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The Development and Secretory Activity of the Pituitary Gland of Cichlids, and a Comparison of the Meta-adenohypophysis with that of other Teleosts.

by

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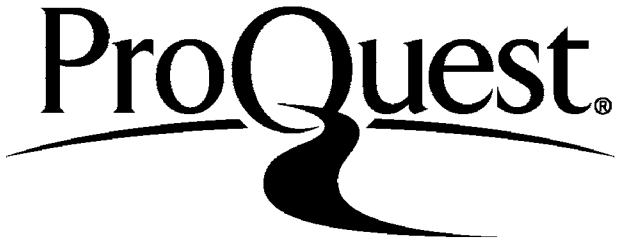


CONTENTS

Abstract.

The structure and histology of the pituitary gland of two species of teleost, Herichthys cyanoguttatus and Cichlasoma nigrofasciatum, is described. Seven cell types can be recognised. Two groups of P.A.S.+ve, A.F.+ve cyanophils in the meso-adenohypophysis are associated with the production of the thyrotrophic and gonadotrophic hormones; it is suggested that the P.A.S.+ve acidophils in the meta-adenohypophysis produce a chromatophorotrophic hormone.

The blood supply of the pituitary in cichlids of different ages has been studied.

The development of the pituitary of Herichthys and the time at which the cell-types become histologically differentiated is described; comparisons are drawn with the developing pituitary of Cichlasoma.

Histological differentiation of the first thyrotrophs coincides with the initial secretion of colloid in the thyroid but the results of experiments in which specimens of Herichthys were reared in thiourea solution indicate that pituitary influence on thyroid activity is not established until a later stage. Thiourea treatment from an early age affects not only the development of the thyroid and thyrotrophs but also that of the swim-bladder, melanophores and skin. The mechanism of these disturbances is discussed.

The effect on the pituitary of long-term adaptation to both white and black backgrounds has been studied in several species of

teleost and in Rana. Chromatic adaptation is accompanied by cytological changes in the pars intermedia of Rana and in certain cells in the meta-adenohypophysis of Phoxinus, Salmo and Cichlasoma but not in Carassius or Ameiurus. The cell types in this region of the pituitary are not histologically comparable in different species; the cellular changes in Phoxinus and Rana can be related to the release of melanophore concentrating- and dispersing hormones respectively but such a correlation has not proved possible for other species. Interpretations of the cytological appearance of the meta-adenohypophysis and intermediate lobe under different conditions are suggested and the functions of the chromatophore-regulating hormone are discussed.

PREFACE

Relatively few workers have directed their attention towards a study of the developing pituitary in teleosts. In the three large volumes edited by Grassé (1958) which cover the anatomy and physiology of Pisces, the subject is dismissed in a single sentence. During the last decade a number of stains, not previously applied to the pituitary, have proved of considerable value for detecting specific cell types and it has therefore seemed worthwhile to study the development of a teleost pituitary taking advantage of these techniques and to investigate especially a problem which has recently received considerable attention in higher vertebrates - that of the pituitary-thyroid relationship during early development. Two cichlid fish, Herichthys cyanoguttatus and Cichlasoma nigrofasciatum, were selected as suitable material for this study since they spawn at frequent intervals throughout the greater part of the year and produce each time a large number of eggs. Moreover, being tropical fish, they develop fairly rapidly.

In order to understand the histological picture presented by the developing pituitary it is necessary to have a clear understanding of the adult gland. This has therefore been studied and an attempt has been made to establish the functions of some of its cells.

By homology with the tetrapod pituitary and from a comparison of bio-assay tests of the anterior and posterior halves of the gland, previous workers have suggested that the posterior region of the teleost pituitary (meta-adenohypophysis) is responsible for the

production of a chromatophore-regulating hormone. During the present investigation on cichlids it was found that one of the cell types from this region showed cytological differences in fish adapted to white and black backgrounds. It was difficult, however, to associate the induced changes in activity with the release of a specific hormone and it was hoped to throw more light on the function of the cells in this region of the pituitary by putting the experiments on to a comparative basis with teleosts in which more is known about the chromatophore-regulating hormones. A considerable part of this work is therefore concerned with the production and release of these hormones under different conditions.

It is convenient to divide the work for this thesis into three parts:

Part I describes the structure of the pituitary in two species of cichlid, its vascular supply and its early development.

Part II deals with the functional relationship between the pituitary and thyroid in Herichthys cyanoguttatus during early development.

Part III includes an account of the cytological variations of the cells of the meta-adenohypophysial in different species of teleosts, with an attempt to interpret these variations in terms of hormone release.

N.B. Throughout this thesis reference is frequently made to some or other aspect of the "cichlid" pituitary. The word "cichlid" is used loosely, to avoid verbosity; it is intended to refer only to Herichthys cyanoguttatus and Cichlasoma nigrofasciatum and not to cichlids in general, about whose endocrine glands almost nothing is known.

PART I

The Histology, Blood Supply and Development of the Cichlid Pituitary.

§ 1. INTRODUCTION

The teleost pituitary, as in all vertebrates, originates from two sources. The cellular portion (adenohypophysis) arises as an upgrowth of cells from the buccal epithelium from which it becomes separated at an early stage in development and makes contact with the floor of the infundibulum. To this area of contact extend the nerve fibres of neurones situated in different regions of the hypothalamus, to form the nervous portion of the pituitary - the neurohypophysis.

In both teleosts and tetrapods the adenohypophysis is divisible into 3 regions. The organisation of the teleost pituitary, however, differs from that of tetrapods in several respects and since there are available only limited data indicating homology between the cellular regions of the pituitary in species from those two groups, the nomenclature used for tetrapods is best avoided for teleosts. Alternative terms have been suggested by Olivereau (1954) but these are based on the histological appearance of the gland and are only applicable to the fish for which they were coined. Recently Pickford & Atz (1957) have reviewed the terminology and have proposed a new nomenclature for the three glandular regions of the teleost pituitary: pro-, meso- and meta-adenohypophysis. These will be adopted throughout the present work.

The structure and histology of the adult teleost pituitary is described in an extensive literature. Several descriptions, with reference to numerous others, are to be found in the papers of Kerr (1933, 1942a & b, 1943, 1948), Scruggs (1939, 1951), Green (1951), Herlant (1954) and Legait (1958), while a comprehensive review of the functions of the pituitary in teleosts is given by Pickford & Atz (1957). Some of these papers also consider the possible homologies between the regions of the pituitary in different classes of vertebrates.

The identification of certain pituitary cells as those responsible for the production of specific hormones has received most attention in mammals, but a certain amount of experimental work, notably that of Atz (1953), Barrington & Matty (1955) and Sokol (1955) has made it possible to identify the cyanophils of the meso-adenohypophysis as thyrotrophs and gonadotrophs. In the following section an attempt has been made to distinguish these two cell types in the cichlid. In addition, the meta-adenohypophysis of both black and white adapted cichlids has been examined; this line of investigation stems from the observations made by Ortman (1954, 1956) who found that in amphibia chromatic adaptation to black and white backgrounds was accompanied by cytological changes in the cells of the pars intermedia.

Accounts of the developing teleost pituitary are less numerous than those of the adult gland. Relevant papers are those by Kerr (1933, 1940), Matthews (1937), Buchmann (1940), Tampi (1953) and Rasquin (1955), but apart from the work of the latter author all the

descriptions are based on material stained with one of the classic trichrome stains.

Balon (1959, 1960) has described the development of both Herichthys cyanoguttatus and Cichlasoma nigrofasciatum but his work is limited to macroscopic observations of external features and such internal structures which could be seen through the transparent skin during the early stages.

§ 2. MATERIAL AND METHODS

The adult pituitary of two species of cichlids, Herichthys cyanoguttatus and Cichlasoma nigrofasciatum, was examined. The following descriptions are based on the pituitaries from 3 specimens of H. cyanoguttatus and 30 specimens of C. nigrofasciatum. Fish were killed by decapitation. The pituitary was then exposed, either by partially dissecting away the overlying tissue or by making a lateral sagittal cut, thus removing the tissues on one side of the pituitary. The remaining adherent tissues were dissected away when the specimen was in the clearing medium prior to embedding in wax.

In a few cases the tissues around the pituitary were left intact, in which case the specimen was decalcified either in Kristensen's fluid or in 10% Versene.

Material was fixed either in aqueous Bouin, formol sublimate (90 parts $HgCl_2$ + 10 parts formalin) or, in a few cases, in Helly's fixative. After fixation in formol sublimate or Helly the material was washed overnight in water. All specimens were dehydrated in alcohol, cleared in methyl benzoate followed by two short rinses in benzene and then placed in a warm benzene-wax mixture for ½-hour. Equal parts of paraffin wax with melting points of 66°C. and 56°C. were used for embedding. This gave a hard wax with a low melting point at about 56°C. Serial sections were cut at 5 μ and stained with one of the following:

1. Heidenhain's Azan (azocarmine, aniline blue, orange G).

2. Gomori-Halmi's Aldehyde Fuchsin (A.F., Halmi,1952) with light green-orange G counterstain.

3. Periodic acid-Schiff (P.A.S.) for muco- and glyco-proteins and mucopolysaccharides (Glegg, Clement and Leblond,1952).

4. McConaill's lead haematoxylin (McConaill,1947) counterstained in some cases with 5% aqueous phloxine solution; in other cases material was stained with P.A.S. before the lead haematoxylin.

5. Gomori's chrome-alum-haematoxylin (C-A-H, as modified by Bargmann & Hild,1949) counterstained with 5% aqueous phloxine solution.

The macroscopical appearance of the ovaries and oöcytes of most fish was noted.

Treatment with thiourea.

Six specimens of Cichlasoma nigrofasciatum were used for these experiments.

Five fish were kept individually in small tanks (20 cm x 20 cm x 30 cm) in 0.05% thiourea solution at 27°C for 3 to 6 weeks.

One fish was kept in a 0.1% thiourea solution for 6 weeks.

Control fish were kept under similar conditions in tap water.

Control and experimental tanks were aerated continuously and both the thiourea solution and tap water were changed weekly.

The lower jaws, containing the thyroids, were fixed in Bouin. Several sections of these were cut at 7 µ.

Development of Herichthys cyanoguttatus.

Several separate batches of Herichthys cyanoguttatus eggs were used for these investigations.

The eggs or newly-hatched young were removed from the parents and kept in a 30 cm square glass tank at 27°C.

The water was aerated continuously and the young fed on Liquifry No. 1 after the 4th day.

At least 5 specimens were fixed from each batch, at first daily and later at less frequent intervals.

Fixation was in Smith bichromate or aqueous Bouin.

The effect of black and white backgrounds on developing and adult fish.

Subsequent treatment and staining was as previously described.

One batch of H. cyanoguttatus eggs and 3 separate batches of Cichlasoma eggs were used for these experiments. In each experiment half the eggs from the batch were reared in a white painted tank (20 cm x 20 cm x 30 cm) while the other half were reared in a similar tank painted black. In other respects the fish were treated as described above. Fish were killed at 2-day intervals and fixed in Bouin.

Adult C. nigrofasciatum were kept individually in similar black or white painted tanks for periods of 1, 3 and 6 weeks, after which they were killed and the pituitaries treated as before. The tanks were aerated continuously and the water changed weekly.

The pituitaries of 9 adult fish from a white background and 6 from a black background were examined.

§ 3. HISTOLOGY OF THE ADULT CICHLID PITUITARY.

The cichlid pituitary is situated some way behind the optic chiasma immediately in front of the saccus vasculosus. It has no well-defined stalk but is closely applied to the infundibulum in direct communication with the 3rd ventricle. A shallow infundibular recess lined by ependymal cells projects slightly into the neurohypophysis.

The disposition of the pituitary cells is illustrated in Figure 1. These are arranged into three distinct regions, the pro-, meso- and meta-adenohypophysis. The regions are not separated from each other by connective tissue, such as is found between the adeno- and neurohypophysis, but blood vessels often occur at their boundaries. Finger-like projections of the neurohypophysis penetrate into all three regions and surround the blood vessels which ramify through the gland. The larger part of the neurohypophysial lobe lies in the posterior portion of the gland and is surrounded by the meta-adenohypophysis.

The Neurohypophysis.

The neurohypophysis is composed mainly of the axons of neurones whose cell bodies are situated in the hypothalamus. Many of the fibres contain variable amounts of neurosecretion which ranges from fine granules to very large droplets and gives a strong staining reaction with A.F. and Gomori's C-A-H. The highest concentrations of of such secretion is seen in the large, posterior portion of the

Figure 1. Diagram of the pituitary gland of Herichthys cyanoguttatus.

Figure 2. Pituicytes in the neurohypophysis. (a. Azan. b. Gomori's C.A.H.) (x90)

Figure 3. Cells of the pro-adenohypophysis (Herichthys cyanoguttatus). (Azan; green filter; x1000).

Abbreviations:

A : acidophil of meso-adenohypophysis.
AM : amphiphil of pro-adenohypophysis.
AP : acidophil of pro-adenohypophysis.
CA : group A. cyanophils.
CB : group B. cyanophils.
H : hypothalamus.
IR : infundibular recess.
NH : neurohypophysis.
NS : neurosecretion.
PA : P.A.S.+ve acidophil.
PB : lead-haematoxylin+ve cyanophil.
PL : large pituicyte.
PS : small pituicyte.

FIGURE 1.

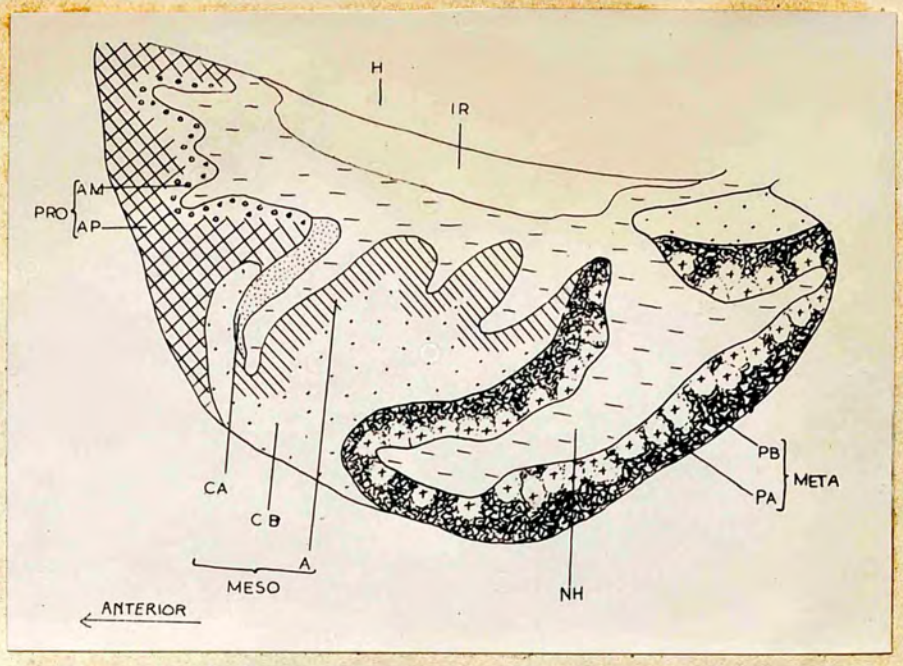
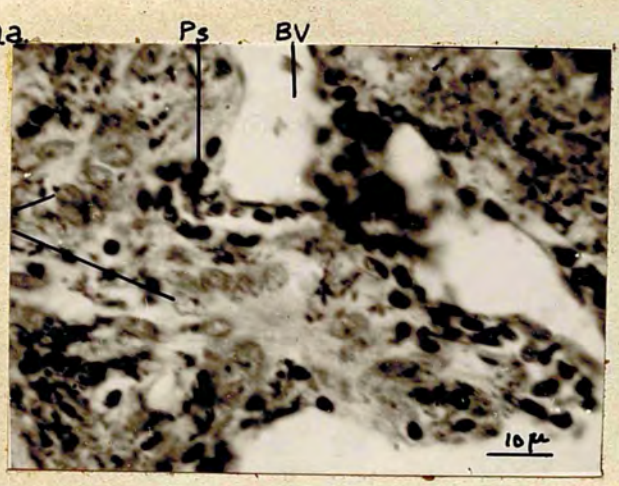


FIGURE 2a.



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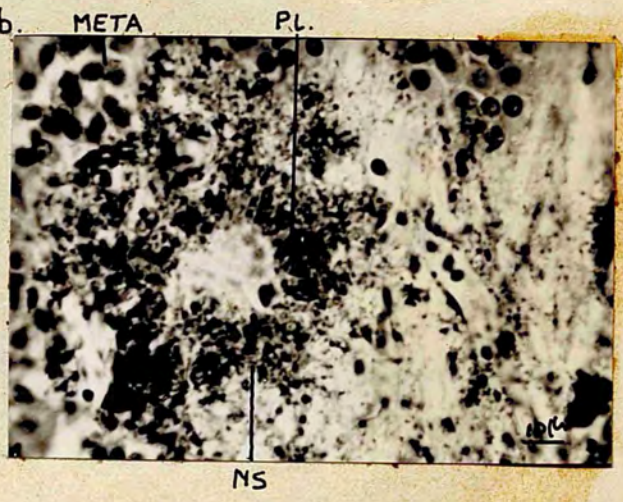
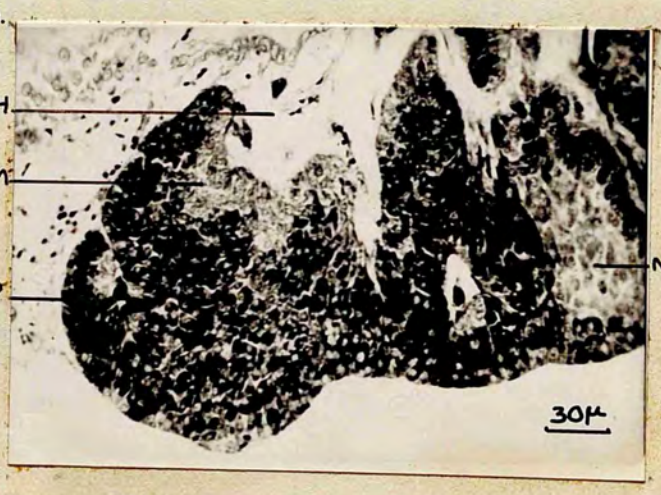


FIGURE 3.



neurohypophysis, surrounding the groups of the larger pituicytes. (Figure 2b) Lesser concentrations of neurosecretion occur in the fibres in the region of the meso-adenohypophysis.

The neurohypophysis is separated from the glandular region by a thin sheath of connective tissue; this is penetrated by nerve fibres which ramify between the cells of the meta- and meso-adenohypophysis.

Two types of pituicytes occur in the neurohypophysis. The larger cells can be seen to originate from the ependymal lining of the infundibular recess. They occur in groups around small central cavities which appear to be derived from the infundibular cavity although the two are no longer in communication. The nuclei of these cells stain only faintly; they are usually a plump, oval shape, although a few may be polymorphic, and they have a distinct nucleolus. The cytoplasm is chromophobic and drawn out towards the central cavity. Occasionally these pituicytes are also clustered around the blood vessels of the neurohypophysis.

The second type of pituicyte has a smaller, frequently polymorphic nucleus which stains with P.A.S. and extremely densely with azocarmine. Very little cytoplasm can be seen around such nuclei. These pituicytes are probably modified glial cells and appear to migrate into the neurohypophysis along the blood vessels from the matrous layer surrounding the brain and pituitary. The number of such cells varies: in some fish they are extremely abundant. (Fig 2a, b.)

The pro-adenohypophysis.

This, the most anterior region of the pituitary, includes two types of cell. The majority of cells are acidophilic, staining brightly with phloxine and orange-red with Azan. These cells are not grouped into follicles, as they are in salmonids and herring, but are often arranged with their axes perpendicular to a blood vessel or finger-like projection of nervous tissue. The cytoplasm is very finely granular and may include a small chromophobic vacuole. The large rounded nucleus occupies no particular position within the cell.

The other cells in this region are pale amphiphils and form a layer between the acidophils and the neurophypophysis. These cells stain pale mauve after Azan and may stain strongly with lead haematoxylin. Small vacuoles often occur in the region of the cell bordering the nervosa, indicating that these are not functionless reserve cells as has sometimes been suggested. The intensity of the staining reaction or the degree of vacuolation of these cells could not be correlated with any particular physiological condition but the strongest staining reactions were observed in small, presumably young fish.

Cyanophils may also occur in groups among the acidophils. These stain in an identical manner to the cyanophils of the meso-adenohypophysis and are therefore not considered to be part of the pro-adenohypophysis.

The Meso-adenohypophysis.

