A Histochemical Investigation of the Pituitary Glands of some Teleost Fish

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With two plates (figs. 2 and 3)

SUMMARY

Some histochemical observations on the pituitaries of three marine and three fresh-water teleost fish have been made. It has been shown that cells of this gland, which are stained identically in different species by Azan, do not always give identical histochemical reactions, and that in the same species cells which after histological staining have similar tinctorial affinities may be differentiated histochemically. These observations are discussed with regard to the function of the gland.

INTRODUCTION

THE histology of the pituitary of teleost fish has been described by a number of authors (Kerr, 1942a, b; Green, 1951; Olivereau, 1954a, b), but these descriptions have added little to the knowledge of the histophysiology of the gland and little is known of the sites of hormone production. Histological studies have indicated that the so-called basiphils or cyanophils are concerned with the elaboration of the gonadotrophic hormone (Rasquin, 1949; Scruggs, 1951), while a second cyanophil type has been identified as a thyrotroph in Astyanax mexicanus (Atz, 1953) and in Phoxinus laevis (Barrington and Matty, 1955). Both basiphils and acidophils have been implicated in the release of ACTH by the teleost pituitary (Rasquin and Stoll, 1955; Chavin, 1956), and acidophils with the release of somatotrophin (Olivereau, 1954b). However, except for the use of the periodic acid/Schiff (PAS) reaction there has been no histochemical investigation of the fish adenohypophysis. Furthermore, as it has recently been shown that the acidophils of the pituitary—generally assumed to be PAS-negative—may be PAS-positive in fish (Matty, 1957), it was thought that a general cytochemical investigation of the pituitaries of a number of teleost fish was desirable in order to provide an additional basis for further investigations of the histophysiology of this gland.

MATERIALS AND METHODS

The pituitaries of three marine teleost fish, the rainbow parrot fish Pseudoscarus guacamaia, the mud-belly Scarus croicensis, and the Bermuda white grunt Bathystoma aurolineatum, and three fresh-water fish, the perch Perca fluviatilis, the roach Rutilus rutilus, and the minnow Phoxinus laevis were
examined. Glands from all the above fish were placed in formaldehyde-saline and in Heidenhain's mercuric chloride / formalin. Other fixatives employed will be referred to later. Material from the minnow was decalcified in 5% versene (Schajowicz and Cabrini, 1956). After overnight fixation and washing in running water, next day both paraffin and gelatin sections were prepared and examined after histochemical and routine histological staining methods (Heidenhain's Azan and Heidenhain's iron haematoxylin with orange G counterstain). In addition the aldehyde-fuchsin method of Gomori (Halmi, 1952), the chrome alum / haematoxylin and phloxine method of Gomori as modified by Bargmann and Hild (1949), and the pyronin / methyl green method for RNA (Brachet, 1953) were applied.

The following histochemical methods were used:

**Method for the detection of carbohydrates**

PAS test (McManus, 1946) with the acetylation technique of McManus and Carson (1950). In addition to the fixatives quoted above, material fixed in Bouin was also examined by this method.

**Methods for the detection of amino-acids**

Demonstration of tyrosine by a modification of the Morel–Sisley diazotization procedure (Lillie, 1957).

Millon’s test for tyrosine as described by Pearse (1953).

Demonstration of cystine and cysteine by the performic acid / Alcian blue method (Adams and Sloper, 1956).

Thioglycollate-ferricyanide reaction for sulphhydril groups (Adams, 1956).

Dihydroxydiphenyl-disulphide (DDD) reaction for disulphide and sulphdryl groups (Barrnett and Seligman, 1954).

Coupled tetrazonium reaction of Danielli (1947, 1950), the technical details being those described by Pearse (1953).

Iodination / coupled tetrazonium reaction for histidine (Landing and Hall, 1956).

Sakaguchi’s 1925 test for arginine, according to the method adapted to histochemical use by Baker (1947).

