The Pituitary in Normal and Parasitized Roach
(Leuciscus rutilus Flem.)

BY
T. KERR
(Department of Zoology, University of Leeds)

With one Plate

INTRODUCTION

The occurrence of the plerocercoid stage of a tapeworm (Ligula intestinalis) in the body-cavity of the roach is accompanied by a marked regression of the gonads, and in view of the relationship of the gonads with the pituitary it seemed possible that this gland might also be involved. Histological changes in the pituitary have therefore been studied; in the first instance to determine whether there are any seasonal changes in the glands of normal roach, particularly in relation to the reproductive cycle, and then to ascertain whether the presence of the parasite induces further change.

The importance of the pituitary in relation to the gonads in teleosts is indicated by the stimulation of the gonads in Pimelodus by pituitary extracts of that species (Cardoso, 1934), and the degeneration following hypophysectomy observed by Vivien (1938, 1939) in Gobius and by Matthews (1939) in Fundulus. The problem could be advanced a further stage if some particular cell type can be associated with these gonad changes in the fish, since in higher vertebrates the acidophils of the distal (anterior) lobe appear to be especially associated with anabolism and growth and the basiphils with thyroid activity and gonad maturation.

MATERIALS AND METHODS

Normal and parasitized roach were collected throughout the year from Thrybergh Reservoir, near Doncaster, where a high proportion of these fish are infected with Ligula, together with other normal fish from various sources in Yorkshire. The pituitary of the roach is attached to the brain by a delicate stalk and largely enclosed by bone and connective tissue, so that removal attached to the brain is impossible; some glands were dissected out separately therefore and their staining reactions compared with those of decalcified specimens still attached to the brain. The differences due to decalcification proved to be slight in this particular species, and to reduce the risk of damage the gland was left in situ and the lower half of the midbrain and the corresponding part of the roof of the mouth were removed together and treated as a unit. Various methods of fixation and staining were tried before the following routine was adopted: fixation in Bouin, decalcification in formol-nitric,
double embedding by Peterfi's method after trimming the tissues, cutting serial longitudinal sections at 6 μ and staining by Mallory's (1936) modification of his own stain. The measurements of fish given are taken from the tip of the snout to the end of the solid part of the tail, not including the tail fin.

**Observations**

*The Pituitary as a whole in Normal Adult Fish*

The general structure of the pituitary can be seen in a median longitudinal section (Pl. I, fig. 1). The three divisions of the glandular (ectodermal) component are named from front to rear the anterior glandular region (anterior lobe of some authors), the middle glandular region (Übergangsteil or transitional lobe), and the posterior glandular region (intermediate lobe), for reasons given elsewhere (Kerr, 1942). As is usual in teleosts the lack of bounding connective tissue allows the cell types of each region to intermingle along the borders but the regions in themselves are histologically quite distinct. A brief description will first be given of these regions and of the cell types in which variation is irregular, followed in more detail by one of the single cell type recognized in this gland whose variations are sufficiently well marked and consistent to be associated with definite physiological states. The nervous lobe shows no changes that need be mentioned.

The anterior glandular region contains acidophils, basiphils, and chromophobes. The first are variable in size, often columnar, and rather solid cells whose closely packed granules hold both acid fuchsin and orange G to a moderate degree and soluble blue to a slight degree and hence stain a dark purplish-brown with Mallory; they have a moderate affinity also for azan and iron haematoxylin. The infrequent chromophobes look like degranulated cells of the same series. Neither show variations throughout the year nor any differences in parasitized fish. The basiphils are small rounded cells, few in numbers compared with the acidophils, usually light in colour but occurring in a complete range to very deep blue and showing considerable differences in numbers and granulation from one fish to another. It has not been possible to correlate these differences, however, with seasonal or other changes and the cells appear to be unaffected by parasitism. After Mallory these cells are similar in colour to the basiphils of the next region but after Anderson's (1929) modification of this stain have a dull amphiphil appearance, suggesting a separate type.