Three cell types may be distinguished in this central region of the pituitary.

Acidophils.

These cells form a palisade layer over the projections of the neurohypophysis. They stain a deeper red with Azan than the cells of the pro-adenohypophysis but usually less brightly with other acidophil stains. In shape they may be cuboidal with a central nucleus or columnar, in which case the nucleus is found at one or other pole of the cell but usually in the same position in the majority of cells. Small vacuoles may sometimes be seen at the proximal pole of the cell bordering the nervosa.

Cyanophils.

These cells compose the greater part of the meso-adenohypophysis. They are organised into two distinct groups, the cells of which differ in size and coarseness of cytoplasmic granules and usually stain with different intensities. The cells from both groups are stained blue after Azan and give a positive reaction with A.F. and P.A.S.

Group A cyanophils.

In the three adult specimens of Herichthys that were examined these occupied a small area in the anterior region of the meso-adenohypophysis. This group of cells abuts directly on to the neurohypophysis and is not separated from it by a layer of acidophils. The cells are small and angular, staining darkly with P.A.S. and aniline blue and a clear purple with A.F. (Figure 4)

Figure 4. L.S. of the pituitary gland of cichlids showing the distribution of the group A. & B. cyanophils (all stained with A.F.).

a) Herichthys cyanoguttatus (green filter; x100).

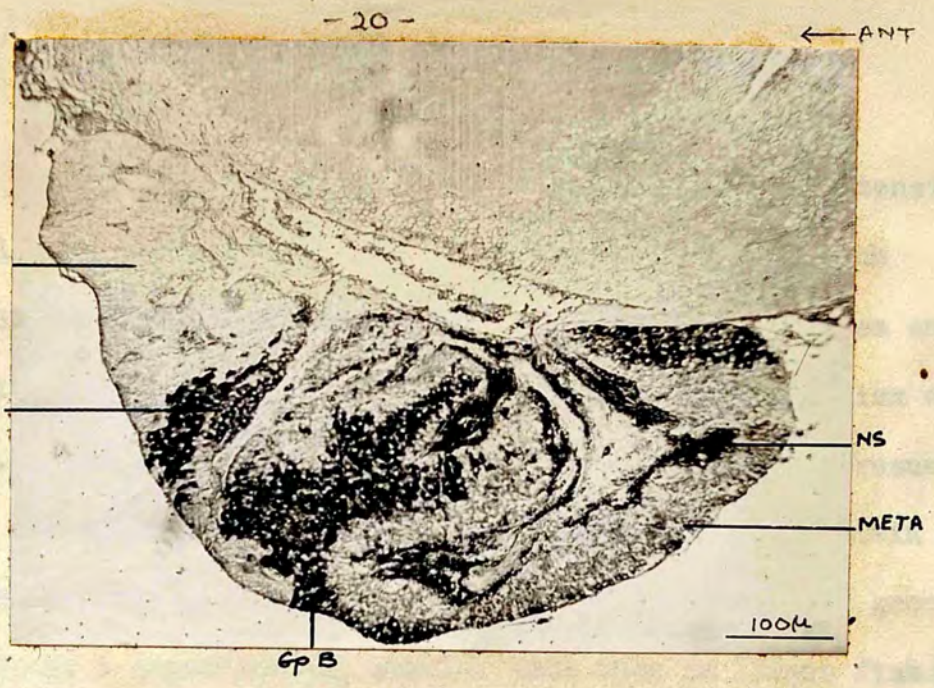
b) Cichlasoma nigrofasciatum, with granulated group A. cyanophils (green filter; x150).

c) Cichlasoma nigrofasciatum, with degranulated group A. cyanophils (green filter; x100).

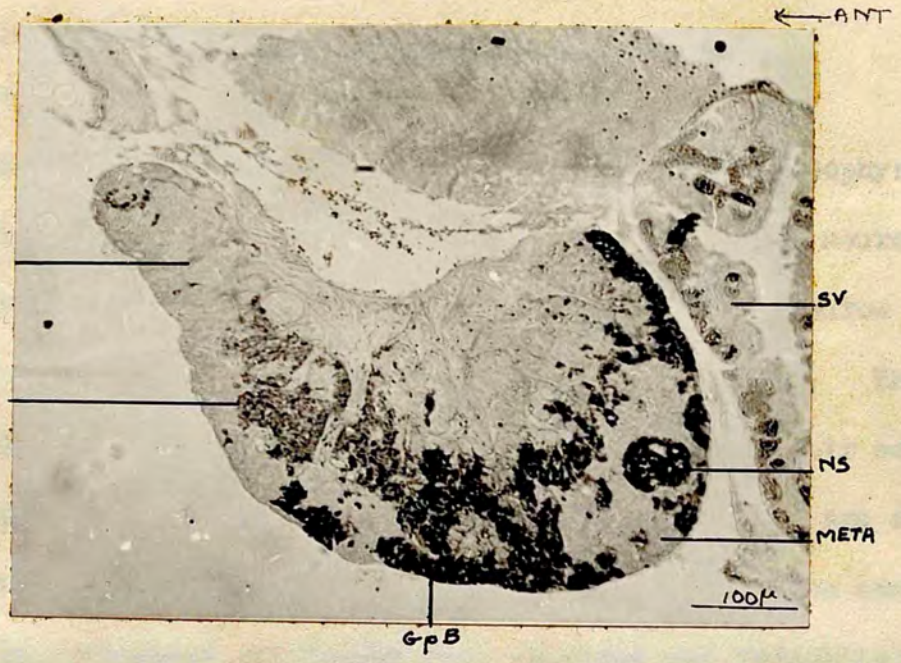
Abbreviations:

Gp.A : group A. cyanophils.
Gp B : group B. cyanophils.
Meta : meta-adenohypophysis.
NS : neurosecretion in neurohypophysis.
PRO : pro-adenohypophysis.
SV : saccus vasculosis.
VC : enlarged and vacuolated cyanophil.

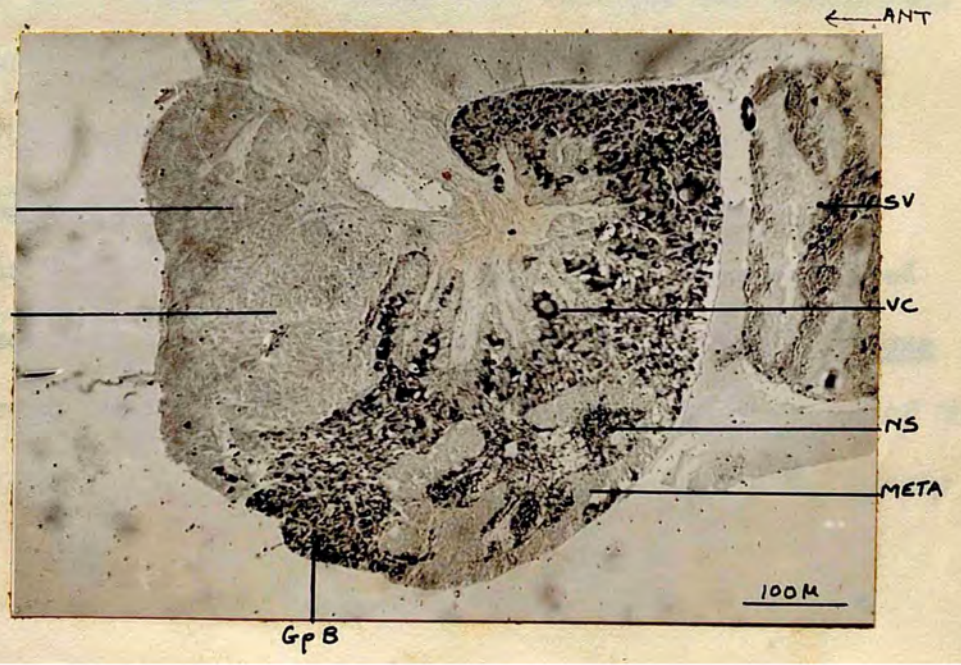
FIGURE 4a.



4b.



4c.



In Cichlasoma this group of cells occupies a more extensive area especially in large mature fish in which the cells are much larger than in Herichthys, with partially degranulated cytoplasm and small irregular vacuoles. Such cells give only a faint reaction with P.A.S. and A.F. and stain grey-blue with Azan. In smaller, presumably younger fish the cells are less enlarged or degranulated and stain more strongly with A.F. and P.A.S. In some of these specimens the group A cyanophils occupy a significantly smaller area than in larger fish. (Fig 4a, b, c.)

Group B cyanophils.

These cells occupy the remaining area of the meso-adenohypophysis and extend laterally around the meta-adenohypophysis. A sheet of acidophils separates this group of cells from the neuro-hypophysis although some of the cells communicate with the neuro-hypophysis by means of extremely fine cellular prolongatrons. The cells can be distinguished from the Group A cyanophils by their coarse granular cytoplasm which stains dark mauve with A.F. They are also usually larger than the Group A cyanophils: those towards the centre of the gland in particular may become very enlarged and vacuolated. In young fish the group occupies a relatively small area of the meso-adenohypophysis.

The cyanophils during different stages of vitellogenesis.

The condition of the gonads of most specimens was noted after macroscopical examination. All the specimens of Cichlasoma examined during these investigations were females and the size of the oöcytes varied from very small in non-gravid specimens to extremely

Figure 5. Cyanophils of group A.

a. Control specimen (Cichlasoma nigrofasciatum) showing degranulation and vacuolisation. (Lead-haematoxylin and phloxine; green filter; x1000).

b. Experimental specimen (Cichlasoma nigrofasciatum) kept in 0.1% thiourea solution for 6 weeks. Vacuolisation appears slightly more pronounced (lead-haematoxylin; green filter; x1000).

c. Control specimen (Cichlasoma nigrofasciatum) with abnormally vacuolated cytoplasm. (Azan; green filter; x1500).

Figure 6. Cyanophils of group B.

a. Enlarged, vacuolated cells of gravid Cichlasoma (lead-haematoxylin; green filter; x1500).

b) Cyanophils from gravid Cichlasoma showing orientation of vacuole towards a blood vessel (lead-haematoxylin & phloxine; green filter; x1500).

Abbreviations:

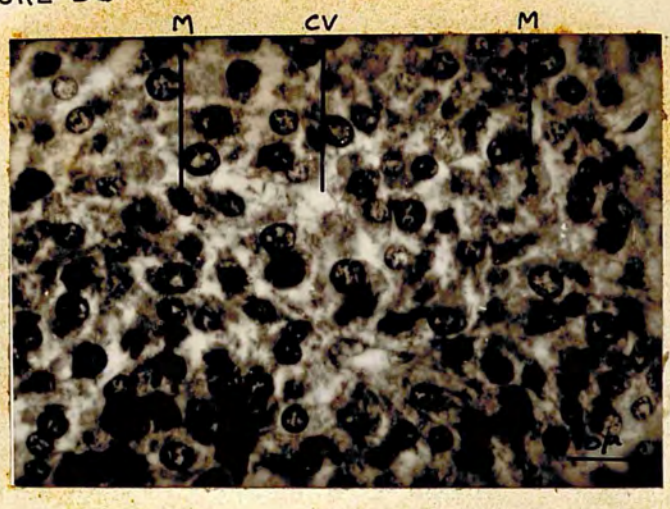
CV : cytoplasm with extensive, irregular vacuoles.

M : mitotic figure.

S : septum containing blood vessel (not seen in photo.)

V : large vacuole.

FIGURE 5a



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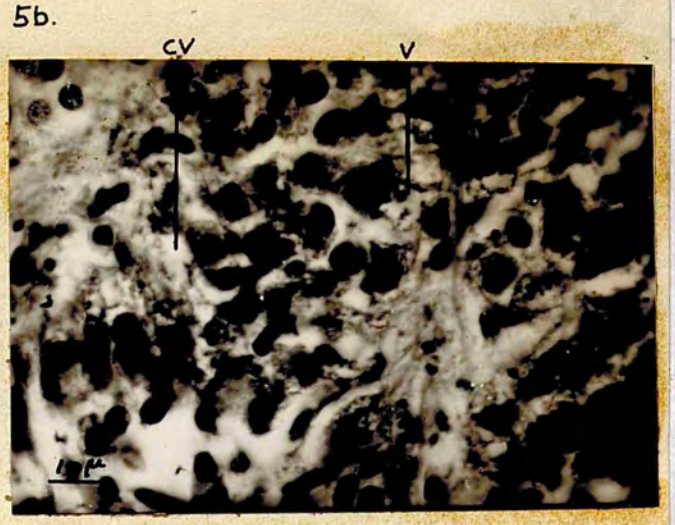


5c

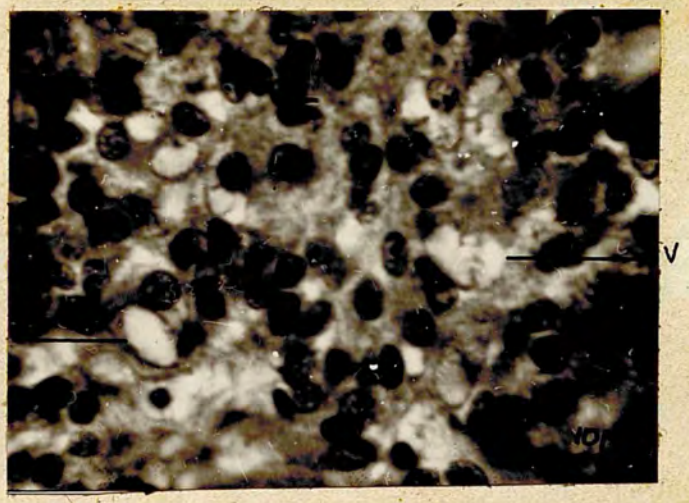
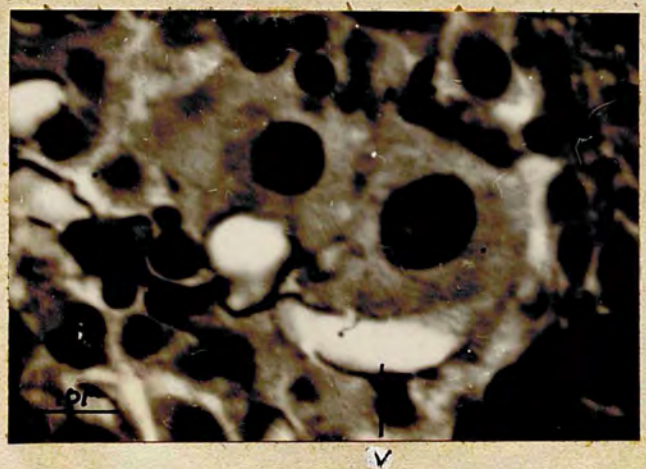
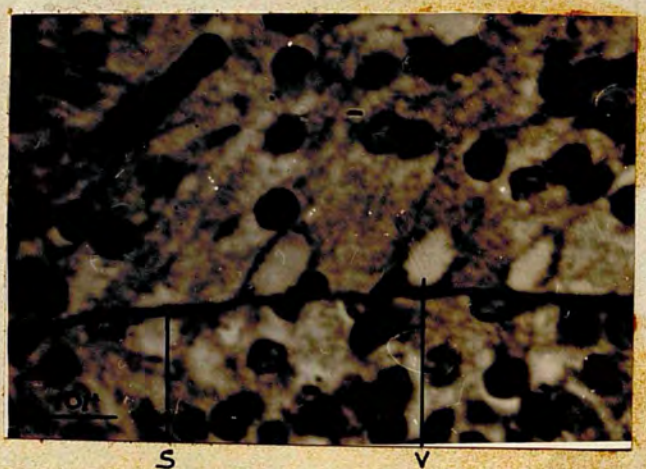


FIGURE 6a



6b.



large in gravid fish. There was a marked correlation between the size of the oöcytes and the size and vacuolisation of the Group B cyanophils. In fish with small or medium sized oöcytes these cyanophils were all fairly small with sparce uneven granulation. This resulted in the cell giving a less intense staining reaction than in gravid fish in which the more centrally-situated Group B cyanophils were enormously enlarged with a dense, even distribution of granules. Such cells stained very strongly with A.F. and P.A.S. and a clear bright blue with Azan; the nucleus of such cells was also greatly enlarged while a single large vacuole occupied a considerable area of the cell (Fig 6) Acidophilic droplets were usually found lining the vacuole; these showed up particularly well after staining with lead haematoxylin. The more peripheral cells of this group were not enlarged and only in a few cases showed any increase in vacuolisation.

Due to an apparently unequal sex-ratio only one male was available for examination; this was a mature specimen of Herichthys. In this specimen all the Group B cyanophils were of equal size and equally vacuolated. These cells gave a strong positive reaction with A.F., P.A.S. and aniline blue. This suggests that in spite of the difference in size and vacuolisation, all the Group B cyanophils in female fish constitute a distinct functional unit. Rasquin and Rosenbloom (1954) noted a similar difference between the central and peripheral cyanophils in Astyanax and suggest that the peripheral cells are older while new cells develop at the centre of the gland.

The Group A cyanophils remained unchanged during oöcytes enlargement.

The effect of thiourea treatment on the cyanophils.

Six Cichlasoma were used for this investigation: three were kept in 0.05% thiourea solution for 3½ weeks, two in 0.05% thiourea solution for 6 weeks, and one in 0.1% thiourea solution for 6 weeks. In all cases the ovaries were found to contain small or medium sized oöcytes while the Group B cyanophils were small and stained unevenly.

After thiourea treatment the Group A cyanophils did not differ greatly from those of the control fish. In both groups these cells were partially or totally degranulated, giving a weak or negative reaction with A.F. and staining grey-blue with Azan. It seemed, however, that these cells became even more vacuolated and "ragged" in appearance after thiourea treatment. (Figure 5)

The Thyroid Gland.

The thyroid gland is composed of follicles which in most teleosts are not grouped together to form a compact gland but are scattered around the blood vessels of the lower jaw. In Cichlasoma there appears to be a continuous process of follicle degeneration and replacement. Small, newly differentiated thyroid follicles encircle the veins of the lower jaw; the epithelium of these follicles in many specimens, but not in all, contained abundant intracellular colloid droplets and was cuboidal or slightly flattened. The colloid was

usually very vacuolated. Larger follicles were situated at a greater distance from the blood vessels. The epithelium of these follicles was very flattened and never contained intracellular colloid droplets while the colloid was never vacuolated. Either red or blue-staining colloid was found in both types of follicles. Apart from these colloid-filled follicles, much of the area in the dorsal and ventral regions of the lower jaw were filled with a network of empty follicles; the walls of these follicles were very thin and not obviously cellular. In some instances the lumen contained remnant of colloid (Figure 7.). The area occupied by this network appeared to increase with age. Empty follicles did not occur in cichlids of 13 weeks, and two small specimens were examined in which only a few such follicles occurred. The area occupied by both the group A and B cyanophils in these fish was very small.

After thiourea treatment the epithelium of those follicles around the veins was hypertrophied and hyperplastic. These changes were especially marked in the fish treated with 0.1% thiourea for 6 weeks. The colloid was reduced in volume and basophilic. (Figure 7)

Two fish kept in tap water and examined in connection with background experiments were found to have extremely vacuolated Group A cyanophils with darkly staining cytoplasm (Fig 5c) The thyroid of these fish did not appear to differ very markedly from that of other fish from tap water but the empty follicles appeared to be more ragged than normal and there were possibly more newly differentiated follicles.

Figure 7. L.S. of lower jaw of Cichlasoma showing thyroid follicles.