**Methods for the detection of lipids**

Gelatin sections fixed as above and also after formaldehyde-calcium and postchroming (Baker, 1949). Some sections were stained in Sudan black B and others in Fettrot (Pearse, 1953).

Baker’s (1946) acid haematein and pyridine extraction test for phospholipids.

Copper phthalocyanin method for phospholipid according to Klüver and Barrera (1953).

**Observations**

As the microanatomy of the pituitaries of *Pseudoscarus guacamaia, Scarus croicensis, and Bathystoma aurolineatum* has not been described, their structure after Azan staining must be first outlined (fig. 1). None has a definite stalk;
that is, they are of the platybasal form, and in shape all are similar to that of the perch (fig. 1, E), having an elongated anterior region and a rounded posterior region. The neurohypophyses are well developed, and in all three species the adenohypophysis, which may be divided conventionally into the

![Diagram of pituitary glands](image)

**FIG. 1.** Diagrams of median longitudinal sections through the pituitary glands of: A, Rutilus rutilus; B, Bathystoma aurolineatum; C, Phoxinus laevis; D, Pseudoscarus guacamaia; E, Perca fluviatilis; F, Scarus croicensis; showing the different cell types after staining with Azan. The distribution of aldehyde-fuchsins positive (AF+) cyanophils (thyrotrophs) is also superimposed on these diagrams.

posterior, median, and anterior glandular zones, surrounds and interdigitates with the neurohypophysis.

Both *Scarus* and *Pseudoscarus* have an anterior glandular zone comprised of a cap of small chromophobes which have immediately posterior to them a region of small amphiphils which contain little cytoplasm. In addition *Scarus* has an anterior-ventral layer of small cells that stain with orange G. The median glandular zone in *Pseudoscarus* consists dorsally of cells staining brightly with orange G; these form a continuous sheet at the boundary with
the neurohypophysis and intermingle ventrally with amphiphils. Along the latero-ventral border of this zone are found patches of cyanophils (the basiphils ('basophils') of earlier authors), of two kinds; small cells staining lightly with aniline blue and larger deeply staining cells with slightly granular cytoplasm and large vacuoles. The posterior half of this zone is composed of very large polygonal orange G cells interspersed with a few very small amphiphils. The median glandular region of *Scarus* is similar except that the sheet of cells at the anterior dorsal border are weakly cyanophil; they do not stain brightly with orange G. Both species have similar posterior glandular zones consisting of small amphiphils and a few dull blue cyanophils (fig. 1, D, F).

*Bathystoma* has a well-marked large anterior glandular zone composed of chromophobes. The median glandular zone has sheets of deeply staining orange G cells adjacent to the neurohypophysis; these extend as strands into the more ventral, predominantly cyanophil region. The cyanophils are of two well-defined types. Patches of cells staining a very light blue lie amongst cells with a deep blue granular cytoplasm; the latter extend posteriorly round the outer edge of the adenohypophysis. The posterior glandular zone is partially enclosed by these deep blue cyanophils of the median glandular zone, which itself encloses the posterior ramifications of the neurohypophysis. The cells lying against the neurohypophysis are weakly staining cyanophils, and between these cyanophils and the peripheral median glandular cyanophils is found a region of chromophobes (fig. 1, B).

The histology of the pituitaries of *Phoxinus*, *Rutilus*, and *Perca* has been described in a similar manner by Kerr (1942). Diagrams of the glands of all 6 species after staining with Azan are shown in fig. 1, and hereafter the nomenclature of the figure will be used in the text. The type 3 cyanophils of *Rutilus* and *Bathystoma* are identified as islands of small pale blue AF-negative cells, but this does not preclude their possible occurrence in other animals examined where small cyanophils appear scattered among larger areas of type 1 cyanophils, nor is it impossible that these type 3 cells are precursors of the type 1 cells.