The middle glandular region again contains acidophils, basiphils, and chromophobes. These acidophils, however, are very small cells, with the granular cytoplasm frequently forming only a little cone on one side of the nucleus, and they have a strong affinity for acid fuchsin, azan, and iron haematoxylin and a very slight one for blue. They appear more brightly staining, therefore, and in that resemble the distal lobe acidophils of higher vertebrates. Their variations appear to be irregular, but their small size and large numbers make them an unsuitable type for study. The basiphils will be described
later. The chromophobes are not numerous and have very scanty and almost colourless cytoplasm. As Bock (1928) and others have pointed out there is nothing 'transitional', in Stendell's (1914) sense, about this region.

The posterior glandular region consists also of cells which from their reactions must similarly be called acidophils, basiphils, and chromophobes. The predominant type is a lavender-coloured basiphil, a cell, however, which has also a considerable affinity for orange G, azan, and iron haematoxylin. Neither they nor the occasional chromophobes show much variation. Scattered amongst them are acidophils with much the same staining reactions as those of the middle region but distinguishable by their larger size; these occur singly or in groups and vary from scarce to quite numerous. The variations appear to be haphazard and may be noticeable even in fish from a single catch. Again, the cells of this region do not appear to be affected by parasitism.

The Basiphils of the Middle Glandular Region

These cells are scattered throughout the region with a general tendency to form irregular groups. They can be found in a complete series from cells with light-blue cytoplasm lacking discrete granules (about 5.5 µ in diameter) through cells of increasing bulk and with increasing numbers of rather small dense blue granules. This series is so complete that it does appear to represent a genuine sequence, in one or both directions; on the other hand, the linking of the degranulated basiphils to the smaller chromophobes (approximately 4 µ in diameter) with their almost colourless cytoplasm may with less certainty be achieved through infrequent cells with reduced light-blue cytoplasm.

In describing the seasonal changes which can be seen in these basiphils a starting-point may be taken in April and early May, when the gonad is at its maximum and the shedding of eggs and sperm is about to begin. The basiphils are now also at their maximum. The light-blue cells are rare, almost all cells are deeply granulated, and the largest are oval or rounded and up to about 13 µ in diameter (Pl. I, fig. 2). The high proportion of these very large cells is most characteristic of this time of year. The size and density of the basiphils give the impression in sections that their relative numbers, as compared with the acidophils, have greatly increased; actually this does not appear to be the case, but the small size and large numbers of the acidophils make accurate cell counts impossible. After breeding there is a regression of the basiphils and by late June or July they are at their least prominent. Then the proportion of degranulated and lightly granulated cells is much higher and the size of the largest granulated cells has fallen to approximately 8 µ in diameter. After July there is a slow increase in granulation and cell size so that although there is some variation, the pituitaries of similarly sized fish in September and October are normally quite distinguishable from those of July, particularly in the size and number of the largest cells. There is a further increase during the winter, culminating in April and May.
The Gonads of Normal Adult Fish

The corresponding changes in the gonads may be outlined briefly (see also Turner, 1919, for the perch, and Bullough, 1939, for the minnow). In the female the eggs are shed in May or June, by July the ovary is producing fresh oogonia, and many of these develop as primary oocytes during the summer. These oocytes in their primary growth phase, with characteristic basiphil cytoplasm, are common by September, and some occur in the secondary growth phase, with yolk droplets in a clearer cytoplasm. By late autumn both stages are abundant and many possess a vitelline membrane; little further change occurs during the winter and the final general maturation only becomes pronounced by spring. In the male in July the germ cells, characterized by their large lightly staining nuclei, are giving rise to numerous little groups of smaller denser spermatogonia; this continues into the autumn; in October primary spermatocytes appear and increase throughout the winter, but the final phases in the production of spermatozoa are again confined to the spring.

The Pituitary and Gonads of Parasitized Fish

These fish have been caught from 7 cm. in length up to 22 cm., but the commonest size throughout two complete years has been 10—12 cm. with few fish ever exceeding 14 cm.