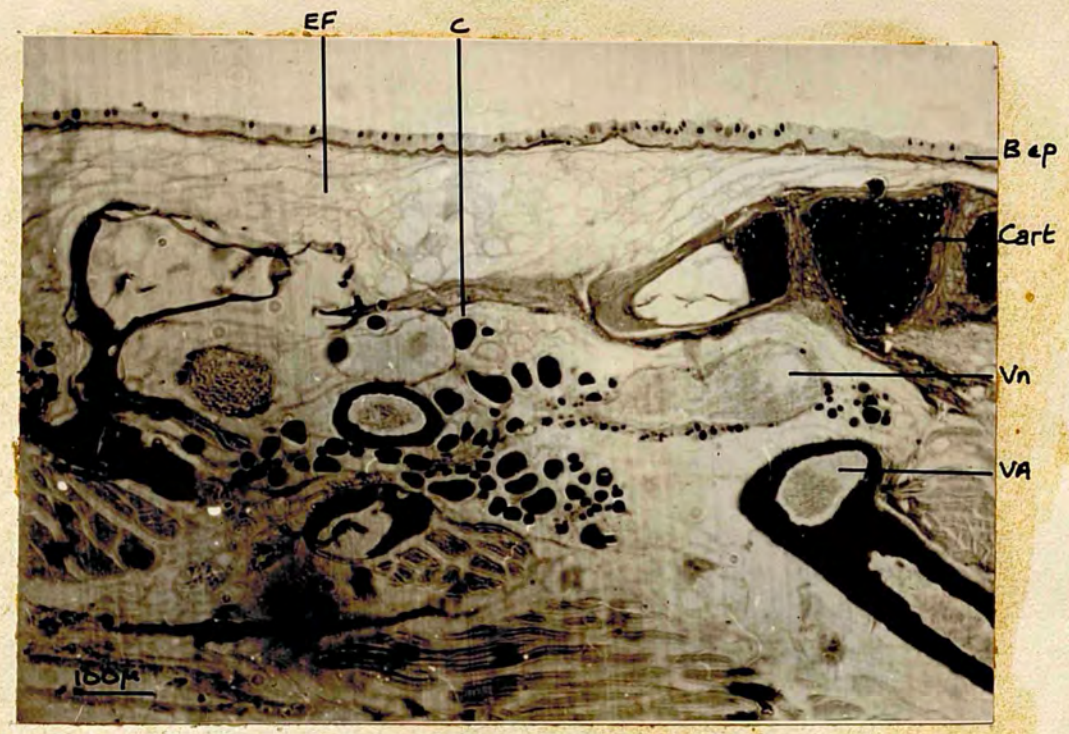
a. Control specimen, kept in water. (A.F. & orange-G; x75).

b. Experimental specimen, kept in 0.1% thiourea solution for 6 weeks (haematoxylin & eosin; green filter; x75).

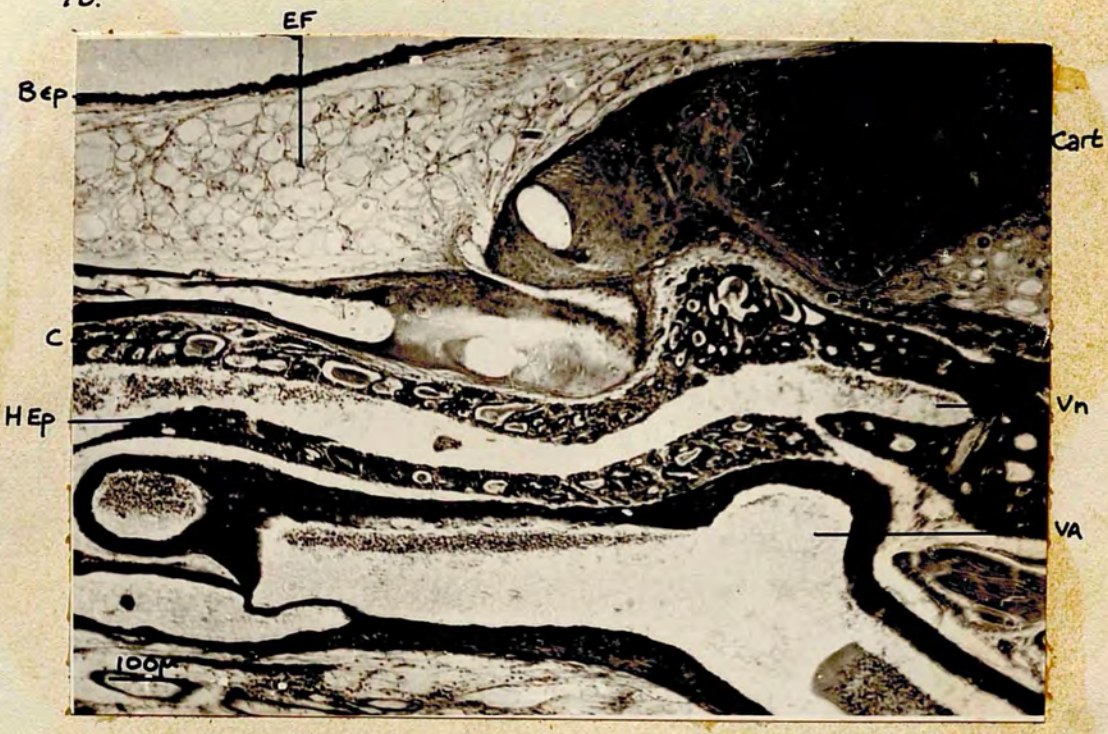
Abbreviations:

B.Ep : buccal epithelium.
C : follicle with colloid.
Cart : cartilage.
EF : region of empty follicles.
H.Ep : hypertrophied epithelium.
VA : ventral aorta.
Vn : vein, surrounded by follicles.

FIGURE 7a



7b.



The colloid in these new follicles was not vacuolated.

The use of the A.F. stain, in the hands of certain workers (Barrington & Matty, 1955; Matty & Matty, 1959) results in a tinctorial distinction between gonadotrophs and thyrotrophs, only the latter giving a positive reaction. Such a differentiation has not been achieved in the teleost pituitary by other workers. In the present investigation this failure is probably due to the A.F. stain used, since in trial observations using this stain on the minnow pituitary fixed in formol sublimate (the fixation employed by Barrington & Matty) all the cyanophils gave a strong positive reaction.

The Meta-adenohypophysis.

The meta-adenohypophysis is clearly distinguished from the other glandular regions of the pituitary and surrounds the large posterior portion of the neurohypophysis. In young cichlids it is relatively small and fails to enclose the neurohypophysis posteriorly; in older fish the meta-adenohypophysis has extended around the nervosa from either side and fused posteriorly and ventrally in the mid-line. This line of fusion is marked by a median sagittal sheet of connective tissue. The meta-adenohypophysis is composed of two cell types: orange-G acidophils and cyanophils.

Acidophils.

These are usually stained by the orange G component in Azan; they are also stained with phloxine and give a positive reaction with P.A.S. They are rounded cells, with a central nucleus, and are

always found bordering the nervosa. Their cytological appearance differs in fish from black and from white backgrounds. In fish maintained on a black background, and also in most fish kept under normal aquarium conditions, these cells are fairly large with dense non-granular cytoplasm (Figure 8.). After only 1 week on a white background these cells give a weaker staining reaction with acidophil stains or P.A.S. while small vacuoles occur in a few of the cells. After 3-6 weeks on a white background the cells are reduced in size, usually very little cytoplasm surrounds the nucleus and this cytoplasm stains extremely weakly or not at all with acidophilic stains or with P.A.S. These cytological changes strongly suggest that the cells have regressed and become inactive on a white background.

Cyanophils.

These cells are stained both by aniline blue and by lead haematoxylin. They are more numerous than the P.A.S.+ve acidophils and do not all abut directly on to the neurohypophysis but achieve connexion with it by means of cytoplasmic "stalks" which penetrate between the acidophils and even occasionally between the cells of the meso-adenohypophysis. The intensity of staining reaction is variable but usually similar in most of these cells in any one pituitary. In some cases they may be stained an extremely dark blue with Azan, when they will also stain densely with lead haematoxylin. Vacuoles may occur within the cells, and large colloid globules are frequent between the cells.

The cytological condition of these cells cannot be correlated with the background on which the fish is kept.

Figure 8. Cells of the meta-adenohypophysis (Cichlasoma nigrofasciatum).

a. The meta-adenohypophysis of a long-term black-adapted specimen, showing distribution of P.A.S.+ve acidophils. (P.A.S. & orange-G; green filter; x 400).

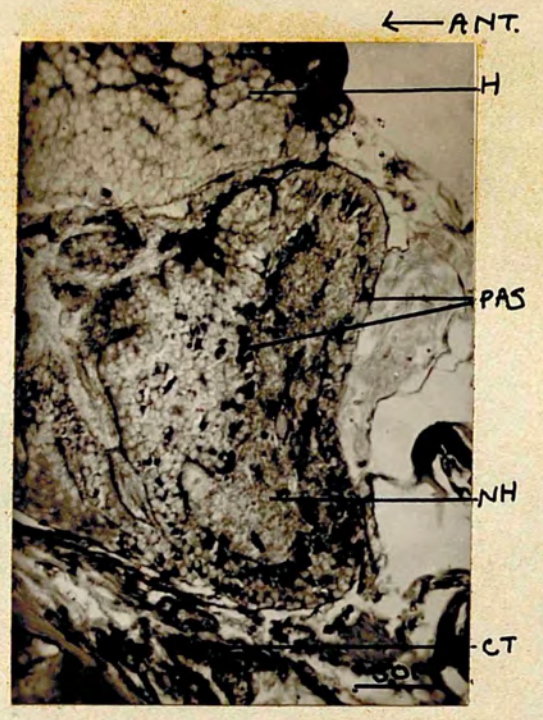
b. Long-term black-adapted Cichlasoma showing large acidophils. These appear chromophobic due to the red filter (lead-haematoxylin & phloxin; red filter; x 1000).

c. Long-term white-adapted Cichlasoma, showing small, degranulated acidophils (lead-haematoxylin & phloxin; green filter; x 1000).

Abbreviations:

CT : connective tissue.
H : hypothalamus.
NH : neurohypophysis.
P : pituicyte.
PAS : P.A.S.+ve acidophils.
Pb : lead-haematoxylin+ve cyanophil.
V : vacuole in lead-haematoxylin cell.

FIGURE 8a



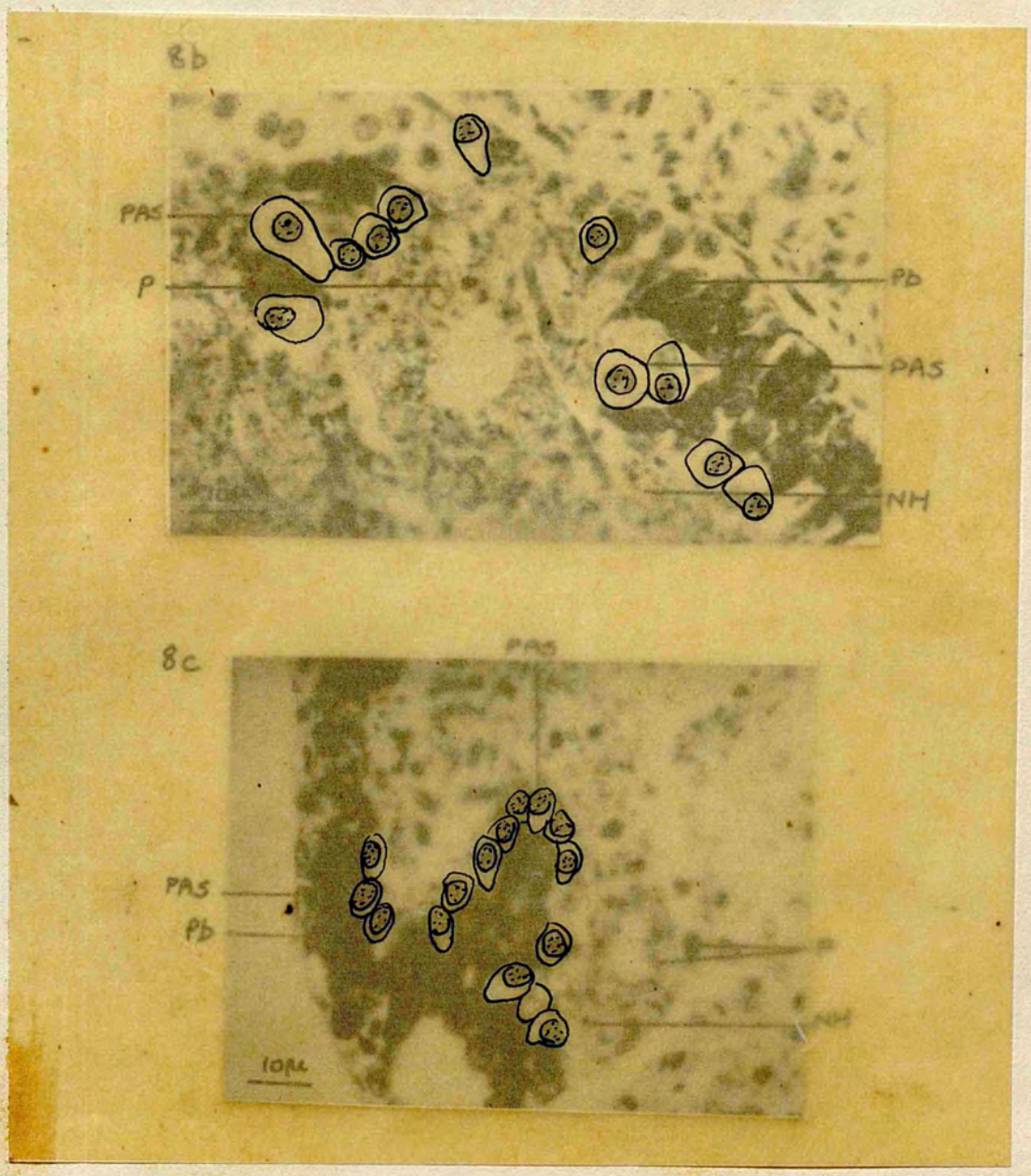
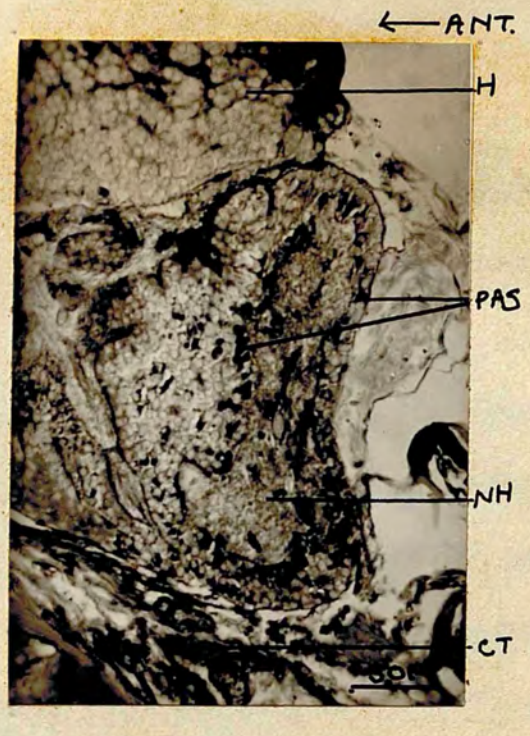
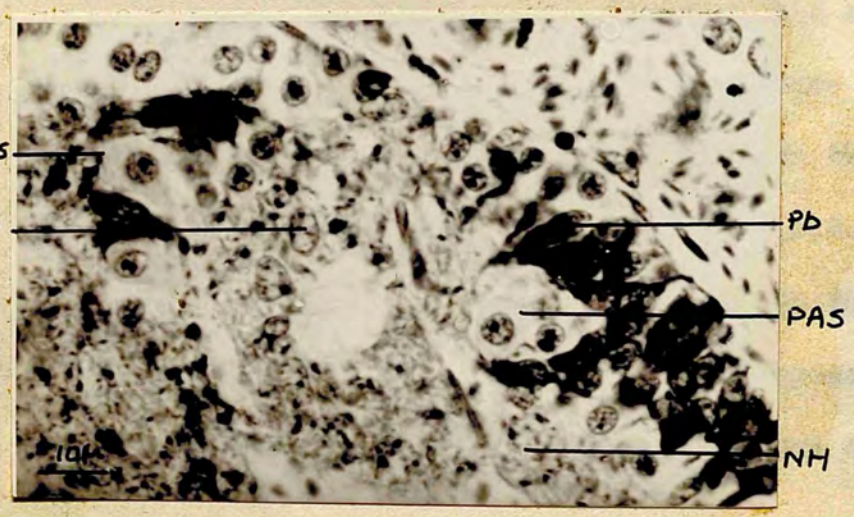


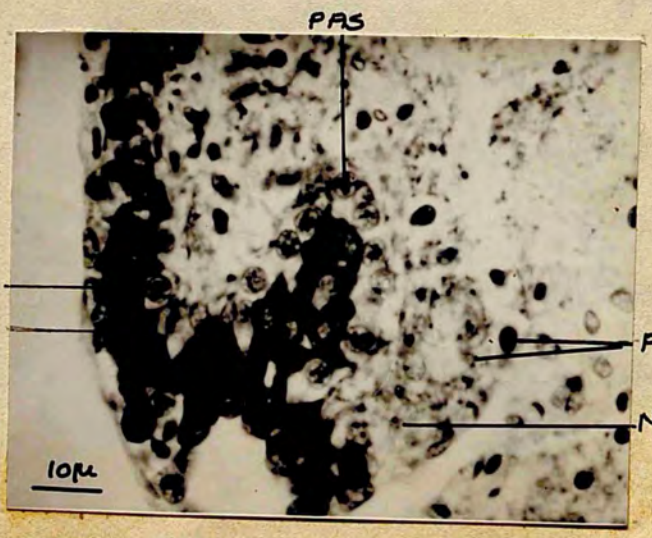
FIGURE 8a



8b



8c



§ 4. DISCUSSION.

Seven distinct cell types may be distinguished in the adult cichlid pituitary gland and it is possible to suggest which are the target organs of the hormones produced by three of these; these three cell types are the cyanophils A and B of the meso-adenohypophysis and the P.A.S.+ve acidophils of the meta-adenophypophysis.

Many workers who have studied the teleost pituitary have identified the P.A.S.+ve cyanophils of the meso-adenohypophysis as thyrotrophs and gonadotrophs; this conclusion is in agreement with the identification of these cells in other classes of vertebrates. Furthermore, the limited biochemical data on the thyrotrophic and gonadotrophic hormones in mammals suggest that they are glyco- or muco-proteins (Li & Evans, 1948; Purves & Griesbach, 1951) and the cells producing them could therefore be expected to stain with P.A.S., which is a histo-chemical stain for glyco- and muco-proteins. On the basis of these previous investigations it seems reasonable to suppose that the P.A.S.+ve cyanophils in cichlids also are the thyrotrophs and gonadotrophs.

In mammals it has proved possible to distinguish between these two types of cell by using the A.F. stain, with which only the thyrotrophs give a positive reaction. A similar differentiation has also been achieved in teleosts by Barrington & Matty (1955) for Phoxinus and by Matty & Matty (1960) for Rutilus. Other workers, however, have had less success with this stain. In Cichlasoma all

the cyanophils stained with A.F. and it is necessary to distinguish between the two cell types by other methods.

Gonadotrophs have been identified in teleosts by the cytological changes which they undergo in response to various experimental procedures such as gonadectomy (Atz, 1953; Sokol, 1955) or injections of steroid hormones (Bretschneider & de Wit, 1947). Most frequently, however, they have been recognised, as in higher vertebrates, by their cytological changes during the reproductive season (Scruggs, 1951; Fontaine & Olivereau, 1949; Olivereau, 1954; Atz & Pickford, 1959, etc.). The changes noted at this time include an increase in the number of gonadotrophs, an enlargement of both the cell and its nucleus, an increased staining reaction and the formation of large cytoplasmic vacuoles. In some species acidophilic granules have also been described in the cytoplasm (Kerr, 1942; Bretschneider & de Wit, 1947).

In Cichlasoma only the group B cyanophils show any change which can be associated with the reproductive cycle; during the later stages of vitellogenesis the more central cyanophils of this group exhibit many of the cytological changes noted above, namely, an increase in both cell and nucleus size, a strong staining reaction and extensive vacuolisation and it therefore seems probable that these cells are gonadotrophs. The peripheral cyanophils, although not showing such marked changes, have also been included in Group B together with the central cyanophils because in non-gravid fish all these cells are histologically similar; also in the mature male Herichthys examined

all the cyanophils of this group were equally, although not enormously, vacuolated and it was not possible to distinguish between central and peripheral elements. If one is correct in classifying all these cells as gonadotrophs, it is necessary to explain the differences between them. Rasquin and Rosenbloom (1949) describe a similar distinction between central and peripheral cyanophils in Astyanax and consider that the peripheral cells, which in Astyanax are the most vacuolated cells and have a small highly chromophilic nucleus, are the oldest cyanophils, new cells becoming differentiated at the centre of the gland near the nervosa. Although a difference in age may also cause the distinction between the Group B cyanophils in Cichlasoma, no mitotic figures were ever seen in those cells near the nervosa and the peripheral cells were less vacuolated than the central ones.

Little is known about the number of gonadotrophins produced in the teleost pituitary but pituitary extracts stimulate both vitellogenesis and ovulation and it has been suggested that more than one gonadotrophin is involved (see reviews by Pickford & Atz,1957; Ball, 1960). In this case it is possible that the peripheral cyanophils produce a different gonadotrophin from the larger central cells. It would be necessary to examine the pituitary of females from more stages of the reproductive cycle to decide this point.