**Aldehyde-fuchsin reaction.** The distribution of cells positive to aldehyde-fuchsin (AF-positive cells) has already been described for the minnow pituitary, and these cells have been identified as TSH-secreting (Barrington and Matty, 1955). Similar cells are identified in all other teleost glands so far examined and their distribution is shown in fig. 1. These cells are always cyanophil and PAS-positive, and are characteristic of the median glandular zone. Furthermore in *Pseudoscarus* these cells seem to be invariably vacuolated (fig. 2, A). In *Rutilus* it appeared to be this type of cell in which the cytoplasm became vacuolated and nucleus pycnotic after immersion in the anti-thyroid drug, thiourea (fig. 2, B). This observation was made on two groups of fish that had been immersed in a $0.1\%$ thiourea solution for periods of 53 and 151 days and provides further evidence that it is the AF-positive cell of the median glandular zone of the teleost pituitary which is responsible for the production of TSH.
Chrome alum / haematoxylin and phloxine procedure. The neurosecretory material of the neurohypophysis was stained by this procedure in all species examined. In all pituitaries, however, there appeared to be a marked tendency for this material to be concentrated in that part of the neurohypophysis which is in intimate contact with the posterior glandular zone of the adenohypophysis; little chrome-haematoxylin positive substance was ever found in the nervosa lying between the cells of the anterior glandular zone.

The type 1 cyanophils of all the species were chrome-haematoxylin positive (fig. 3, d, opposite p. 263), and in Rutillus the occasional cyanophil found in the anterior glandular zone of carminophils also stained by this procedure. However, the type 2 cyanophils of the posterior glandular zone of Phoxinus, Perca, and Bathystoma were not stained by this procedure. The AF-positive cyanophils of all species were intensely chrome-haematoxylin positive.

Pyronin G / methyl green procedure. RNA was present in the nucleoli of all cells of the adenohypophysis but few of the different Azan cell types showed any visible difference in amounts of extra-nucleolar RNA or in nucleolar size. In Rutillus, however, the chromophobes of the posterior glandular zone showed RNA concentrated in one or more eccentric patches outside the nuclei in greater amounts than is found elsewhere in this pituitary (fig. 2, c). Also the small orange G cells of Perca showed evidence of a higher RNA concentration than other cell types.

Carbohydrates. Both the AF-positive cyanophils and the type 1 cyanophils of the median glandular zone of Rutillus, Phoxinus, and Perca were strongly PAS-positive. The AF-positive cells showed a fine magenta granulation closely embracing the nucleus after the PAS procedure, whereas the type 1 cells contained coarse granules of PAS-positive material scattered throughout the cytoplasm. In Rutillus there were also a few lightly staining cyanophils of the anterior glandular zone which were PAS-positive, whereas in Phoxinus and Perca a third group of PAS-positive cells were the type 2 cyanophils of the posterior glandular zone. In Bathystoma the cyanophils which morphologically and topographically resemble the type 1, type 2, and AF-positive cells of the above species were also PAS-positive, but in addition the cyanophils of the median glandular zone which appear in patches between the type 1 cells and the AF-positive cells also reacted to the PAS procedure. In these four species all the cyanophils were PAS-positive and no other cell of the adenohypophysis appeared to contain carbohydrate.

In Pseudoscarus the AF-positive cyanophils and the AF-negative cyanophils which surround them were PAS-positive, the former staining deeply. Also the large orange G cells of the median glandular zone were PAS-positive. Finally, in Scarus the large orange G cells were PAS-positive, but the cyanophils of the median glandular zone adjacent to the nervosa were PAS-negative. The amphiphils of the posterior glandular zone were also PAS-positive, but somewhat weakly so; this cell type may correspond to the type 2 cell of other species.