The effect of this parasitism on the pituitary is shown only by the basiphils of the middle glandular region, but there it is distinct and consistent. These cells differ from those of normal fish in the smaller maximum size that they normally attain (up to about 6.5 μ in diameter), in their much lower general level of granulation, and in the high proportion of small specimens with more or less clear blue cytoplasm. These differences are, of course, most marked in April and May (Pl. I, 3) when the normal basiphils are at their maximum, but they remain clear throughout the year. There is some variation from fish to fish even in the same catch, such as the appearance of an occasional more heavily granulated cell, but it is not sufficient to overlap the normal condition. Seasonal changes also are either absent or so reduced that they are obscured by this individual variation. The impression given by complete sections under lower power is that there has been a great reduction in the proportion of basiphils, but detailed observation under high power does not support this and it seems more probable that the proportion is not significantly altered.

The gonads of parasitized fish show a very uniform level of development at all times of the year, and this is true not only of the very small gonad which is typical of these fish but also of the occasional larger examples that do occur. The ovaries contain oogonia and primary oocytes which have developed up to about the end of their primary growth phase, the features of the secondary phase (loss of basiphility in the cytoplasm and the appearance in it of vacuoles and yolk droplets, and finally the vitelline membrane) have not been seen in the ovaries cut. In the testes the vast majority of the cells are germ cells with large clear nuclei and only a few small groups of the darker spermatogonia appear amongst them. The nuclei of the germ cells are more uniformly large
in size than in unparasitized fish, possibly owing to the lack of transformation stages towards spermatogonia, and the internal connective tissue framework gives only a very indefinite indication of a lobular organization. In both sexes, therefore, the gonads are comparable to those of spent fish in which all the later maturation stages are missing.

**Conditions in Immature Fish**

The largest roach whose gonads are still immature in May are 7–8 cm. in length, and they show some features complementary to the foregoing. The ovaries with their oogonia and early primary oocytes resemble in sections those of parasitized or spent fish, although the gonad as a whole is very small; the testes, too, show a corresponding level of development except that the groups of spermatogonia are more numerous. The pituitary in both sexes is characterized by the small size of the basiphils of the middle region. These, with their reduced and lightly granular cytoplasm, closely recall the cells of parasitized fish; they occur in definite little clusters, foreshadowing the larger and less well-defined groupings seen in the adult.

**Thyroids of Adult Fish**

The thyroids of normal and parasitized fish were cut in May, July, October, and January. The diffuse nature of the gland and the variations in different vesicles, even indeed in different parts of the wall of the same vesicle, make comparisons unusually difficult. Only very marked differences would show convincingly and such were not observed, but it must be emphasized that a considerable range of activity is possible without the manifestation of really distinct histological effects.

**Sticklebacks infected with Schistocephalus**

The case of the stickleback (*Gasterosteus aculeatus* L.) infected by *Schistocephalus solidus* closely resembles that of the roach and *Ligula*—in the life-histories of the tapeworms concerned, in the occurrence of the plerocercoid in the body-cavity of the fish, and in the relatively enormous bulk of tapeworm to fish—and, since no information was elsewhere available, a brief examination has been made of such infected and normal fish. Bock (1928) did not find any typical middle-region basiphils in the normal pituitary at all; this may have been due to his employment of Susa since the use of this fixative, like decalcification, causes the basiphils to stain much like chromophobes. If the gland is fixed in Bouin, or better still corrosive formol, and dissected out with needles before sectioning, the basiphils can be seen as small, moderately staining, but not numerous cells, less well suited to the observation of changes than those of the roach, yet sufficiently clear to show any marked variations. No differences could be seen, however, between those of normal and parasitized fish. In the testes of infected fish cut in May there are appreciably more germ cells and spermatogonia still present than in normal fish, but there are at the same time large numbers of spermatozoa filling the lobules of the gland; full
sexual coloration also develops in parasitized fish and there seems little reason to doubt that they breed. In the ovaries the secondary growth phase of the oocyte, with yolk and vitelline membrane, certainly proceeds, but amongst the largest eggs there is a very marked degree of atresia (compare Vivien, 1939) and it is not possible to say whether eggs capable of fertilization are produced. However, it can be said that this is a case of much more balanced parasitism in which a pituitary of not noticeably altered appearance can carry the gonads towards the end of their maturation at least.