Sokol (1955) found that the gonadotrophs and thyrotrophs in Lebistes, which she identified by their respective reactions to castration and thiourea treatment, formed two topographically distinct

groups and in view of the identification in Cichlasoma of the Group B cyanophils as gonadotrophs it seems possible that the Group A cyanophils are the thyrotrophs. Such a view is supported by the observation that the gonadotrophs are usually large and rounded with coarse granulation while the thyrotrophs are smaller and angular with fine granulation (Atz, 1953, Astyanax; Barrington & Matty, 1955, Phoxinus; Farquar & Rinehart, 1954, Rat.). Also while active gonadotrophs develop a single large vacuole and retain darkly staining cytoplasm, the thyrotrophs usually develop several small irregular vacuoles and become degranulated during periods of increased activity (Purves & Griesbach, 1951; Farquar & Rinehart, 1954). These distinctions exist between the Group A and B cyanophils in Cichlasoma.

A more valid identification of the thyrotrophs has been achieved in several teleosts by adding an antithyroid drug to the aquarium water. These drugs inhibit the production of thyroxine and the resultant hypersecretion of T.S.H. is accompanied by the enlargement, vacuolisation and degranulation of the thyrotrophs. During the course of the present investigations, however, experiments made with thiourea on adult cichlids failed to yield satisfactory results. In numerous teleosts the thyroid becomes hyper-active during the breeding season. This season in Cichlasoma nigrofasciatum extends over the greater part of the year (February to November) and in all the mature fish examined the thyroid showed signs of active follicle formation while the colloid in the small follicles was usually vacuolated, a condition associated with thyroxine reabsorption. (See § 3). All the fish

examined in connection with thyrotroph identification were mature and this activity of the thyroid may explain why, if the Group A cyanophils are thyrotrophs, these cells were vacuolated and degranulated in control fish as well as these fish treated with thiourea. The fact that these cells were active and degranulated prior to the experiment may explain why thiourea treatment caused no significant further cytological change. A previous observation on the cichlid pituitary has been made by Stolk (1956) who noted that a thyroid tumour in Cichlasoma biocellatum was accompanied by a considerable enlargement of the meso-adenohypophysis. Unfortunately no cytological details of either pituitary or thyroid are given. The localisation of the early A.F.+ve cells during development of Cichlasoma nigrofasciatum (§6) also suggests that the Group A cyanophils are thyrotrophs but thiourea experiments on cichlids in which these cells are not yet vacuolated are necessary to confirm this identification.

The meta-adenohypophysis has been homologised with the intermediate lobe of tetrapods (reviews by Green, 1951; da Lage, 1958) which is known to produce the melanophore-regulating hormone, and it therefore seems probable that the cytological changes in the P.A.S.+ve acidophils in the cichlid meta-adenohypophysis, associated with adaptation to white and black backgrounds, reflect the release of a chromatophore hormone. These cells become reduced in size and staining affinity in white-adapted fish and this can be interpreted as the result of diminished activity. Although a reduction in staining reaction may also occur in hyperactive pituitary cells, for instance

in thyroidectomy cells, this is accompanied by an increase in cell size and by cytoplasmic vacuolisation; the larger, darker staining P.A.S.+ve acidophils in black-adapted fish have more the appearance of active cells than the smaller, pale cells in fish kept on a white background. Both melanophore dispersion and melanogenesis appear to be under pituitary control in teleosts (Pickford & Atz, 1957) and since both activities occurred in cichlids maintained on a black illuminated background it is impossible to say with which particular function the hormone from the P.A.S.+ve acidophils are concerned.

§ 5. BLOOD SUPPLY OF THE CICHLID PITUITARY.

The blood supply of the pituitary was established from transverse and longitudinal sagittal sections of the entire head region of Herichthys cyanoguttatus in specimens of 10 and 13 weeks. The blood supply of adult cichlids was also examined; except for a few minor variations the arrangement of vessels was the same as in the younger fish.

The vascular supply of the pituitary has been described for a number of teleosts (Corydora - Miller, 1944; Salmonids and Cyprinids - Florentin, 1936; Salmo - Olivereau, 1954; Tinca - Lenys, 1960; Rhodeus - Bretschneider & de Wit, 1947; Phoxinus - Barrington, 1960), while Green (1951) gives a comparative review for both tetrapods and teleosts.

A plan of the pituitary vascular system of Herichthys is given in Figures 9a,b,c. The gland receives arterial blood from the internal carotid arteries. Branches from these arteries enter the neurohypophysis in the anterior region of the pituitary and give rise to an inter-communicating system of longitudinal vessels which form a vascular network, or plexus, within the nervosa. Vessels radiate out from this plexus, following the contours of the glandular regions, and pass through the meso - and meta-adenohypophysis to unite with capillaries on the surface of the gland. Blood from these surface vessels drains into a pair of closely apposed efferent vessels leaving the pituitary ventro-median region of the meta-adenohypophysis. These communicate with the anterior cardinal veins.

Figure 9. Diagrams to show the blood supply of the cichlid pituitary.

a. L.S. , 13 week old Herichthys.

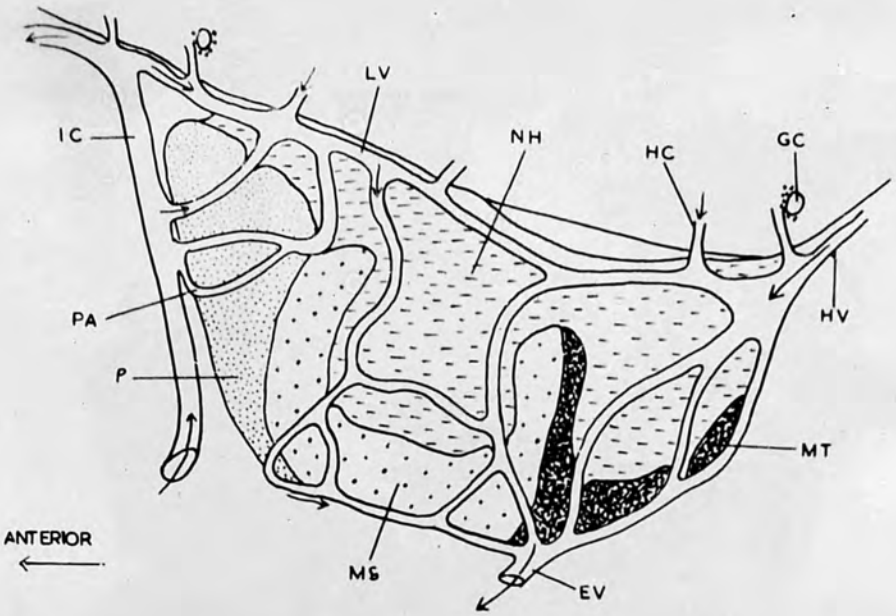
b. T.S. , through meso-adenohypophysis, 13 week old Herichthys.

c. Plan of the blood vessels in the posterior region of the pituitary of an older cichlid.

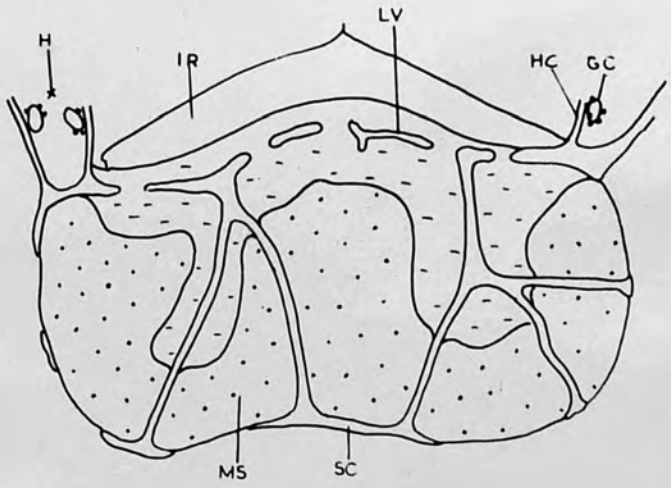
Abbreviations:

EV : efferent hypophysial vein.
GC : granular cell of the hypothalamus.
H : hypothalamus.
HC : capillary from the hypothalamus.
HV : median hypocranial vein.
IC : internal carotid artery.
LV : longitudinal vessel.
MS : meso-adenohypophysis.
MT : meta-adenohypophysis.
NH : neurohypophysis.
P : pro-adenohypophysis.
PA : arteriole to pro-adenohypophysis.
SC : capillary network on the surface of the pituitary.
SV : saccus vasculosus.

FIGURE 9 a



9b



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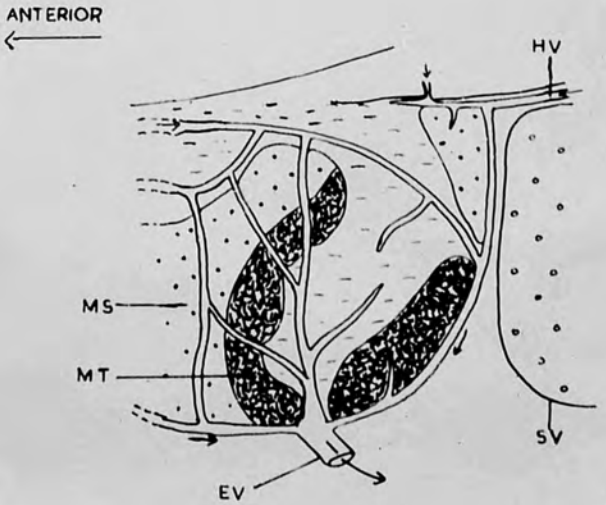


Small branches from the internal carotids also supply the pro-adenohypophysis directly with arterial blood.

The pituitary also receives a supply of venous blood. Capillaries from the meninges around the brain and from the saccus vasculosus drain into a median hypocranial vein, posterior to the pituitary. In young cichlids this penetrates the gland at its posterior junction with the hypothalamus and communicates with the vascular plexus in the neurohypophysis: at this point of entry also arises a pair of vessels which penetrate and subdivide within the main lobe of the neurohypophysis. These branches also drain into the ventral efferent vessels. In the adult gland, the afferent veins are relatively smaller than in younger fish and appear to have shifted their point of entry into the pituitary due to the backward growth of the meso-adenohypophysis. The efferent veins are relatively large and receive vessels from all regions of the pituitary before leaving the neurohypophysis (figure 9c). The main posterior lobe of the neurohypophysis thus does not possess a vascular supply independent of that in the glandular region, such as one finds in tetrapods. Consideration of this vascular arrangement suggests that the blood flows in the direction indicated by arrows in figures 9a,b,c. It is of interest to compare this with the pituitary vascular system in Rhodeus which has been described by Bretschneider and de Wit (1947). In this fish the direction of blood flow appears to be opposite to that suggested for cichlids; blood from the median hypocranial vein is distributed over the surface of the gland whence it flows through the

adenohypophysis before draining into efferent vessels in the dorsal region of the neurohypophysis.

In addition to the venous blood entering the pituitary via the median hypocranial vein, small vessels from the meninges - receiving blood from the capillary network in the hypothalamus - drain directly into the vascular plexus of the neurohypophysis. In many teleosts there appears to be no vascular connexion between the diencephalon and the pituitary (Salmo - Olivereau, 1954; review by Green, 1951), although other reports suggest that a vascular connexion does exist in some fish (Salmonids - Florentin, 1936).

In adult cichlids the meningeal vessels form a vascular link between the hypothalamus and the pituitary, while in young cichlids very small capillaries in the walls of the 3rd ventricle appear in some cases to pass directly into the pituitary. Although there is no hypophysial portal system, such as exists in tetrapods, it is possible that the capillaries may transport secretions of the hypothalamus to the pituitary. Vessels from the region of the pre-optic nucleus pass posteriorly in the walls of the 3rd ventricle to communicate with a system of fine capillaries in the ventral hypothalamus. Associated with these capillaries are cells containing numerous granules which stain with the neurosecretory stains - A.F. and Gomori's C.A.H. (Figure 16 , page 55). These cells differ from the usual neuro-secretory neurones and are possibly not even of neural origin; the cell body never appears to be extended into an axon or fibre and the granules are always grouped closely around the nucleus. These cells are usually found near the meningeal surface of the ventral

hypothalamus pressed closely against a capillary vessel. The capillaries either drain into the meningeal vessels or end in the ependymal lining of the 3rd ventricle while in young cichlids (stages up to 13 weeks examined) a few capillaries also appear to communicate directly with the neurohypophysial plexus. Granular cells were never seen in adult cichlids, but occur in the ventral hypothalamus of young goldfish (Carassius auratus), adult Tiger-Barbs (Barbus partipentazona) and adult minnow (Phoxinus phoxinus) in which they have been described by Barrington (1960). This author also describes their close connexion with the blood capillaries in this region and has found granules, similar to those found in the cells,within the capillaries thus emphasising the possibility that these cells have a secretory function and exert their influence via the blood system. As Barrington has pointed out, this arrangement is analogous to the median eminence of tetrapods, in which neurosecretory matter passes into the capillaries prior to being transported to the pituitary. In minnows, as in cichlids, many of the capillaries associated with the granular cells end in the ependymal lining of the 3rd ventricle. It is uncertain to what extent secretions in this ventricle may influence the pituitary but Stahl (1958) has found that the activity of the nucleus lateralis tuberis, whose secretions also pass into the 3rd ventricle, coincides with that of the gonadotrophs and with the maturation of the gonads and he suggests that these events are all correlated and that the secretions in the ventricular fluid may influence pituitary function.

§ 6. DEVELOPMENT OF THE CICHLID PITUITARY.

The progressive changes in shape which the pituitary undergoes during its development are illustrated in figure 10. The primordium of the adenohypophysis grows backwards from the roof of the buccal cavity as a solid rod of cells and at the time of hatching it is closely applied to the infundibular recess. At this stage it is composed of numerous cells with sparse chromophobic cytoplasm surrounding a large oval nucleus with a distinct nucleolus. Cell boundaries cannot be seen. By the second day the adenohypophysis as seen in longitudinal section has assumed an ovoid shape, the anterior end being drawn into a stalk which is attached to the roof of the buccal cavity. At this stage a vascular supply has been established; the paired internal carotids lie along the ventro-lateral surface of the gland and the anterior cardinals drain blood away from it. During the following days the adenohypophysis elongates and the stalk is reduced to a narrow strand of cells which has disappeared completely by the 5th day. The first indication of a neurohypophysis is seen on the 4th day when nerve fibres containing neurosecretion are visible between the adenohypophysis and the ependymal cells of the infundibulum. The neurohypophysis enlarges with the penetration of more nerve fibres, especially into the posterior region of the gland, so that by the end of the first week a distinct, small neurohypophysial lobe is established into which migrate ependymal cells from the infundibular recess. Further changes in the shape of the pituitary during the following weeks are the result of differential growth of its regions. Finger-

Figure 10. Diagrams showing the development of the pituitary of Herichthys cyanoguttatus.

1. At hatching.
2. Beginning of the second day (about 24 hours after hatching).
3. 3rd day after hatching.
4. 6th day after hatching.
5. 10 days after hatching.

Figure 11. The hypophysial blood supply at the end of the 2nd day after hatching.

Abbreviations:

ACH : anterior cardinal vein.
AH : adenohypophysis.
BV : blood vessel.
E : ependymal cells.
FI : floor of infundibulum.
HV : median hypocranial vein.
IC : internal carotid artery.
IR : infundibular recess.
N : notochord.
NH : neurohypophysis.
OV : ophthalmic vein.
RB : roof of buccal cavity.

FIGURE 10.

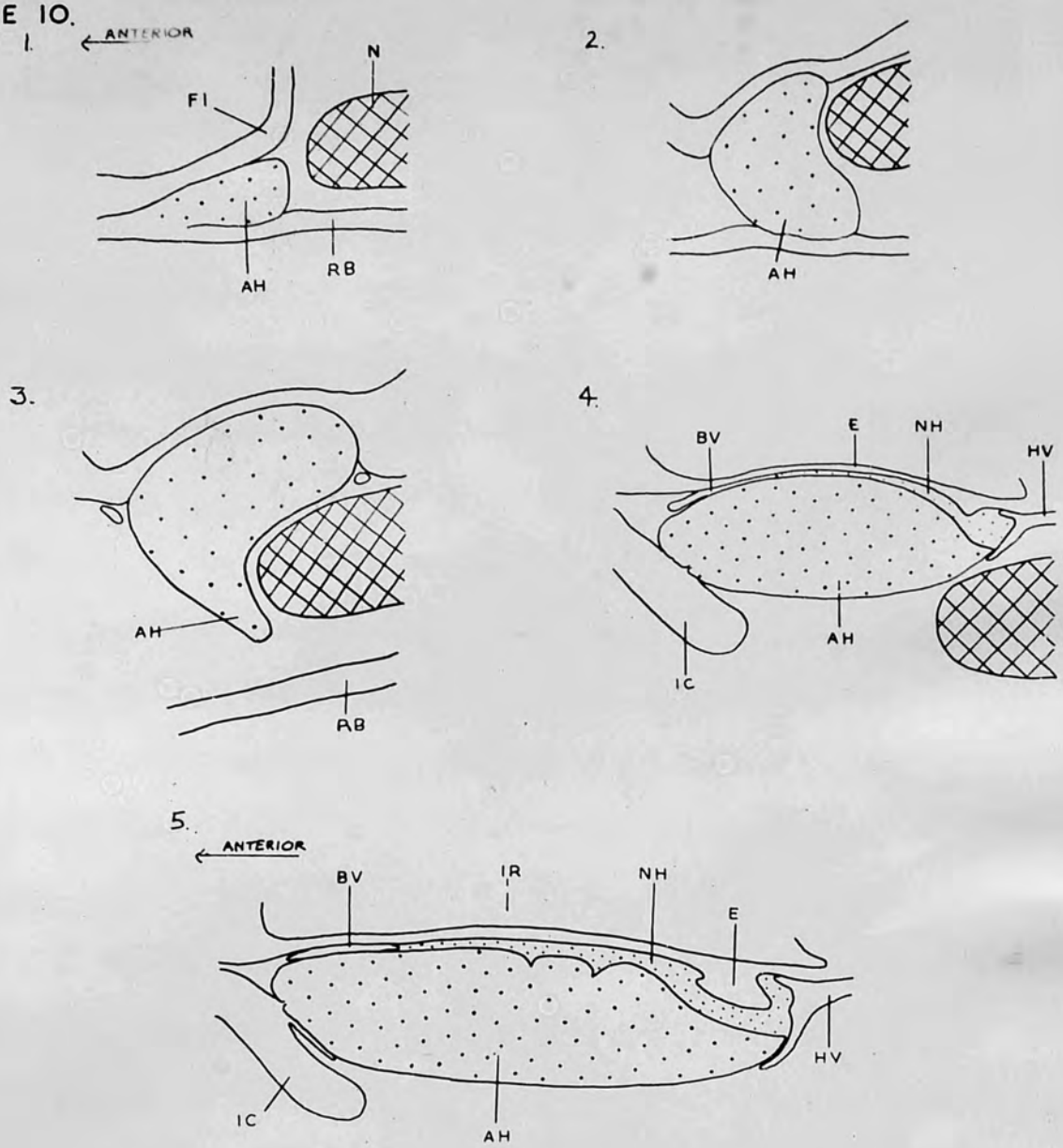
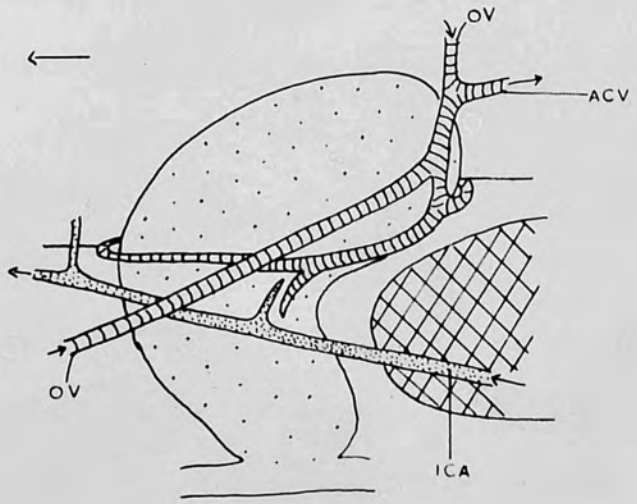


FIGURE 11.



like branches of the neurohypophysis, accompanied by blood vessels, project into all regions of the adenohypophysis while the main posterior portion of the neurohypophysis enlarges and becomes surrounded by the glandular tissue.

i. Differentiation of the pituitary cells.

Three distinct cellular regions, pro-, meso- and meta-adenohypophysis, are not clearly established until the second week, by which time the majority of the cells characteristic of these regions can be distinguished tinctorially. The first chromophilic cells, however, can be detected as early as the 2nd day when the adeno-hypophysis is still connected to the buccal epithelium by the hypophysial stalk.