The nervosa in all fish examined was PAS-negative.
Tyrosine. After applying the Morel-Sisley diazotization procedure it was found that a strong reaction can be obtained in certain cell types. The small orange G type cells of *Perca* showed intense orange-red coloration whereas no other type in this species showed other than the general faint pink-orange background reaction. The small orange G cells of *Bathystoma* and *Pseudoscarus* and the weakly cyanophil cells of the median glandular zone of *Scarus* also gave an orange-pink coloration after this technique, but it was not as intense as that seen in *Perca*. *Rutilus* and *Phoxinus* also have a cell type which gave a much stronger reaction than any other type but in these pituitaries it is shown by making careful comparison with adjacent sections stained in Azan that it was the cyanophil type 1 cell (fig. 2, e). Thus all the pituitaries examined appeared to have one cell type which has a high tyrosine content, although after Azan staining these cells may differ tinctorially.

The Millon reaction did not colour the cells as intensely as the above method and its localization and the differentiation of cell types after this technique was difficult to interpret.

*Performic acid / Alcian blue reaction.* Cystine and/or cysteine was localized by a deep blue coloration in the cytoplasm of the cyanophil type 1 cells of all the fish that were examined. In *Rutilus* and *Phoxinus* there was a pale blue granular colouring of the other cyanophil types. In *Perca* the cyanophil type 2 cell, which can clearly be differentiated histologically, gave a definite blue coloration and a somewhat weaker reaction was seen in the small orange G cells (fig. 3, b). The neurosecretory material of the neurohypophysis in all species gave a positive but variable reaction.

*Thioglycollate-ferricyanide reaction.* Sulphhydryl groups were demonstrated by a deep blue colour after this reaction. The cyanophil type 1 of *Rutilus*, including the scattered cyanophils of the anterior carminophil zone, reacted intensely but a weaker reaction was shown by other cyanophils (fig. 3, a). In *Phoxinus* both cyanophil 1 and 2 gave positive reactions. This was also true of *Perca* but in this case the reaction in the cyanophil type 1 was not strongly marked and the small orange G were slightly positive. In *Pseudoscarus* and *Scarus* the large orange G cells gave a pale but distinct reaction. Again the neurosecretory material gave a positive but variable reaction.

*Dihydroxydinapthyldisulphide reaction.* The results obtained by this method were in agreement with the thioglycollate-ferricyanide reaction but with some minor quantitative differences. For example, a strong reaction colouring both the small orange G cells and the 'sphere cell' droplets of *Perca* a deep brown was given after applying this method, but both cyanophil type 1 and 2, although positive, were coloured only a light brown (fig. 3, c). The cyanophil
FIG. 3
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type 1 cells again were positive in all species and again the other cyanophils of *Rutilus* demonstrated the presence of —SH or S—S groups. The cyanophil type 2 in *Phoxinus, Perca,* and *Bathystoma* gave pale but positive reactions. The cytoplasm of the large orange G cells of *Pseudoscarus* and *Scarus* contain more DDD-positive material than any other cell type in these pituitaries. The small orange G cells of *Pseudoscarus* are weakly positive. The neurosecretory material was unreactive.

**Coupled tetrazonium reaction.** The brown coloration indicating the presence of histidine, tryptophane, or tyrosine was variable in intensity. In *Rutilus* and *Bathystoma* the cyanophil type 1 gave a definite positive reaction, although not all the cyanophil type 1 of *Phoxinus* were coloured. The cyanophil type 2 cells of both *Phoxinus* and *Bathystoma* were stained deep brown. The small orange G cells gave a strongly positive reaction in *Perca, Bathystoma,* and *Pseudoscarus,* although somewhat paler in the latter where the large orange G cells showed the strongest reaction. The large orange G cells of *Scarus* were also positive. In all species examined the nervosa gave a positive but variable response.

**Iodination / coupled tetrazonium reaction for histidine.** This method gave an over-all background coloration to all the pituitaries stained, and only in *Perca* and *Bathystoma* was there any positive reaction. In both species the small orange G cells were strongly positive and in *Perca* the anterior cap of carminophils also gave a definite positive reaction, while the cyanophil type 2 cells were slightly stained (fig. 3, ε). In all species the nervosa remained unstained.