**DISCUSSION**

The roach pituitary has its glandular component divided into the usual three regions found in teleosts—here called anterior, middle, and posterior glandular regions—and the division is based upon the cell types contained and not upon any precise connective tissue or other anatomical separation. Each region contains cell types which for descriptive purposes must be grouped under the three general headings of acidophils, basiphils, and chromophobes, but differences in size, shape, and staining reactions of the chromophils permit a clear differentiation. In the glands examined more or less distinct variations have been found amongst these chromophil cells from fish to fish and from season to season, but with the exception of those in one cell type they have been considered as too erratic or indefinite to sustain any conclusions.

The exceptional cell type is the basophil of the middle glandular region (*Übergangsteil*, transitional lobe). When stained with Mallory these cells can at all times be found as a complete sequence from examples with non-granular pale-blue cytoplasm through stages with increasing amounts of small bright-blue granules up to large heavily loaded cells. Just before breeding in April and May the number of very large dense cells is at a maximum; after the breeding season the size and granulation decrease to a minimum in July with an increase in the proportion of degranulated cells; from then on there is a slow building-up until the pre-breeding condition is again attained. On this evidence an association between these basiphils and the maturation of the gonads can be suggested, and this suggestion is supported by the condition of the pituitary in fish parasitized by *Ligula*. Here the gonads are permanently reduced to about the level found in freshly spent fish—in the ovary oogonia and early primary oocytes, in the testis germ cells and some spermatogonia, with all the later maturation stages missing in each case—and the middle region basiphils in the pituitary are smaller and much less densely granular than those of normal fish at any time of year. Such variations as these cells show in parasitized fish appear to be individual and not related to seasonal changes, nor are they so extensive as to overlap those of normal fish. The remaining cell types in the gland show no changes as a result of the parasitism. The conditions found in the largest normal fish still immature in May are in good agreement; their gonads resemble those of parasitized and spent fish in the level of maturation attained, and their basiphils are of reduced size and granulation.
Comparisons with other fish cannot easily be made, since seasonal changes so far recorded for teleost pituitaries are few and difficult to relate to particular physiological states. The most complete is by Matthews (1936) for Fundulus; he divides the glandular component of the pituitary into two regions only, though Scruggs (1939) finds it composed of the usual three, and there is also some doubt as to which region Matthews's cell types actually belong. The cycles he finds, however, appear to be as follows, using the nomenclature for the regions adopted in the present paper: (a) a winter increase of middle region acidophils; these are large cells in Fundulus well adapted to show changes, whereas in the roach they are numerous and very small and regular changes were not detected; Matthews found middle region basiphils on the other hand rare and no changes were noted; (b) a more indefinite summer increase in the posterior region acidophils; these cells in the roach show considerable variations but of a kind too haphazard to be reliable; and (c) a summer and autumn increase in large basiphils which Matthews assigns to the posterior region but Scruggs, more plausibly, to the middle. Even so no parallel to the condition in the roach can be drawn. Comparison with Bock's (1928) results is even more difficult since in the middle region of the stickleback gland he found no typical basiphils at all and his seasonal changes are restricted to a spring increase in acidophil activity; finally, no comparison can be made with the results of Evans (1937). In the change from the yellow to the silver form of the eel, however, with the corresponding development of the gonads, Bernardi (1943) finds an increase in the middle region basiphils. In vertebrates higher than the fish there is some reason to associate the distal (anterior) lobe basiphils with the gonads. In seasonal changes (e.g. Hartmann, 1944, in the garter snake; Kayser, 1940, in the hedgehog) an increase in these cells coincides with gonad ripening; in development (e.g. Schooley and Riddle, 1938, in the pigeon) their final differentiation may await sexual maturity.