Cyanophils.

At the beginning of the 2nd day (about 24 hours after hatching) it is possible to detect in some fish a few cells which give a weak positive reaction with A.F. and Gomori's C.A.H. At this stage the internal carotid arteries and the anterior cardinal veins lie close to the pituitary and by the end of the 2nd day these vessels are associated with small capillaries on the surface of the pituitary (figure 12). No well-defined capillaries are found within the gland at this stage but small indentations and breaks in the thin surrounding connective tissue capsule are associated with minute but distinct channels between the cells. These become more clearly defined during the following days. The A.F.+ve cells become gradually stronger staining and may be found bordering the vascular channels. Large

globules of colloid-like secretion, which stain pale mauve with A.F. and pink with P.A.S. occur in the cephalic veins and occasionally also in the ventricle of the heart. These are first seen on the 2nd day after hatching, while on the 4th day small, darker staining droplets appear in some specimens in the capillaries over the ventral surface of the pituitary. Droplets similar to this latter secretion have been noted by Phillips & Schmidt (1957) in the hypophysial vessels of the fetal rat, and interpreted by them as droplets of thyrotrophic hormone.

By the 5th day some cells can be seen to possess cytoplasmic prolongations or stalks which extend towards the neurohypophysis. By this stage also, well-defined longitudinal blood vessels are established within the neurohypophysis. It is not possible to say whether these two features are in any way connected. The cell stalks do not appear to make direct contact with the blood vessels but end at the boundary between the nervosa and adenohypophysis (figure 13). No cyanophils can be located with Azan staining until after the 6th or 7th day.

It seems, from the observations of several workers, that thyrotroph differentiation precedes that of the gonadotrophs (Jost & Taverier, 1956, rat; Grignon, 1957, chick; etc.) and it is probable that in Herichthys all the A.F.+ve cells during the early stages of development are thyrotrophs. It is not possible to state the exact age at which gonadotrophs become differentiated, since they could not be distinguished from the thyrotrophs by their staining reactions. At about 2 weeks numerous A.F.+ve cells are scattered throughout the

Figures 12, 13, 14 & 15 showing the distribution of A.F.+ve cells (=Gomori's C.A.H. cells) in the developing pituitary.

12. T.S. pituitary gland of Herichthys, 2 days after hatching (Gomori's C.A.H. & phloxin; yellow filter; x600).

13. L.S. pituitary gland, 14 day old specimens.

a. Herichthys (A.F.; green filter; x600).

b. Cichlasoma (A.F.; green filter; x600).

14.a. L.S. pituitary gland, 10 week old Herichthys (A.F. & orange-G; green filter; x400).

b. L.S. pituitary gland, 5 week old Cichlasoma. (A.F.; green filter; x400).

15. T.S. pituitary gland, 10 week old Herichthys. (A.F. & orange-G; green filter; x400).

Abbreviations:

AC : tributary of anterior cardinal vein.
AF : A.F.+ve cells; note long stalks to neurohypophysis.
G : granular cell of hypothalamus.
H : hypothalamus.
IC : internal carotid artery.
NH : neurohypophysis with neurosecretion.

FIGURE 12

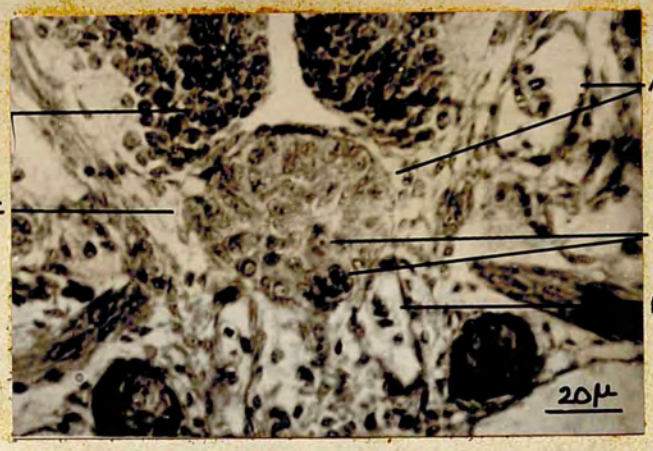
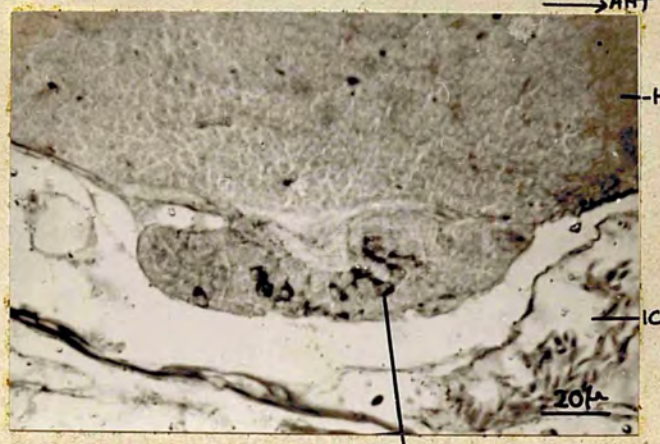


FIGURE 13a



13b

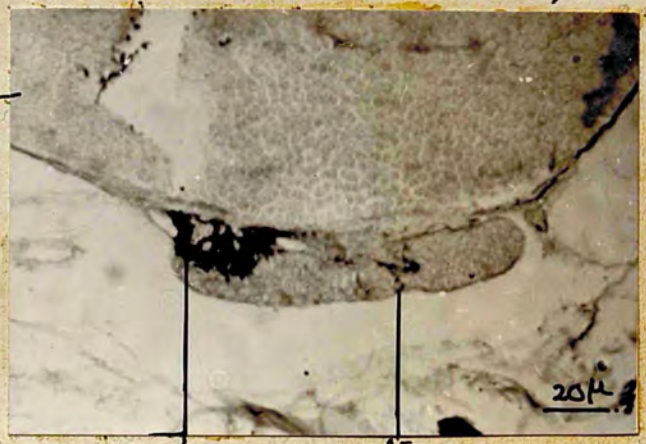
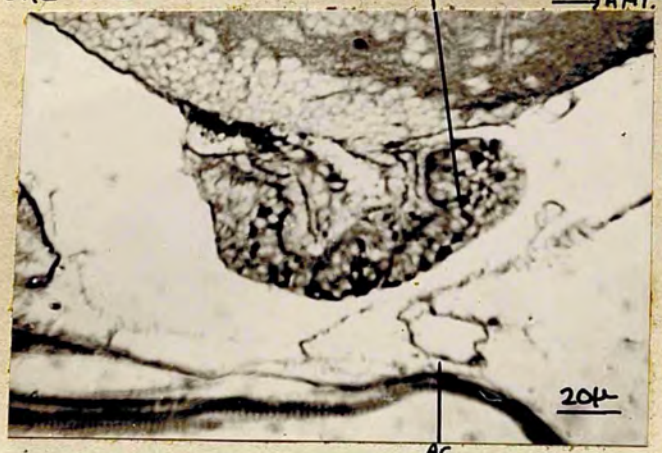


FIGURE 14a



14b

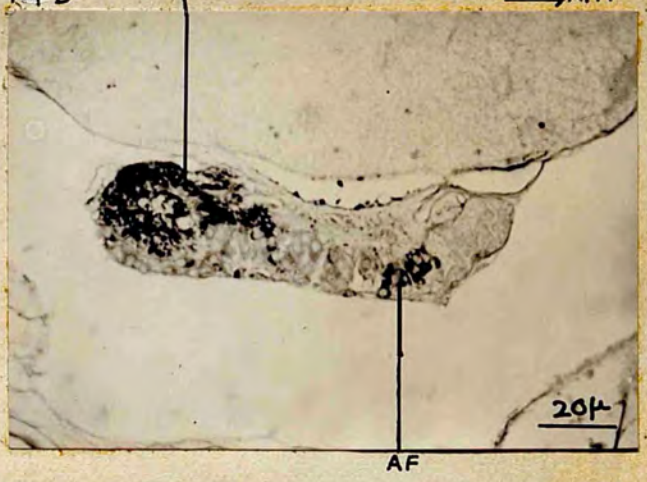
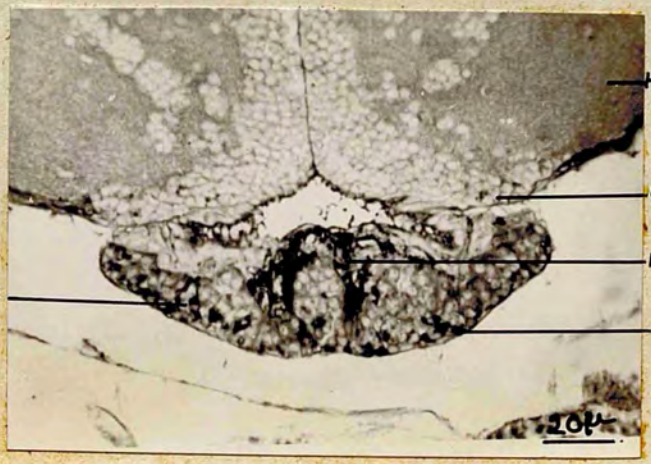


FIGURE 15



pituitary and reference to the distribution of cells in the adult gland suggests that those cells situated at the periphery of the gland are probably gonadotrophs. By 10 weeks the number of A.F.+ve cells has greatly increased, especially in the lateral regions of the pituitary. A few of these cells in the anterior meso-adenohypophysis are by this time situated in direct contact with the neurohypophysis, that is, in the position occupied by the Group A cyanophils of the adult gland (presumed to be thyrotrophs). The majority of the A.F.+ve cells (probably all gonadotrophs) are separated from the nervosa by the acidophils but usually still maintain contact with it through their cellular "stalks", although these may be very fine or absent in a few of the more peripheral cells.

An interesting difference exists in the pituitary of the cichlid, Cichlasoma nigrofasciatum. In this species very few A.F.+ve are present at 2 weeks. At 5 weeks these cells have increased in number but are nearly all concentrated in the region of Group A cyanophils and are only very rarely found at the periphery of the gland. (Figure 14b).

This difference both in the number and arrangement of the A.F.+ve cells of Herichthys and Cichlasoma may be significant in view of the difference which also exists in the rate of oöcyte proliferation in these two genera. In Herichthys at 1 week very few oöcytes are present; these are arranged in two single rows ventral to the kidneys. At 5 weeks the number and size of these primary oöcytes has not significantly increased. In Cichlasoma, on the other hand, the oöcytes

are much more numerous than in Herichthys one week after hatching and by 5 weeks, although the number of oöcytes was not counted, it is obvious that they have increased still further in number but not in size (fig.18).

Acidophils.

Large acidophils, both in the anterior region of the pituitary (pro-adenohypophysis) and in the central region (meso-adenohypophysis), give a faint positive reaction with azocarmine or phloxine by the 6th or 7th day. The nuclei of these cells are usually large and pale and often slightly polymorphic, especially those of the meso-adenohypophysis. These latter nuclei tend to be orientated with their elongated axes at right angles to the nervosa, an arrangement which they may already show on the 5th day.

The acidophils of the pro-adenohypophysis increase in number and extend forward tending to spread around the internal carotids which fuse with one another at the anterior limit of the pituitary and come to lie immediately ventral to the gland.

Cells of the meta-adenohypophysis.

The cells of the meta-adenohypophysis are the last to become tinctorially differentiated. At the end of the 2nd week some of the cells which surround the now much enlarged posterior neuro-hypophysial lobe, give a positive reaction with P.A.S. They do not react with Gomori's C.A.H. but stain with phloxine by which they may be distinguished from the cyanophils of the meso-adenohypophysis.

The intensity of the staining reaction of these cells is

Figure 16. Detail from figure 15 showing granules in certain of the hypothalamic cells (A.F. & orange-G; green filter; x 1500).

Figure 17. L.S. pituitary gland of 9-day old Cichlasoma from a black background showing P.A.S.+ve acidophils in the meta-adenohypophysis (P.A.S.; green filter; x 1200).

Figure 18. L.S. of developing gonads.

a. 2 week old Herichthys (haematoxylin & phloxin; green filter; x 150).

b. 2 week old Cichlasoma (haematoxylin & phloxin; green filter; x 150).

c. 5 week old Cichlasoma (haematoxylin & phloxin; green filter; x 150).

Abbreviations:

BV : blood vessel.
G : granular cell of hypothalamus.
N : neurohypophysis.
O : ovary with primary oöcytes.
PAS : P.A.S.+ve acidophil of meta-adenohypophysis.

FIGURE 16

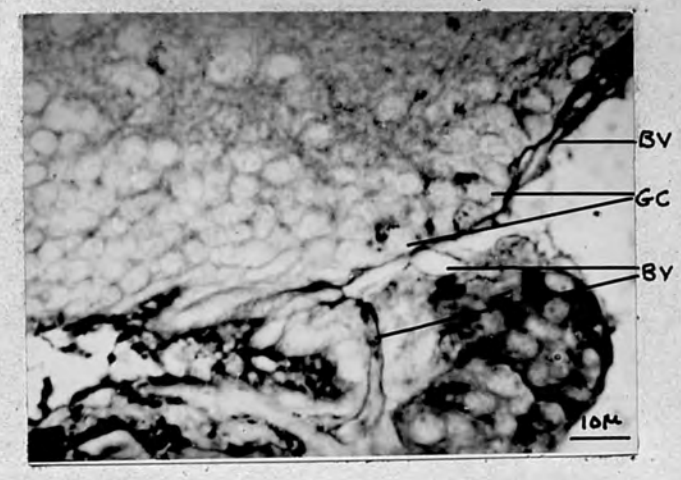


FIGURE 17

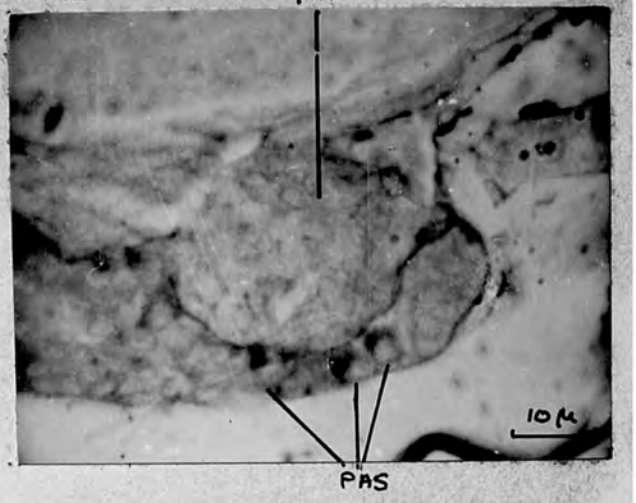
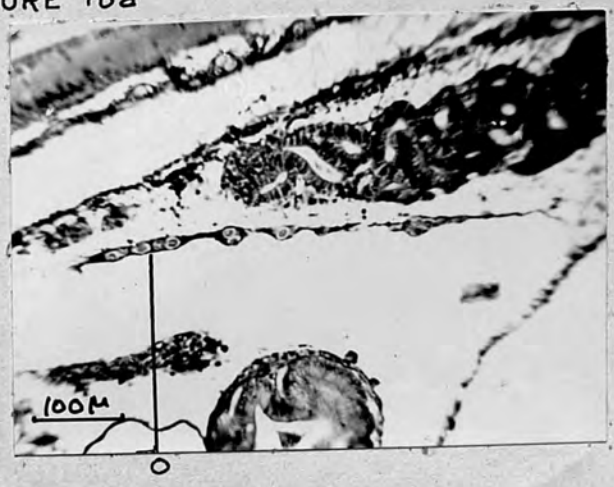
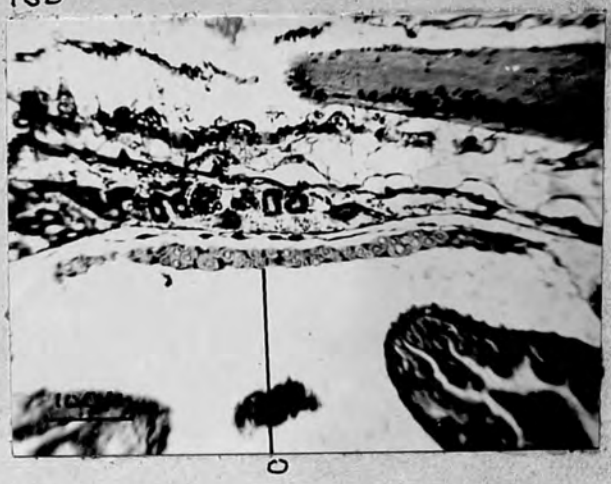


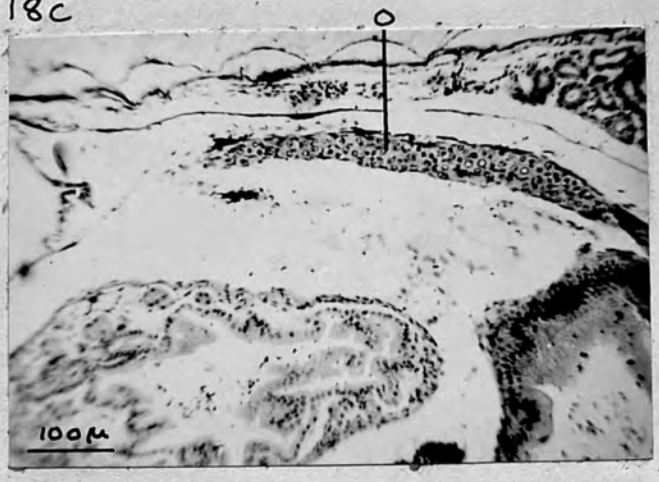
FIGURE 18a



18b



18c



markedly affected by the background on which the fish are kept. In those fish which have been reared on a black background the cells give a slight positive reaction with P.A.S. or phloxine on the 14th day and a stronger reaction by the 19th day. On the other hand, in fish reared on a white background these cells fail to give a positive reaction with either P.A.S. or phloxine. These results agree with those obtained in the adult cichlid, Cichlasoma nigrofasciatum.

A similar result was usually obtained in young Cichlasoma nigrofasciatum which had been reared on black or white backgrounds. Three experiments were done with this species. P.A.S.+ve acidophils can first be detected on the 9th day after hatching and in two of the experiments these were present in fish from a black background but not in those from a white background (Fig 17) In a 3rd experiment they could not be detected in fish from either background. In all three experiments the tanks containing the fish were placed near a window where they received good daylight illumination. In the two experiments (made during October and March) from which positive reactions were obtained in black-adapted fish, the laboratory in which the tanks were kept was artificially illuminated until 10 p.m. During the third experiment, done in March, the laboratory was not illuminated during the evenings and the tanks therefore received daylight illumination only. It is possible that the amount of illumination may be an important factor controlling the activity of these cells.

The basophils of the meta-adenohypophysis (lead haematoxylin cells) differentiate only gradually. No definite positive staining

reaction was given by these cells until about 5 weeks, after which they became gradually more strongly reactive. At 13 weeks this lobe of the adenohypophysis is still small relative to the size in adults and the main neurohypophysial lobe is not yet completely surrounded by the cells of the meso- and meta-adenohypophysis, as it is in the adult.

ii. The hypothalamus.

In most of the developing stages of Herichthys cyanoguttatus cells containing large A.F.+ve granules were seen in the ventral region of the hypothalamus (fig.16). The granules, which also give a strong positive reaction with Gomori's C.A.H., occur grouped closely around the cell nucleus and would appear to be a form of secretion. They are first seen in developing fish about 1 week after hatching. Such granules were present in all young fish fixed in Smith's bichromate but were often less well preserved in Bouin-fixed material and in some cases could not be detected at all.

Similar cells were also noted in the hypothalamus of adult minnows (Phoxinus phoxinus), goldfish, Carassius auratus) and tiger-barbs (Barbus partipentazona), but were never found in adult cichlids. The nature of these cells is discussed further in § 5.

TABLE 1. NORMAL TABLE OF THE DEVELOPMENT OF HERICHTHYS CYANOGUTTATUS.