**Sakaguchi's (1925) test for arginine.** In no cell in any pituitary examined was it possible to demonstrate any concentration of arginine.

With reference to the observations on amino-acids quoted above it was not possible to differentiate AF-positive cyanophils always from surrounding type 1 cyanophils after these histo-chemical procedures, and we therefore have been unable to make specific references.

**Lipids.** Staining gelatin sections with Sudan black B or Fettrot gave no positive indication of the presence of fat-droplets in any pituitary cells or in the nervosa. However, after Sudan black the nervosa appeared blue, which may have indicated a very fine deposit of fatty material. Neither the neurosecretory substance or any adenohypophysial cells showed a marked concentration of phospholipid after acid haematein. The copper phthalocyanin reaction, however, although not demonstrating any structures corresponding to Gomori-positive material, did stain with varying intensity both nuclei and cytoplasm of adenohypophysial cells in all species. In view of the negative

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**Fig. 3 (plate).** Photomicrographs of pituitaries of: A, *Rutilus,* with thioglycollate-ferricyanide positive cyanophils type 1 and 2 (c1 and c2) and neurosecretory material (nsm); B, *Perca,* with performic acid / Alcian blue positive cyanophil 2 (c2) and neurosecretory material (nsm); C, *Perca,* with DDD—positive small orange G cells (og) and ‘sphere cells’ (s); D, *Rutilus,* longitudinal section showing chrome-haematoxylin positive cyanophil (c1) and neurosecretory material (nsm); E, *Perca,* longitudinal section showing presence of histidine in anterior carminophils (ac) and in small orange G cells (og) of the median glandular zone.
DISCUSSION

The problems of homologizing the zones of the teleost pituitary with those of other vertebrates and the dependent problem of correct terminology for these zones has exercised investigators for many years (Stendall, 1914; Pickford and Atz, 1957). Kerr (1948) has suggested that the anterior glandular region and the median glandular region of the adenohypophysis (the pro- and meso-adenohypophysis of Pickford) are homologous with the pars distalis of higher vertebrates and the posterior zone with the whole or part of the pars intermedia. Reviews by Charipper (1937) and Pickford and Atz (1957) have demonstrated how extensively the histology of the teleostean pituitary has been studied and how variable have been the descriptions of cell types and their distribution in the gland. The gland is probably highly labile in form, as previous authors have suggested, but much of the apparent variance is possibly due to the describing of pituitary cells after the use of different stains. In the present work the glands were examined histologically after staining with the Azan method, for the reason that this triple stain gives clear, non-fading, repeatable results and differentiates a number of cell types in the teleost pituitary, and furthermore has been used in a number of the most recent morphological accounts of this gland. However, it is fully realized that other staining methods might have given a somewhat different tinctorial picture which could equally well have been used to give a histological description for comparison with the histochemical investigation.

Although most of the cell types found in the mammalian pituitary have been identified in teleosts, only two types have been related directly to specific hormone production, namely the thyrotrophs and gonadotrophs, which are cyanophils of the median glandular region (Pickford and Atz, 1957). Even here much of the evidence is circumstantial and the FSH cell has not been distinguished from the LH cell, although Olivereau (1954b) has associated the acidophils of the pituitary of Salmo salar with the luteinizing hormone, a view for which there is little evidence in mammals and even less in fish. As in mammals the somatotroph has been assumed to be an acidophil but the evidence, based upon observations of pituitaries during development and growth, is casual, and recently Olivereau and Francotte-Henry (1956) noted that the acidophils of Caecobarbus geertsi may be abundant when growth is slow. Other cells, both of the anterior and median glandular zone, undergo changes during growth, seasonally, and during migratory periods, but these observations (reviewed by Pickford, 1957) have not demonstrated specific hormone-producing sites.

