pituitary in 2-year-old hybrid. The section shows the separation of the zones of the pituitary, R, rostral pars distalis; P, proximal pars distalis; PI, pars intermedia; NH, neurohypophysis (pars nervosa). There is no enlargement of the proximal pars distalis at this stage. Paraffin section, AB-PAS-OG, × 100.

**Fig. 12.** Enlarged area of the neurointermediate lobe in Fig. 11. The darkly stained cells (arrows) are stained with AB and represent the non-PAS-staining cells in Fig. 3. The PAS-stained cells appear light grey in the micrograph. Paraffin section, AB-PAS-OG, × 190.

**Fig. 13.** Enlarged area of proximal pars distalis in Fig. 11. Dark-staining basophils and clear acidophils (A) are apparent, but there is no sign of the proliferation of the gonadotrophs found in older hybrids. Paraffin section, AB-PAS-OG, × 190.

**Fig. 14.** Prolactin cell of the rostral pars distalis of a nontumorous fish. Note the content of electron-dense cytoplasmic granules and active Golgi complex. × 10,580.

**Fig. 15.** ACTH cells in the same animal as in Fig. 14. Note the smaller granules in this cell compared with the prolactin cell in Fig. 14. The ACTH cells commonly abut the basement membrane (M), which separates the rostral pars distalis and neurohypophysis (NH). × 10,580.

**Fig. 16.** Area of rostral pars distalis in nontumorous carp, showing ACTH cell (A) and prolactin cell (P) adjacent to the electron-dense basement membrane complex, which separates the neurohypophysis (NH) from the rostral pars distalis. × 10,580.

**Fig. 17.** Nongranulated cell in nontumorous carp among a group of prolactin cells. × 10,580.

**Fig. 18.** Rostral pars distalis cells in nontumorous carp, showing a type 2 basophil (thyrotroph) (large arrow) and 2 type 1 basophils (small arrows) adjacent to a somatroph. × 10,735.

**Fig. 19.** Similar to Fig. 20, showing the extensive endoplasmic reticulum in somatotrophs. × 10,175.

**Fig. 20.** Part of 2 somatotrophs, showing the rod-shaped electron-dense granules of these cells. × 10,735.

**Fig. 21.** Part of a type 1 basophil in a nontumorous carp, showing the large vacuole (V) in these cells. Note also the electron-dense cytoplasmic inclusions and the larger, less electron-dense droplets. × 13,500.

**Fig. 22.** Similar to Fig. 21, showing the small electron-dense granules (which vary in electron density) and a larger droplet adjacent to a vacuole. In addition, membrane-bound organelles filled with a fibrous matrix are present. × 17,730.

**Fig. 23.** Similar to Figs. 21 and 22, showing "fibrous" organelles, which also contain a core of material similar to that found in the droplets. × 13,575.

**Fig. 24.** Adjacent basophil cell types 1 and 3. Note that the electron-dense granules in type 1 cells (f) are markedly larger than in type 3 (III), and also that the cell vacuolation in type 3 cells takes the form of small clumps of vacuoles rather than the single large vacuole (V) of the type 1 cells. × 13,470.

**Fig. 25.** Apparently degenerating vacuolated type 1 basophil cell (V) adjacent to a nongranulated cell (NG) in the proximal pars distalis of a nontumorous carp. × 10,971.

**Fig. 26.** Globular inclusion in the cytoplasm of a basophil type 3 cell of a tumor hybrid. × 17,440.

**Fig. 27.** Pars intermedia cell corresponding to the non-PAS-staining cell in light micrographs. Note the variation in electron density of the cytoplasmic granules and the marked formations of endoplasmic reticulum. × 10,580.

**Fig. 28.** Similar to Fig. 27, showing cells corresponding to PAS-staining pars intermedia (PI) cells. NH, neurohypophysis. × 10,580.
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