The removal of the pituitary in fish (Matthews, 1939, in Fundulus; Vivien, 1938, 1939, in Gobius) results after a time in an inability to form the later maturation stages of the reproductive cells or to maintain those already formed. Since this lack of later maturation stages characterizes the gonads in both sexes of these parasitized roach, it appears that here too the influence of the pituitary has been suppressed. Comparable results have been obtained from hypophysectomized mammals. In the male (e.g. Smith, 1930, in the rat) there is a clear similarity to the fish; in the female (e.g. Desaive, 1940, in the rabbit) stages in the ovary do not develop beyond that of the earliest liquid-producing follicles, a comparison with the fish is not here so simple, but both reproductive cells appear to be primary oocytes. The opposite experiment of removing the gonads to determine the effect upon the pituitary does not seem to have been performed on fish; in mammals, however (Ellison and Wolfe, 1934, 1935, in the rat), the result is an increase in the proportion of distal lobe basiphils, possibly followed by a characteristic degeneration. The reduced basiphils of the parasitized fish can hardly represent either of these
effects; in other words, the condition of the fish basiphils can hardly be itself a result of the regression of the gonads. In higher types also thyroid functioning appears to be largely under the same basophil control, and the effects of thyroidectomy upon the pituitary resemble those of gonadectomy (Grobstein, 1938, in the newt; Brolin, 1946, in the rat), but it has not been possible to find definite parasitic effects on the roach thyroids.

The actual cause of the reduction of the basiphils in the roach is still undetermined, except that it is connected with the presence of the tapeworm or its waste products, but the effect is the virtual elimination of the gonad-stimulating hormone of the pituitary. From a brief investigation of the closely parallel case of parasitized sticklebacks it is clear that the influence of *Schistoscephalus* on this fish is very much less severe, though there are indications in the ovaries of what may prove to be a slight effect of a similar nature. In the teleost, therefore, it is suggested that the middle region basiphils are particularly concerned in the maturation of the gonads—their influence becoming effective from the level of spermatogonia onwards in the male and of early primary oocytes in the female—and their maintenance at breeding-point, whether or not other factors determine the actual discharge of the gametes. These cells then would correspond physiologically to the basiphils of the distal lobe of higher types, a lobe to which the entire middle glandular region of the fish has a strong histological resemblance.

I am again in debt to Professor E. A. Spaul, D.Sc., for reading the manuscript and for his comments on the interpretations involved, and to Mr. G. Withem and Mr. W. H. Price of Doncaster and Mr. R. W. Ward of the Yorkshire Fishery Board for facilities and help in the collection of the fish.

**Summary**

1. A short description of the glandular component of the roach pituitary is given, from the point of view of the various cell types.

2. The seasonal variations are described in the basiphils of the middle glandular region (transitional lobe), the only cell type in this fish whose variations are sufficiently regular to be reliable, and a correspondence between these changes and the sex cycle is pointed out.

3. In roach parasitized by the plerocercoid of the tapeworm *Ligula* these basiphils are markedly reduced in size and granulation, whilst other cell types are not affected, and the gonads are also in a condition with all the later maturation stages missing.

4. The facts presented and a discussion of other work lead to the suggestions that it is these later stages of maturation which are under pituitary control in fish as in higher types, that the middle region basiphils are the principal cell type involved, and that these basiphils are comparable to the histologically similar basiphils of the distal lobe of later vertebrates.
REFERENCES

—— 1935. Ibid., 19, 160.
—— 1939. Ibid., 208, 948.

EXPLANATION OF PLATE

PLATE I

Fig. 1. Longitudinal section of pituitary of normal roach, 14 cm. in length. May. 6 μ. Mallory. ×90.

A filter has been used to accentuate the groups of bright blue basiphils in the middle glandular region. In a corresponding photograph of the gland of a parasitized fish the reduced basiphils do not show up at all.

Fig. 2. Small group of full-sized basiphils in the middle glandular region of a normal fish, 13 cm. in length. May. 6 μ. Mallory. ×550.

Fig. 3. Small group of reduced basiphils in the middle glandular region of a parasitized fish, 13 cm. in length. May. 6 μ. Mallory. ×550.