Age	ADENOHYPOPHYSIS	NEUROHYPOPHYSIS	THYROID(See PART 11)	OTHER MORPHOLOGICAL FEATURES	CICHLASOMA
1st day	Solid rod of cells wedged between diencephalon and buccal epithelium. Cells chromophobic.	–	–	–	–
2nd day	Ovoid. Hypophysial stalk broad. A few very faint A.F.+ve cells.	–	Colloid granules and empty vescicles around root of ventral aorta.	Beginning of melanin deposition in eye cup. Appearance of dispersed melanophores over ventral region of body.	–
3rd day	Hypophysial stalk narrower.	–	Further secretion of colloid.	Appearance of dispersed melanophores over dorsal region of body.	–
4th day	Hypophysial stalk very narrow.	A few neurosecretory fibres present.	Follicles with homogeneous colloid formed.	–	–
5th	Hypophysial stalk disappeared. A.F.+ve cells slightly darker staining and more numerous. Some with "stalks" to nervosa. Nuclei of presumptive acidophils show orientation towards the nervosa.	Further penetration of nerve fibres. Longitudinal blood vessels penetrate neurohypophysis.	Follicles increased in number and also slightly in size.	Oral plate perforated.	–
6th day	–	–	–	–	Melanophores of fish kept on a white background become concentrated.
7th day	Gland elongated. Acidophils in pro- and meso-adenohypophysis.	Further increase in size, especially in posterior region of gland.	–	Swim-bladder dilates. Fish becomes free-swimming. Secretory granules appear in cells in the ventral hypothalamus.	–
8th day	–	–	–	Melanophores of fish kepton a white background become concentrated.	–
9th day	–	–	–	–	P.A.S.+ve acidophils present in fish reared on a black background.
14th day	A.F.+ve cells in position of gonadotrophs. P.A.S.+ve acidophils present in fish reared on a black background.	–	–	–	–

§ 7. DISCUSSION.

The first cells to become histologically differentiated are the A.F.+ve cyanophils which can be detected with A.F. but not with Azan as early as the 2nd day after hatcing (4th day after laying). The early appearance of these cells is in contrast with the observations of previous workers on other teleosts, in which cyanophils could not be detected until late in development, and in some instances not until after the differentiation of the acidophils (Buchmann, 1940, Clupea; Kerr, 1940, Salmo & Perca; Evropeitzeva, 1948, Coregonus; Tampi, 1953, Chanos; Rasquin, 1955, Albula). With the exception of Rasquin, all these investigators employed one of the classic trichrome stains which have been found in the present investigation to be relatively insensitive for the detection of cyanophils during the early stages of their differentiation.

In the adult pituitary gland both group A and B cyanophils, including the thyrotrophs and the gonadotrophs, are stained with A.F. It is probably correct to assume, however, that all the A.F.+ve cells in the pituitary during the first few days are thyrotrophs. This seems probable both because of the relative importance of the thyroid and the gonads during these early stages and from the observations of other workers, who have been able to distinguish between thyrotrophs and gonadotrophs either on the basis of their staining reaction or by their distribution in the gland (Cordier, 1954, Xenopus; Jost & Taverier, 1956, rat; Grignon, 1957, chick). Although the appearance of the thyrotrophs on the second day coincides with the first secretion

of colloid by the thyroid, it would be incorrect to assume that functional differentiation, that is, the release of hormone, necessarily accompanies histological differentiation. The work of Jost et al. (1952, 1953) on the rabbit, and of Phillips and Schmidt (1958, 1959) on the rat suggests that in these species histological differentiation of the thyrotrophs precedes by several days the release of thyrotrophic hormone. Moreover, Iakovleva (1949), working on Acipenser, has suggested that the thyroid gland in this fish is capable of secretory activity independent of pituitary stimulation; colloid secretion by the thyroid is thus not in itself adequate evidence for the release of thyrotrophic hormone. This problem is considered further in Part II.

In Cichlasoma during the first few weeks of development, A.F.+ve cells occur only in the position of the group A cyanophils of the adult pituitary, thus lending support to the suggestion that the group A cyanophils are indeed thyrotrophs.

Since the neurohypophysis and neurosecretory material do not appear until the 4th day of development, it seems possible that, as in chick (Tixier-Vidal, 1958) and Xenopus (Witschi & Chang, 1959), the thyrotrophs can produce although not necessarily release hormone without stimulus from the central nervous system.

Rebel and Marescaux (1960) were unable to detect A.F.+ve cells in the pituitary of Rana temporaria until thyroid activity had reached its peak during metamorphosis; there is little doubt, however, that metamorphosis in Amphibia is dependent on pituitary stimulation

and that thyrotrophs are functionally active before this time (Adler, 1914; Allen, 1916, 1938; Eakin, 1950). In this instance, therefore, functional differentiation precedes histological differentiation. It is possible that in Herichthys, although only A.F.+ve cells were stained before the 7th day, other cells may be functionally differentiated before this time; the orientation of the acidophil nuclei with respect to the nervosa in the meso-adenohypophysis on the 5th day is suggestive of cellular activity.

The distribution of the A.F.+ve cells in the developing pituitary of Herichthys suggests that the gonadotrophs are differentiated before the end of the 2nd week. As for the thyroid, little is known about gonad-pituitary relationship in teleosts during early development. The results of hypophysectomy experiments have shown that the time at which gonadotrophin is first secreted varies enormously for different species of vertebrates; thus in the rabbit gonadotrophin is essential for normal gonad development during foetal life at about the same time that thyrotrophin is first secreted (Jost & Danysz, 1952) while in the mouse, gonadotrophins do not become essential for gonadal development until much later in life (Raynaud, 1959). In chick gonadotrophin is secreted at a later stage than thyrotrophin although still early in development, before hatching (Fugo, 1940). In Amphibia, on the other hand, it seems that gonadotrophs become both histologically and functionally differentiated late in development, during metamorphosis, although it should be pointed out that in all these examples it is possible that gonadotrophin is released

some time before it becomes essential for normal gonad development (Kerr, 1939; Willier, 1955). The effects of gonadotrophin on developing teleosts are also very variable. Pituitary extracts are said to stimulate sexual development in some young, immature species of teleost (Lebistes, Xiphophorus, Fundulus, Carassius - see Pickford & Atz, 1957), but not in others (Gobius, Vivien, 1941). Although gonadotrophins have been shown to be necessary, in adult teleosts, for vitellogenesis and ovulation, they appear to have little effect on newly-formed primary oöcytes. (Reviews by Pickford & Atz, 1957; Ball, 1960). Primary oöcyte production is rapid during the post-spawning period when the gonadotrophin content of the pituitary is low, and there is some evidence that the hormone may actually inhibit oöcyte differentiation (Ball, 1960). In Herichthys during the first few weeks of development there are few primary oöcytes but the gonadotrophs appear to be already differentiated; in Cichlasoma, on the other hand, primary oöcytes are relatively numerous at 2 weeks and, unlike the situation in Herichthys, continue to be produced during the following weeks, while few or no gonadotrophs (group B cyanophils) can be detected in the pituitary. Although the difference in gonad development between these two species may not have an hormonal origin, it is interesting to consider the possibility that in Cichlasoma the production of primary oöcytes reflects the absence of gonadotrophic hormone.

PART II

Pituitary-Thyroid Relationship during Development

§ 1. INTRODUCTION.

The control which the pituitary exerts over the thyroid gland was first demonstrated in Amphibia, when it was found that the thyroid failed to secrete colloid after hypophysectomy but that this activity was restored by injections of pituitary extract or by pituitary implants (Adler,1914; Smith,1916; Allen,1918). The presence of a thyroid stimulating hormone (TSH) has since been demonstrated in the pituitary of numerous species from all classes of vertebrates with the possible exception of the elasmobranchs.

The pituitary and thyroid regulate each other's control by a feed-back system: the release of TSH into the circulation stimulates the rate of thyroxine production by the thyroid while an increase in the level of thyroxine in the blood restrains the release of TSH. The thyrotrophs in the pituitary, responsible for the production of TSH, have been identified in many animals and shown to be P.A.S.+ve cyanophils.

Many workers, using in most cases the classic trichrome stains, have been unable to detect cyanophils in the pituitary during the early stages of development at a time when the thyroid follicles are already differentiated and actively engaged in colloid production. It has therefore been suggested that either the thyroid can function independently of pituitary stimulation during its early stages of

activity, or that pituitary cells other than the cyanophils can produce T.S.H. In Amphibia, for instance, the pioneer work of Adler (1914) and that of numerous subsequent investigators has shown that the pituitary is necessary for thyroid activity during early development, while Etkin (1939) demonstrated the presence of T.S.H. in the pituitary at the initial stages of thyroid differentiation. Kerr (1939) studying the developing pituitary of Rana temporaria therefore suggested that the acidophils, which were the only cells to be stained by Azan at the onset of thyroid activity, might be the site of T.S.H. production. A similar view was expressed by Buchmann (1940) who studied the teleost Clupea. On the other hand, the work of Rumph & Smith (1926) on the pig suggests that in this species the thyroid is independent of pituitary control during the early stages of its secretion; thyroxine was shown to be present in the 90 mm. embryo, but T.S.H. could not be detected by bioassay methods until the 260 - 280 mm. stage. This suggests that the thyroid is autonomous during its early development. Such an idea is not untenable, since it has been shown that in the adult the thyroid may possess a residual secretory activity after hypophysectomy (Randall & Albert, 1951; D'Angelo, 1955).

During the last decade the problem has received considerable attention in tetrapods, where further experiments and the use of new staining techniques have shown that typical thyrotrophs become both histologically and functionally differentiated at a far earlier stage than was previously supposed.

Relatively few investigators have studied the problem in fish. Since views on pituitary-thyroid relationship in fish must inevitably be influenced by the conclusions reached for other vertebrates, data from some of the more recent work on both tetrapods and fish will be reviewed before describing the results of the present investigation.

A. PITUITARY-THYROID RELATIONSHIP DURING DEVELOPMENT IN TETRAPODS

The problem of establishing at what stage in development the pituitary begins to influence the activity of the thyroid has been investigated in several ways. These may be considered under three headings: histological, experimental and biochemical.

i) Histological data.

Comparison of accounts giving the time at which cyanophils first appear in the pituitary shows that these cells can be detected much earlier by staining with P.A.S. or A.F. than with Heidenhain's Azan (Man - Aron, 1931; Brewer, 1957; Waterman, 1959. Chick - Grignon, 1957. Amphibia - Saxen, 1958. See also Part I, 6).

Work on the rat (Jost & Taverier, 1956; Phillips & Schmidt, 1958, 1959), rabbit (Jost & Danysz, 1952; Jost & Gouse, 1953; Waterman & Gorbman, 1956), chick (Tixier-Vidal, 1958) and Xenopus (Saxen et al., 1957) has shown that thyrotrophs are histologically detectable before the thyroid begins to secrete colloid. In the case of both rat and rabbit colloid secretion is accompanied by a degranulation of these cells, indicating the sudden release of T.S.H. and its participation in thyroid activity.

In the case of the mouse (Raynaud & Frilly, 1948), Pleurodeles waltlii (Pasteels, 1960) and Rana (Saxen, 1958) the appearance of P.A.S.+ve cells in the pituitary coincides with the first secretion of thyroid colloid. Rebel and Marescaux (1960) using the A.F. stain were unable to detect thyrotrophs in the pituitary of Rana temporaria until some time after the beginning of metamorphosis. Since it has been frequently shown, in other Amphibia, that the hypophysis is essential for thyroid activity and metamorphosis (Allen, 1916, 1918; Smith, 1916; Pasteels, 1954; Cordier, 1953) these results were interpreted as an indication that thyrotrophs may be functionally differentiated before they are detectable histologically.

ii. Experimental data.

More satisfactory evidence of thyroid-pituitary interaction may be obtained from experiments designed to alter the level of either thyroxine or T.S.H. The effect which this has on the reciprocal gland indicates the extent of the latter' s dependence on the hormone whose level has been changed.

When the thyroid is allowed to develop in the absence of T.S.H., either after hypophysectomy or when it is cultured in vitro, any retardation in its development may be assumed to be due to lack of T.S.H. Thus, hypophysectomy of the fetal rat (Carpenter, 1957; Jost, 1957) and rabbit (Jost & Danysz, 1952; Jost, 1953) shows that while the thyroid is capable of self differentiation into follicles and begins to secrete colloid at the normal age, the rate of secretion is significantly reduced so that far less colloid than normal has

accumulated after several days. Tixier-Vidal (1958), in her work on chick, finds that when thyroid rudiments are cultured alone normal follicles fail to develop and little or no colloid is secreted. These effects become more pronounced the younger the embryo from which the thyroid rudiment is taken. She found, however, that colloid secretion could be stimulated in such thyroid rudiments by culturing them with pituitaries from chicks as young as 6 - 7 days. This stimulus from the pituitary precedes by 1 - 2 days the appearance of histologically differentiated thyrotrophs and by 3 - 4 days the first secretion of thyroidal colloid under normal conditions. Experiments on hamster (Petrovic & Aron, 1955), in which the developing pituitary was grafted near the thyroid, show that in this species also thyrotrophs are functionally and histologically differentiated some time before the intial secretion of colloid.

In Rana T.S.H. may first be detected in the pituitary, by similar methods, at the same time as the thyroid begins to secrete colloid (Etkin, 1939) while in vitro experiments have shown that T.S.H. is produced at a very early age in many species. It may be argued that the hormone is not normally released into the circulation until a later stage. In overcoming this difficulty, experiments with anti-thyroid drugs are of considerable value. These drugs inhibit the synthesis of thyroxine; if a thyroid-pituitary relationship is already established, the thyrotrophs will respond to the lack of thyroxine by liberating increased quantities of T.S.H.; the presence of this hormone is revealed by the characteristic response of the thyroid.

It is obvious that these experiments can only demonstrate the presence of T.S.H. once the thyroid has begun to secrete thyroxine.

Numerous experiments have been made involving the use of antithyroid drugs on developing tetrapods but in relatively few has the effect of the drug been observed during the early stages of thyroid activity; species in which these early stages have been studied include the chick (Tixier-Vidal, 1958), rat (Jost, 1957), mouse (Kauffman et al.,1948) and guinea-pig (Logothetopoulos & Scott, 1956). Among the thyroidal changes noted are cellular hypertrophy and hyperplasia, a loss of colloid and an enlargement of the follicle lumen. In most instances some of these changes were noticeable during the first stages of colloid secretion, indicating that T.S.H. is already secreted at this time. In the guinea-pig Logothetopoulos & Scott were unable to detect any changes one or two days after the beginning of colloid secretion; they consider that T.S.H. secretion and thyroid secretion are probably synonymous but that the delay in the effects of antithyroid drug on the thyroid may reflect the time required for the pituitary to release increased quantities of T.S.H. and for the thyroid to respond. Since in the other species listed above the response to antithyroid drugs did not appear to involve a time lag, it is possible that T.S.H. in the guinea-pig does not significantly affect thyroid activity until a few days after the first secretion of colloid. The matter requires further investigation.

iii. Biochemical data.

Included under this heading are experiments involving the use of radio-active iodine I^{131}. The use of this isotope enables a determination of thyroid activity prior to colloid secretion. Iodine is concentrated by the thyroid for a greater (pig, man) or lesser (chick) length of time before the appearance of organic thyroxine precursors (Waterman, 1959). This function of the thyroid appears to be to a large extent independent of pituitary stimulus. Jost et al. (1952) state that hypophysectomy in the fetal rabbit does not significantly affect the uptake of iodine by the thyroid until about the 28th day of gestation, although T.S.H. is released from the pituitary by the 22nd day (Jost & Gouse, 1953; Jost & Danysz, 1952). This agrees with observations on adult animals in which the influence of the pituitary on iodine fixation appears to be a quantitative rather than a qualitative one (Randall & Albert, 1951).

It is clear that two problems are involved in the question of thyroid-pituitary interaction: that of the autonomous ability of the thyroid and that of the time at which T.S.H. is released. In some cases the thyroid has been shown to be capable of a certain degree of autonomous activity, although the pituitary releases thyrotrophic hormone at the onset of colloid secretion, providing "double-assurance" for thyroid activity. The histological data show that it is unnecessary to assume that the acidophils are involved with T.S.H. production.

B. PITUITARY-THYROID RELATIONSHIP IN DEVELOPING FISH

Histological descriptions of the developing teleost pituitary and thyroid are found in papers by Kerr (1940, Salmo and Perca), Buchmann (1940, Clupea), Evropeitzeva (1949, Coregonus), Irikhimovitch (1948, Abramis), Tampi (1953, Chanos) and Rasquin (1955, Albula); several Russian workers have studied the development of these glands in Acipenser (Olifan, 1945; Irikhimovitch, 1948; Iakovleva, 1949). With the exception of Rasquin (1955), who employed the A.F. stain, all investigators used a trichrome stain in studying the pituitary. In no species, including Albula, were cyanophils detected in the pituitary until after the thyroid had been actively secreting colloid for some time. In some cases, however, acidophil differentiation preceded that of the cyanophils (Buchmann, 1940; Rasquin, 1955). The suggestion has been made, as for tetrapods, that either the thyroid is independent of pituitary control during its early development or the thyrotrophs are not cyanophils (Buchmann, 1940; Rasquin, 1955). Several attempts have been made to clarify the problem using anti-thyroid drugs.

Fortune (1955) found that the thyroid of Phoxinus fry, which have been reared in a 0.05% solution of thiourea from the egg stage, is affected from the beginning of its secretory activity - the thyroid epithelium hypertrophies and the colloid is reduced and stains differently from that of the controls. It may therefore be concluded

that T.S.H. is secreted by the pituitary during these early stages, although Fortune did not study the pituitary of this fish.

The interpretations of the Russian workers are rather different. On the basis of histological observations on Acipenser stellatus Irikhimovitch (1948) suggested that the thyroid can produce but not liberate thyroxine until the pituitary cyanophils are differentiated; the sudden increase of thyroid activity, when the fish migrates seawards, coincides with the histological differentiation of the thyrotrophs. These results are contradicted by Iakovleva (1949) who reared Acipenser fry in a 0.033% solution of thiourea from the second day after hatching at which stage the thyroid was just beginning to secrete colloid. By the fifth week the growth rate was considerably retarded and there had been no further formation of dermal scutes, indicating that thyroxine is normally released before this time and affects morphogenesis. Thiourea treatment also inhibited any further differentiation of thyroid follicles and there was a complete absence of colloid; the thyroid epithelium was not hypertrophied, however, and Iakovleva concludes that no T.S.H. is secreted at this stage of development. This conclusion is accepted by Pickford & Atz (1957) in their recent review of the subject. It is assumed however that the loss of colloid is due to the direct inhibitory action of thiourea on the secretory activity of the thyroid. This point is discussed further in II,§ 4.

Evropeitzeva (1949) came to a similar conclusion for the teleost Coregonus. Her conclusion that the thyroid is free from

pituitary control during early development is rather surprising in view of the fact that, besides a loss of colloid, the thyroid showed clear signs of cellular hypertrophy.

Thus three views of pituitary-thyroid interaction during early development in fish have been advanced:

a) The thyroid is able to produce and release thyroxine without stimulus from the pituitary (Iakovleva, 1949; Evropeitzeva, 1949).

b) The thyroid is able to secrete colloid without pituitary stimulus, but release of thyroxine must await thyrotrophic differentiation (Irikhimovitch, 1948).

c) The thyroid is under the influence of the pituitary from the earliest stages of colloid secretion, although the thyrotrophic hormone may not be produced by cyanophils (Fortune, 1955; Rasquin, 1955).

§ 2. MATERIAL AND METHODS.

Developmental stages of the teleost Herichthys cyanoguttatus (blue cichlid) were used in the following experiments. Young fish were reared in the laboratory in white plastic tanks (20 cm. x 20 cm. x 30 cm.) containing 8 litres of 0.033% thiourea solution while controls were reared in an equivalent volume of tap water. Both the control and experimental tanks were placed in a single large tank of water thermostatically controlled at either 26° or 27°C.

Three similar experiments (A, B and C) were made, using three separate batches of eggs. The batches were laid at different times and were at different stages of development at the beginning of the experiment.

Experiment	Month	Age at beginning of experiment	Temperature
A	May	2 days before hatching, i.e. eggs on day of fertilisation	27°C
B	March	4 days after hatching	26°C
C	March	Approximately 1 week after hatching	27°C

The thiourea solution was changed weekly, except for a few fish from experiment A. which were left in the original solution throughout the experiment. Both control and experimental tanks were continuously aerated and the young fish were fed with Liquifry No. 1 after the 4th day, by which time the oral plate had perforated.

During experiment A specimens were fixed at the following times.

Time after hatching	Number of control fish	Number of thiourea-treated fish
Beginning of 2nd day (24 hrs.)	5	6
End of 2nd day	4	5
3rd day	5	5
4th day	5	5
5th day	7	6
6th - 10th day	8	13
14th day	Numerous	Numerous

At least 4 specimens were examined from both control and experimental groups. For experiments B and C specimens were examined at the beginning of the experiment, and subsequently at two-day intervals. Fish were removed from the tanks with a wide-mouthed pipette and fixed in Bouin. They were dehydrated in alcohol, cleared in methyl benzoate followed by benzene and embedded in a mixture of equal parts of paraffin wax M.P. 55° & 65°. Sections were cut at 5 μ and stained by one of the following methods.