The evidence for a site of ACTH production, i.e. a corticotroph, is confused. Rasquin and Stoll (1955) have claimed that a basiphil which was not a gonadotroph (although no evidence for this statement was given) produced ACTH; while Rasquin and Atz (1952) and Chavin (1956), after treating fish
with saline, DOCA, or ACTH, have demonstrated changes in both the acidophils and basophils of the median glandular zone. Intermedin, or the melanophore-dispersing hormone, is certainly produced in the posterior glandular zone of the teleost adenohypophysis (Hewer, 1926), but again the cellular site of this hormone has not been revealed and Chavin (1956) could find no change in any cells of this zone after treatment with ACTH and intermedin.

Argyrophils have been associated in the mammal pituitary with ACTH-release or gonadotrophin production (Knigge, 1955a, b), but have hitherto not been described in teleost fish. However, during this study it was found that cyanophils of the posterior glandular region of the adenohypophysis of Phoxinus were argyrophil after applying the method of Ranson as modified by Smith (1956) or Bodian (1936), whereas the weakly staining amphiphils or chromophobes were not. Also a clearly marked arygyrophil is found in Pseudoscarus, but here it is identified as the large deeply staining vacuolated cyanophil of the posterior-ventral region of the median glandular zone.

Although it has been shown in the present work that the teleost neurohypophysis contains neurosecretory material which is histochemically similar to that of the rat (Howe and Pearse, 1956), functionally the neurohypophysis is an enigma. No 'water balance principle' has been demonstrated (Fontaine, 1956), nor have studies using mammalian posterior pituitary hormones revealed that they have any unequivocal effect upon salt metabolism (Sexton, 1955; Burden, 1956; Matty and Morris, unpublished). Some evidence that this part of the teleost pituitary may be concerned with osmoregulation comes from the work of Arvy, Fontaine, and Gabe (1954) and Arvy and Gabe (1954), who observe changes in the amount of neurosecretory material contained in the neurohypophysis upon altering the salinity of the water in which fish are immersed. From the present work and from the examination of a great number of other teleost pituitaries it does appear that the amount of neurosecretory material is greatly variable between fish of the same species. Thus such qualitative results as have been obtained from salinity experiments should be repeated in a more quantitative manner.

It has become evident from our observations that histological cell types differentiated by Azan and histochemical cell types do not always correspond in different species. For example, orange G cells are PAS-negative in Phoxinus but some orange G cells are PAS-positive in Pseudoscarus, and except for the fact that they are performic / Alcian blue negative, they are histochemically identical to type 1 cyanophils. Also in Rutilus although all cyanophils are PAS-positive, some have little or no sulphydryl groupings present in their cytoplasm, whereas other cyanophils have abundant —SH radicles. Again in Perca it is the small orange G cell that has a high tyrosine content, little or no tyrosine being found in the cyanophils, but in Rutilus it is a cyanophil which has the greatest tyrosine content. All these observations indicate the difficulty of endeavouring to identify a specific hormone-producing cell in all teleost fish upon tinctorial grounds alone. Furthermore the unresponsiveness of the chromophobes and amphiphils to histochemical procedures in these fish
again raises the question whether they are reserve cells without secretory function and if under certain circumstances they are able to differentiate into obvious secretory cells.

From the work of Pickford and of Wilhelmi (Pickford and Atz, 1957) it is now well established that the teleost pituitary contains a growth-promoting hormone; however, as mentioned previously, there has been no unequivocal demonstration of the somatotroph although it has been suggested that acidophils, as in mammals, are responsible for the production of the growth-hormone. If the assumption is made that the fish and mammalian growth-hormones are chemically similar (Wilhelmi, 1955) and pituitary cells containing such a hormone are PAS-negative and DDD-positive, then from our observations, since acidophils are less DDD-positive than cyanophils (except for the small orange G cells of the median glandular zone of Perca), there are no cells in the fish pituitary which are both PAS-negative and DDD-positive. Thus it may be that the growth hormone is produced in cyanophils along with other glycoprotein hormones. This problem may be answered, along with a recognition of the site of production of ACTH, intermedin, and possibly LH, when histochemical and experimental investigations of the fish pituitary are made together.

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