1. Halmi's aldehyde fuchsin (A.F.) counter-stained with light green-orange G.
2. Heidenhain's Azan (azocarmine, aniline blue and orange G).
3. Masson's trichrome.

§ 3. RESULTS

The thyroid of all vertebrates is composed of numerous follicles, each follicle consisting of a central lumen surrounded by an epithelium of follicle cells. The central lumen is usually filled with a colloid, secreted by the follicle cells, which incorporates the precursors of thyroxine. Unlike the arrangement found in other vertebrates, where the follicles are aggregated to form a compact gland, the follicles in most teleosts occur singly, scattered around the arteries and veins in the lower jaw. Some workers (e.g. Baker-Cohen, 1958) have reported the presence of such follicles around the eye, brain, heart and kidney. This was not observed in cichlids.

(1) The effect of thiourea treatment on thyroid development.

(a) Controls.

Thyroid cells containing fine granules of secretion are first distinguishable about 24 hours after hatching, when they form a compact group around the base of the ventral aorta (fig.19). At the beginning of the 2nd day three phases of colloid accumulation may be distinguished: (Fig.21).

(i) Homogenous intracellular globules of colloid which stain weakly with A.F.

(ii) Coarse, intracellular granules which stain strongly with A.F. These form granular masses of varying sizes, the larger of which are probably extracellular, but the cell boundaries are not visible. These masses are probably an early stage of follicle formation.

Figure 19. L.S. of anterior region of 2-day old Herichthys cyanoguttatus.

Figure 20. L.S. of lower jaw of 3-day old Herichthys showing distribution of thyroid follicles (A.F. & orange-G; green filter; x 480).

Figure 21. 4-day old Herichthys (control) showing follicles with homogenous colloid. (A.F. & orange-G; green filter; x 1200).

Abbreviations:

ii	: granular colloid mass	see text
iii	: empty follicle	see text
BC	: buccal cavity	
HF	: follicle with homogenous colloid	
IR	: infundibular recess	
N	: notochord	
NT	: nucleus of thyroid cell	
OR	: optic recess	
P	: pituitary gland	
PN	: pineal gland	
RBC	: red blood corpuscle	
TH	: position of thyroid	
V	: ventricle of heart	
3rd V	: 3rd ventricle of brain	
4th V	: 4th ventricle of brain	
VA	: ventral aorta	
Vn	: vein of lower jaw	
YS	: yolk sac.	

FIGURE 19.

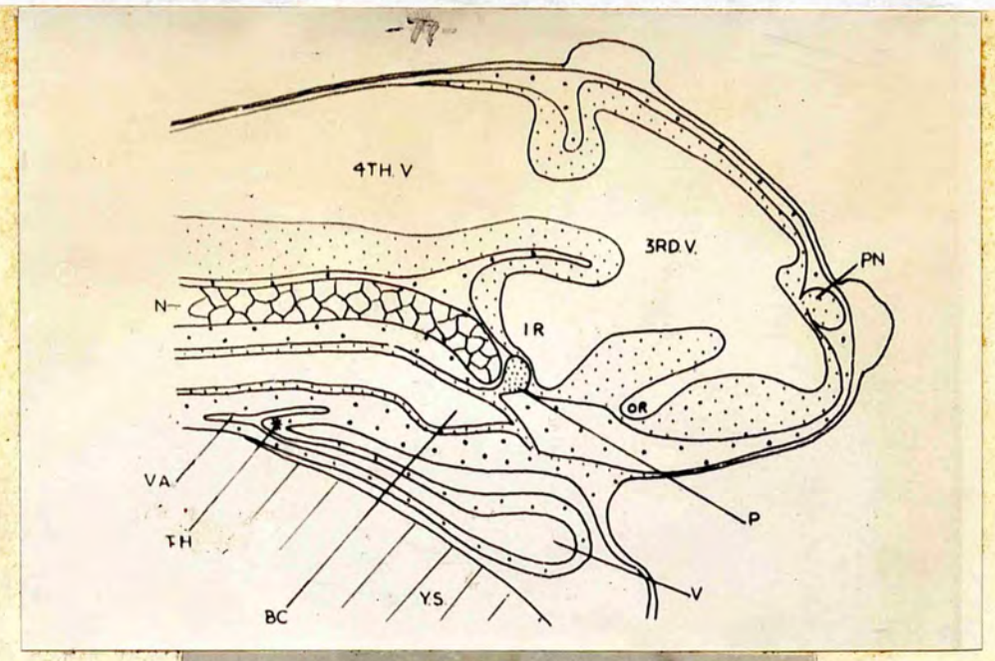


FIGURE 20.

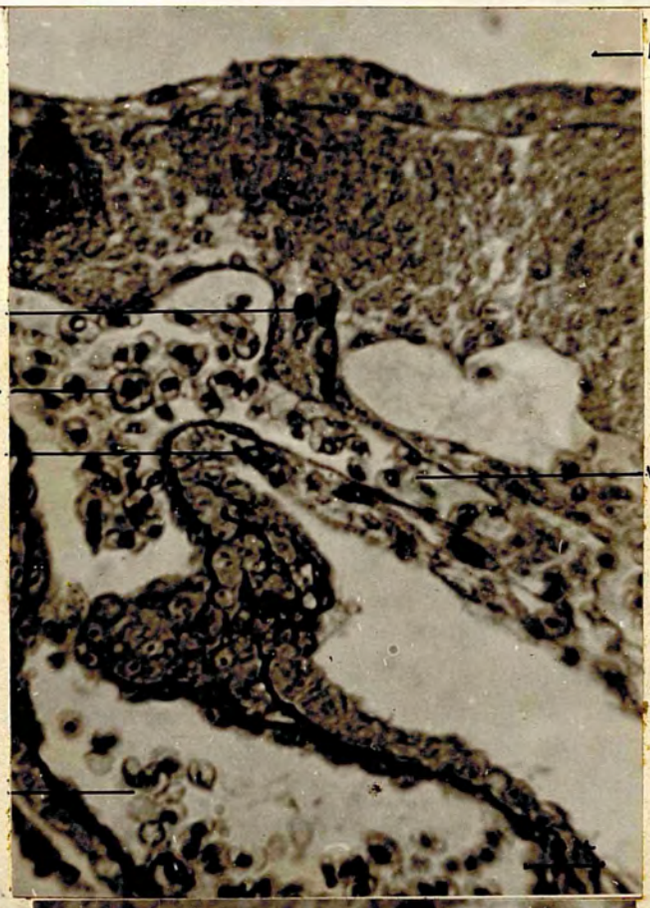


FIGURE 21.

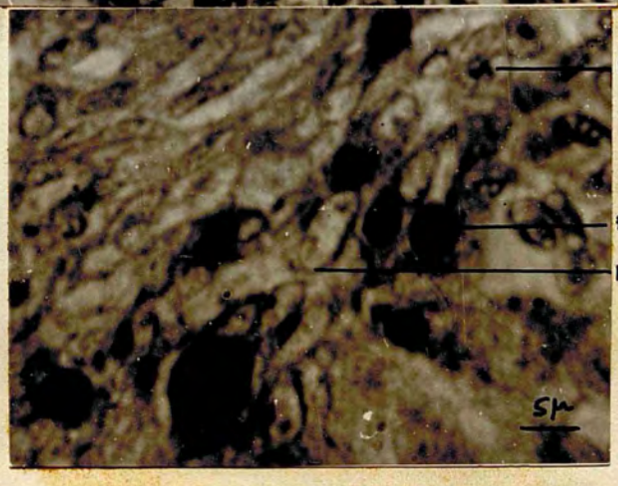


Figure 22. Diagrams to show the development of the thyroid in control and thiourea-treated Herichthys (experiment A).

a. 2nd day after hatching.

b. 3rd day after hatching.

c. 4th day after hatching.

i) control fish

ii) experimental fish.

Abbreviations:

i : large mass of homogeneous, pale staining colloid
ii : granular colloid mass
iii : empty follicle
} see text

BC : bulbus chordis of heart

FH : follicle with densely stained homogeneous colloid

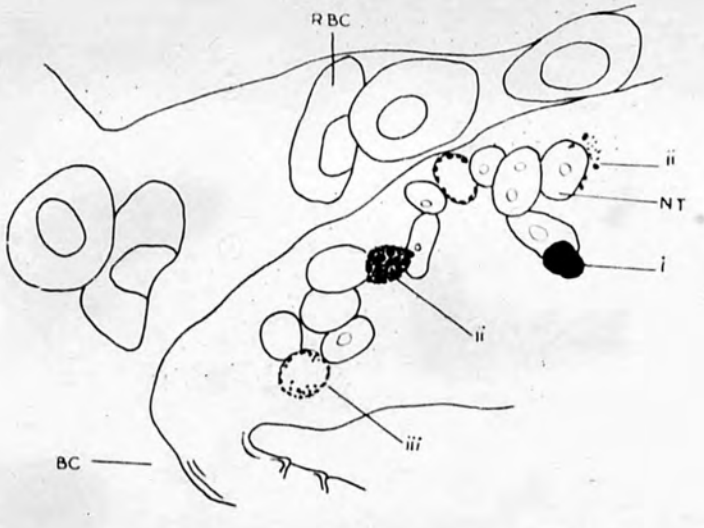
GC : granular cytoplasm of follicle epithelial cells

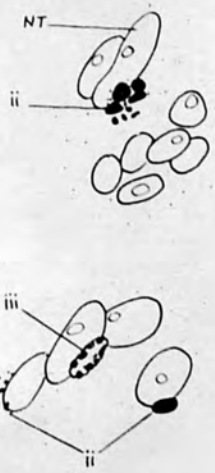
NT : nucleus of thyroid cell

RBC : red blood corpuscle.

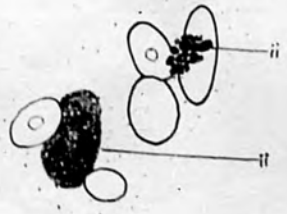
FIGURE 22

a.

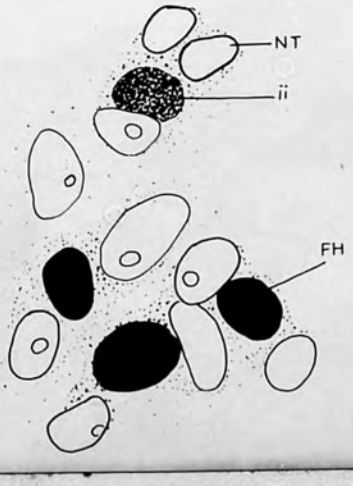




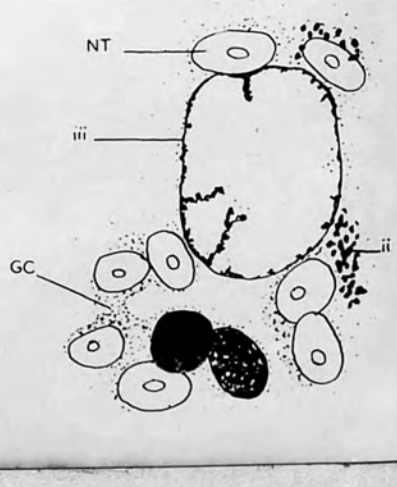
b.



c i)



c ii)



(iii) Intracellular vacuoles with coarse peripheral A.F.+ve granules. Intermediate stages may be found between these and the granular colloid masses (ii) indicating that one develops from the other. The larger of the vacuoles appear to be extracellular and are associated with several cells. These are therefore true but empty follicles.

By the third day there has been an increase in the amount of the granular colloid secretion (ii) while a careful estimation of the number and size of the empty follicles shows that these remain unchanged (Table 2). By the fourth day the thyroid cells are filled with extremely minute secretory granules, giving the cytoplasm a mauve tint after A.F. staining. Many follicles are now filled with a colloid of dense homogeneous texture, replacing the granular colloid which was observed in the earlier stages. (Fig.21,22). A few enlarged empty follicles also occur. By the 5th day very few empty follicles are to be found, while follicles containing smooth, homogeneous colloid are slightly larger than before, and have doubled in number. The cytoplasm of the follicle cells is rather sparse and contains few fine secretory granules, while the colloid, at first A.F.+ve and basophilic, becomes more acidophilic during the following days and loses its affinity for both A.F. and P.A.S.

(b) <u>Experiment A. Fish reared in 0.03% thiourea solution from 2 days before hatching</u>.

The thyroid of these fish did not differ from that of the controls until the 4th day after hatching. At this stage there was

TABLE 2. THE EFFECT OF 0.033% THIOUREA SOLUTION ON THE DEVELOPMENT OF THE THYROID⊕

AGE	CONTROL FISH					EXPERIMENTAL FISH (A)				
	Granular colloid masses*	Follicles with homogeneous colloid †		Empty follicles		Granular colloid masses	Follicles with homogeneous colloid		Empty follicles	
	Number	Number	Size (μ)	Number	Size (μ)	Number	Number	Size (μ)	Number	Size (μ)
End of 2nd day	40	-	-	12	4.8	30	-	-	17	5.0
	14	-	-	12	4.0	27	-	-	17	4.8
	37	-	-	9	4.8	48	-	-	10	6.4
	22	-	-	6	5.6	28	-	-	8	4.8
						27	-	-	8	2.4
3rd day	67	-	-	8	4.8	66	-	-	15	6.4
	41	1	-	7	4.8	22	-	-	10	4.8
	31	-	-	4	5.6	32	-	-	7	4.0
	52	1	-	3	5.2	38	-	-	5	5.6
4th day		22	9.5	10	9.6		7	4.8	15	10.4
		18	9.5	7	8.0		5	4.8	14	12.0
		32	9.5	4	8.8		4	4.8	13	12.0
		26	7.0	0	0		7	4.8	13	8.8
		-	-	-	-		7	9.5	5	14.4
5th day		41	12.0	4	12.0		-	-	18	15.2
		50	8.0	2	8.0		-	-	15	16.5
		40	13.7	1	7.2		2	9.6	15	26.4
		43	12.8	1	9.6		2	4.8	10	20.0
		57	12.8	1	8.8		3	4.0	9	16.5
		-	-	-	-		12	6.4	7	15.2
14th day		54	17.0	-	-		40	14.0	41	76.0
		58	17.0	-	-		9	9·2	49	62.0
		60	14.0	-	-		8	14·0	51	83·0
		49	11.6	-	-		20	16.5	43	95·0

⊕ Eggs placed in thiourea solution the same day on which they were fertilised.

*The term "granular colloid masses" includes both intracellular accumulations of colloid granules and follicular granular colloid. It is impossible to distinguish clearly between the two in many cases. The masses were of various sizes. All the colloid masses in every section from a continuous series were counted, no attempt being made to avoid counting twice a colloid mass which extended over two sections. These counts show that the amount of colloid has increased from the 2nd to the 3rd day, and are not comparable with other counts in this table.

† Care was taken to avoid counting more than once any follicle which extended over two sections. The size recorded is the diameter of the largest follicle.

little accumulation of homogeneous colloid but the empty follicles (fig.22) had increased in both number and size. This increase in size was even more marked by the 5th day but at this stage there appeared to be no further addition to their number. Unlike the controls, follicles with homogeneous colloid were rarely seen (see Table 2), but intracellular colloid granules were abundant. By the 9th day dense homogeneous colloid was still rare but many of the follicles had become filled with flocculent or granular colloid which was strongly A.F.+ve.

The majority of experimental fish, but not all, were placed in fresh thiourea solution at the end of the first week (8th day after hatching). There was a difference in the further development of the thyroid in fish from these two groups which suggests that the accumulation of colloid observed in 9-day old fish may have been due to a decline in the anti-thyroid properties of the thiourea solution.

By the end of the second week the fish placed in a fresh solution of thiourea possessed only a few follicles with dense homogeneous colloid; these follicles were small, and the colloid acidophilic, with very little affinity for A.F. The majority of follicles in these fish were enormously enlarged with sparse flocculent colloid which stained strongly with A.F. (Fig.24). The total number of follicles was counted in four fish. This was done by making camera-lucida drawings of all the sections from a continuous series and then comparing successive drawings so that each follicle could be traced over several sections and the total number of follicles

Figure 23. The thyroid of 6-day old Herichthys.

a. Control; fish reared in water. (A.F. & orange-G; green filter; x 480).

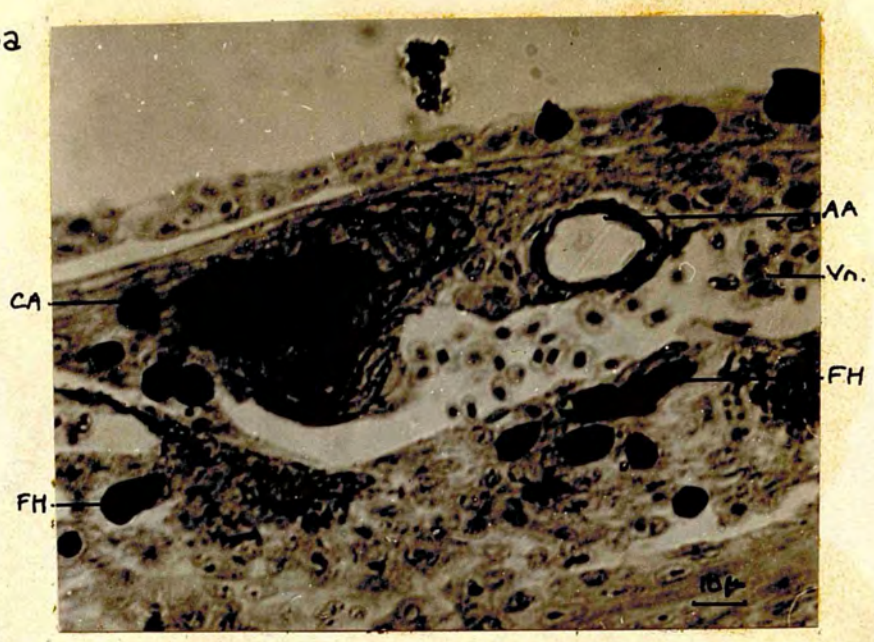
b. Experiment A.; fish reared in 0.033% thiourea solution. (A.F. & orange-G; green filter; x 1200).

Figure 24. The thyroid of 14-day old Herichthys reared in 0.033% thiourea solution (experiment A.); solution renewed after 8 days (A.F. & orange-G; green filter; x 480). Note difference in magnification of figs. 23 & 24.

Abbreviations:

AA : aortic arch vessel.
CA : cartilaginous branchial arch.
EH : enlarged follicle with sparce colloid.
FH : follicle with homogeneous colloid.
V : vein of lower jaw.

FIGURE 23a



23b

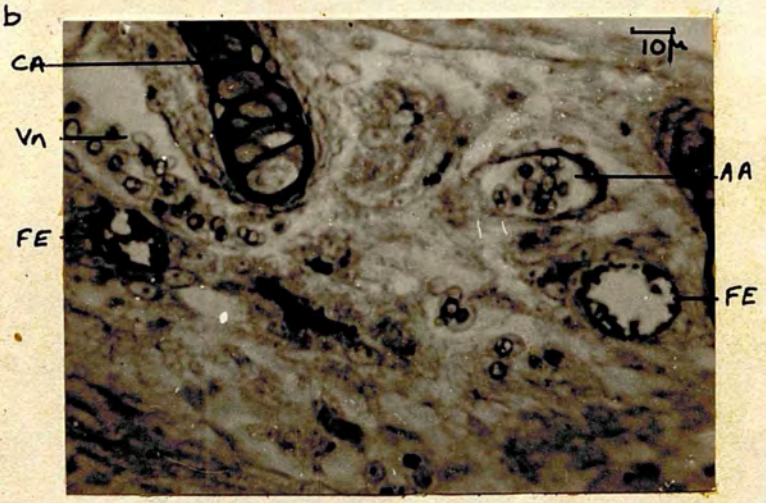


FIGURE 24

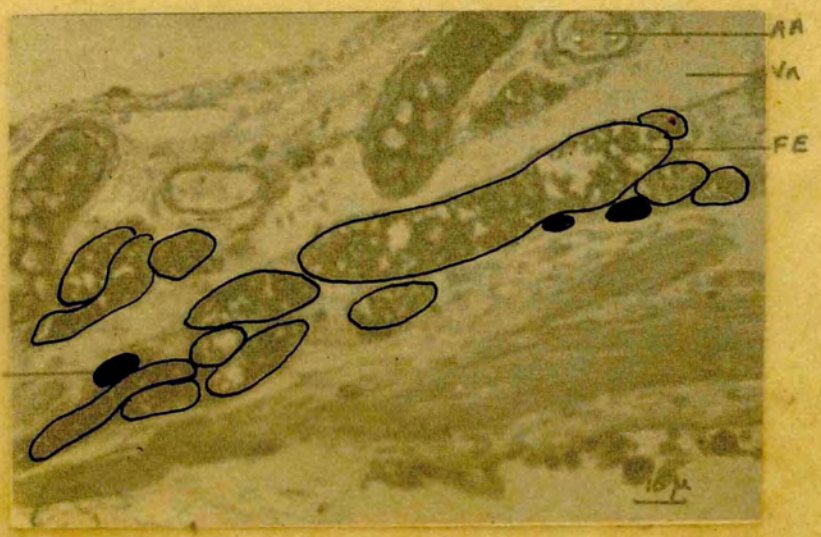
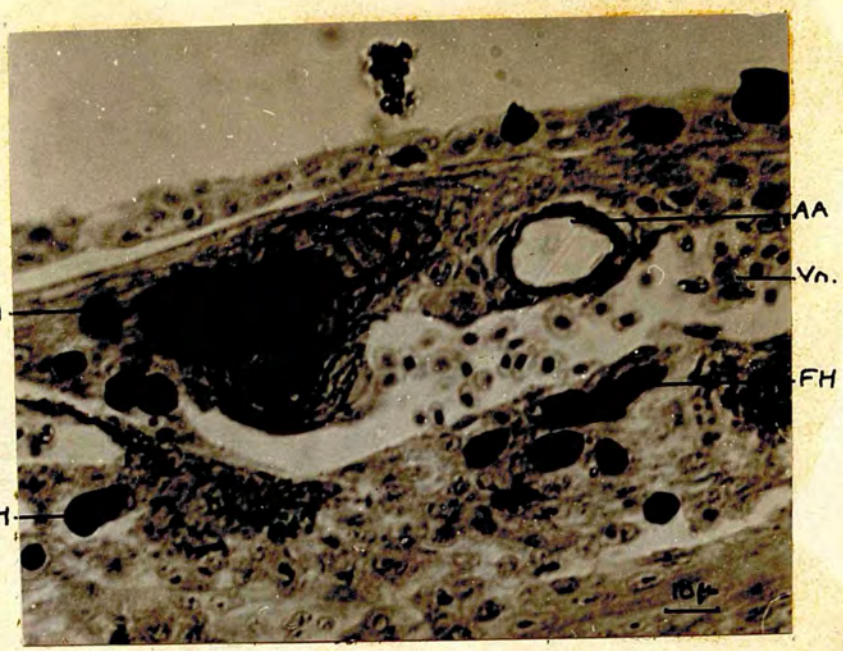


FIGURE 23a



23b

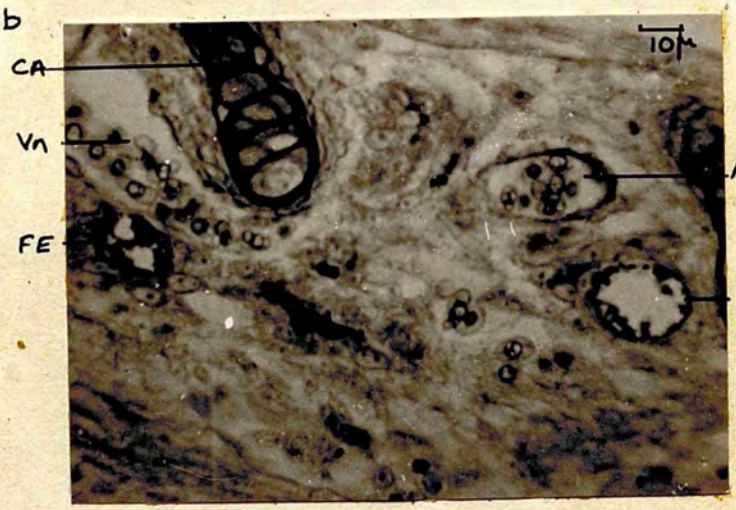
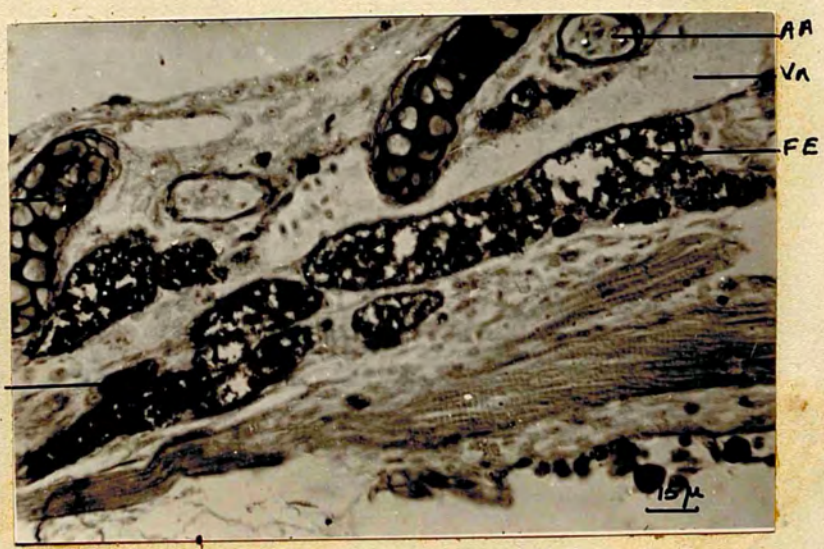


FIGURE 24



estimated. The number of thyroid cells composing these enlarged follicles exceeded that composing the smaller follicles of control fish. In a longitudinal section of an experimental fish cut at 5μ, 16 cells were counted around a follicle 74μ long. This may be compared with a follicle, fron a control fish, 12 μ long around which only 5 cells were counted in a single longitudinal section. It is obvious therefore that although the total number of follicles in experimental fish does not exceed that in control fish, there must have been an increase in the number of thyroid cells (hyperplasia). Cellular hypertrophy, on the other hand, was not observed; it is possible that hypertrophy is masked by the extension of the cells around the enlarged follicle lumen.

The thyroid follicles of fish which were not subjected to a renewed dose of thiourea were less enlarged and in these cases the epithelium appeared slightly hypertrophied (fig.25). The lumen of these follicles appeared collapsed and slit-like and the epithelial cells were crowded together, each with an oval nucleus which was orientated with its axis at right angles to the lumen. The follicle colloid was dense and A.F.+ve.

(c) Experiment B. Fish placed in 0.03% thiourea solution 4 days after hatching.

On the 4th day after hatching follicles with either homogeneous or granular colloid were present.

After 2 days in thiourea the experimental fish differed little from the controls except for the presence, in some follicle cells, of large intracellular colloid droplets.

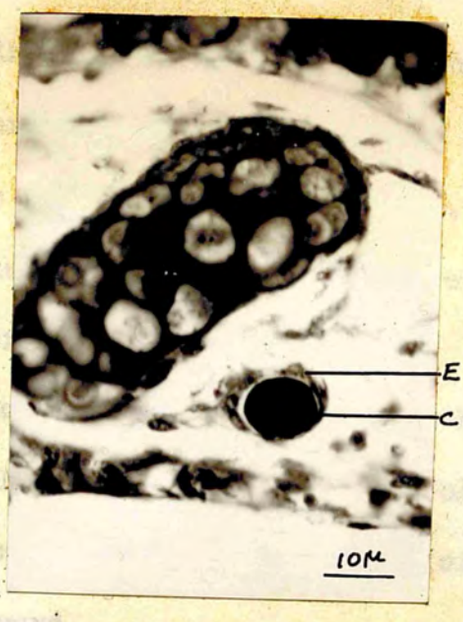
Figure 25. Thyroid follicles of 14-day old Herichthys.

a. Control specimen. (Gomori's C.A.H. & phloxin; yellow filter; x 1000).

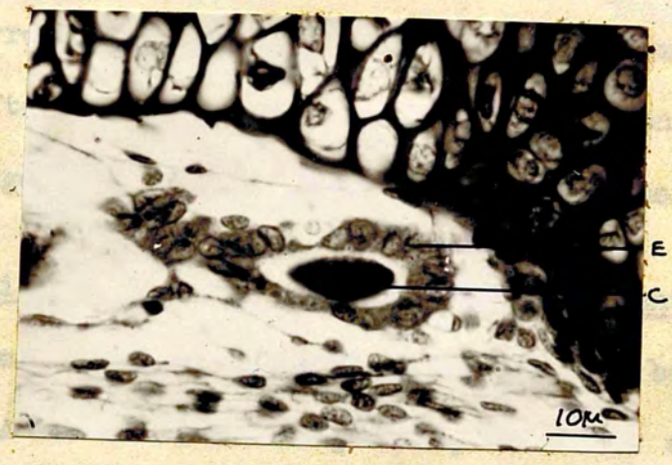
b & c. Specimen reared in 0.033% thiourea solution (experiment A.); solution not renewed after 8 days. (Gomori's C.A.H. & phloxin; yellow filter; x 1000).

Abbreviations: C : colloid
E : follicle epithelium.

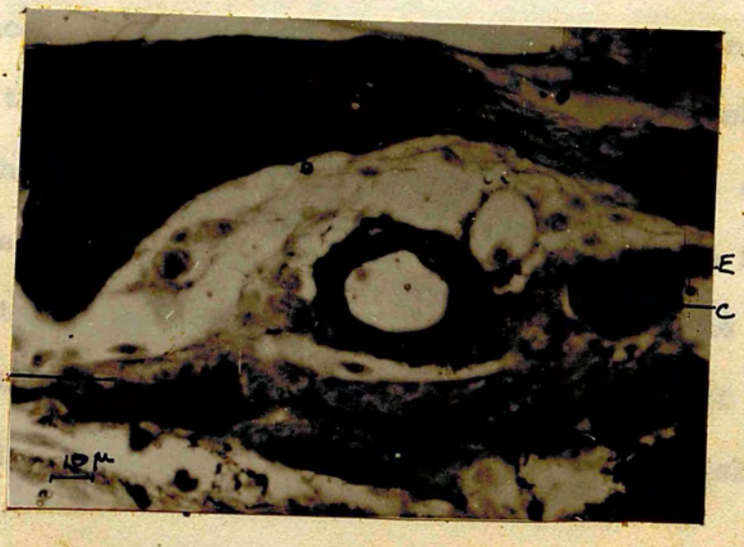
FIGURE 25a



25b



25c



After 4 days in thiourea (8 days after hatching) the follicle cells were hypertrophied and packed with large colloid droplets. The follicle lumen was, in some cases, small and contained dense colloid, while other follicles were almost empty and the colloid reduced to small peripheral droplets.

After 9 days in thiourea (13 days after hatching) the appearance of the follicles was very variable; follicles with normal homogeneous colloid, or with coarse granular colloid, or almost empty follicles could all be found.

(d) Experiment C. Fish placed in 0.03% thiourea solution 1 week after hatching.

The thyroid of these fish did not differ from that of the controls until after they had been in thiourea solution for 6 days, when similar changes occurred to those seen in Experiment B.

2). Effect of thiourea treatment on pituitary differentiation.

The normal development of the pituitary has been described in Part I, §6. A few cells staining weakly with A.F. are present in the pituitary of both control and thiourea-treated fish on the 2nd day and no difference can be detected between the pituitaries of fish from these two conditions during the early stages of development. However, by the end of the second week after hatching, the volume of the pituitary in the thiourea-treated fish from Experiment A is noticeably larger than that of the controls (fig.26). To determine whether this enlargement is the result of an increase in the number of A.F. cells or an increase in their size, the total number of A.F. cells was

counted in the pituitaries of both control and experimental fish. Only the cell nuclei were counted to avoid counting twice any cell which extended over two sections. The maximum dorso-ventral depth of the meso-adenohypophysis was measured and the number of sections over which the pituitary extended was counted to indicate the increase in pituitary volume. These results are shown in Table 3 .

Table 3. The effect of 0.033% thiourea solution on the developing pituitary.

Control			Experimental		
Total no. A.F.+ve cells	Depth	Width	Total no. A.F. cells	Depth	Width
1. 175	24 μ	85-90 μ	1. 230	32 μ	85-90 μ
2. 140	20 μ	85-90 μ	2. 195	33.5 μ	85-90 μ
3. 139	20 μ	75-80 μ	3. 188	32 μ	85-90 μ
4. 135	24 μ	75-80 μ	4. 174	28 μ	85-90 μ
			5. 154	32 μ	85-90 μ

Two facts emerge from these observations. While there is considerable variation in both the size of the pituitary and the number of A.F. cells present in fish from similar environmental conditions, it is nevertheless clear that thiourea treatment results in a significant increase in the number of thyrotrophs. It must be remembered that at this stage it is not possible to distinguish between thyrotrophs and gonadotrophs although, judging by the distribution of A.F.+ve cells, gonadotrophs are probably present; both types of cell have therefore been included in the cell counts and, assuming the number of gonadotrophs to be the same in both control

Figure 26. L.S. of the pituitary of 14-day old Herichthys (A.F. & orange-G; green filter; x 480).

a. Control specimen.

b. Specimen from experiment A; solution renewed after 8 days.

Figure 27. Section through the skin of 14-day old Herichthys (Gomori's C.A.H. & phloxin; yellow filter; x 600).

a. Control specimen.

b. Specimen from experiment A; thiourea solution renewed after 8 days.

Abbreviations:

AF : A.F.+ve cell

AMG : atrophied mucus gland

B : brain

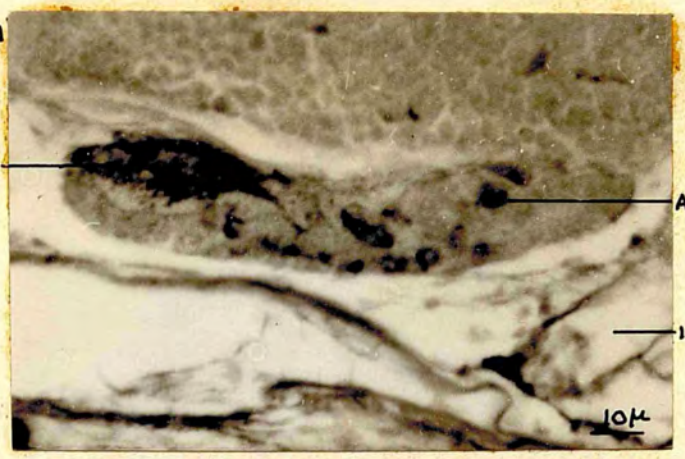
IC : internal carotid artery

M : melanophore

MG : mucus gland

NH : neurohypophysis with neurosecretion

FIGURE 26a



26b

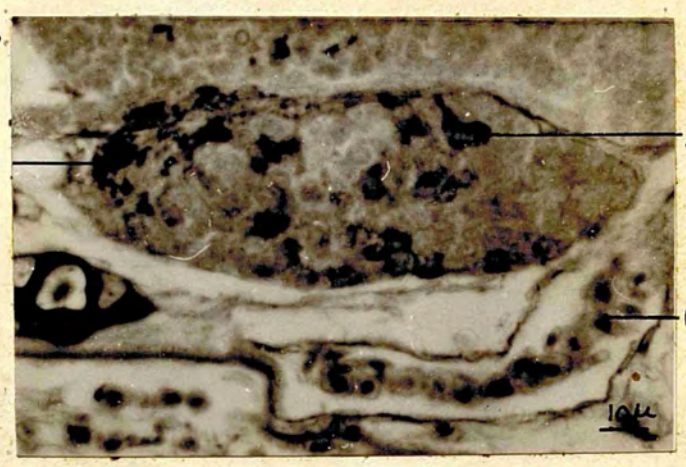
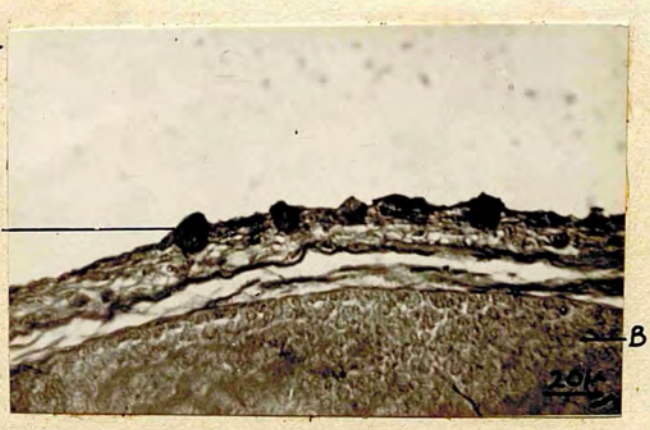
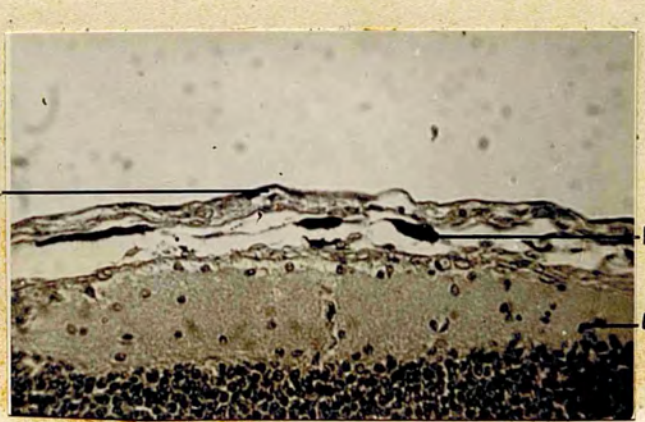


FIGURE 27a



27b



and experimental fish, it is probable that the number of thyrotrophs may be even greater in the thiourea-treated fish than the counts indicate. Where there is an overlap between the number of cells from the two groups, the size of the pituitary is still considerably larger in the experimental fish. This suggests that the increase in pituitary size may also be due to an increase in the size of the cells. The cell boundaries are indistinct and an accurate measurement of the cell diameter to confirm this deduction is thus not possible; any such measurement is also hampered by the fact that the cells are irregular in shape. There is no apparent difference in the cytological appearance of thyrotrophs from control and experimental fish.

3) Effect of thiourea treatment on morphogenesis.

The following features were affected by treatment with thiourea:

i. Swim bladder.

Normally this expands on the day after hatching, at which time the young fish leave the bottom to become free swimming.

When fish are reared in thiourea solution from the egg stage onwards (Experiment A) expansion of the swim bladder does not occur, and the fish fail to become free swimming.

In Experiment B, in which thiourea treatment began 4 days after hatching, the swim bladder expanded normally on the 7th day.

ii. Melanophores.

Melanin pigment appears in the eye cup on the 2nd day after hatching and in the melanophores on the ventral surface of the body

shortly afterwards. Dorsal melanophores appear on the 3rd - 4th day. During the first week the melanophores are fully dispersed in fish from both black and white backgrounds, but on the 8th day, apart from those over the yolk sac, all the melanophores of fish kept on a white background become fully concentrated; the melanophores of fish on a black background remain dispersed. When fish are transferred from one background to another, the melanophores become fully concentrated or dispersed in about 1½ hours. This observation, together with the fact that the yolk sac melanophores do not concentrate, suggests that chromatic changes at this stage of development are under nervous control. In fish from experiment A melanophore concentration was delayed until the 13th day. In fish from experiments B and C melanophore concentration occurred at the normal time.

iii. Skin.

The skin became very thin and the mucus glands degenerated after treatment with thiourea. This effect was noticeable in fish from all three experimental groups (A, B and C) about 9 days after immersion in thiourea solution (fig.27).

iv. Yolk sac.

The resorption of the yolk sac was slightly retarded in fish from Experiment A.

There was no apparent effect of thiourea treatment on morphogenesis prior to the 7th day.

Mauthner's cell, which in Amphibia has been shown to be affected by the level of thyroxine (Weiss & Rossetti, 1957) appeared identical in control and experimental fish. Perforation of the oral membrane and the appearance of melanin in the melanophores occurred at the normal time in fish from experiment A.

§ 4. DISCUSSION.

Before attemtping to interpret the effects of thiourea on the development of the thyroid and pituitary it is important to differentiate between those effects which are directly caused by the drug and the secondary effects attributable to the thyrotrophic hormone (T.S.H.).

It has been shown that thiourea prevents thyroxine production by its inhibitory effect on the activity of the peroxidases concerned with the conversion of inorganic iodine into bound, organic iodine from which thyroxine is formed (review by Pitt Rivers, 1950; Leloup, 1952; Astwood, 1955). The lack of thyroxine stimulates the increased production and release of T.S.H. and injections of thyrotrophin will therefore bring about the same changes in the thyroid as anti-thyroid drugs, namely, loss of colloid with the consequent appearance of empty follicles, hypertrophy and hyperplasia of the follicular epithelium, and an increase in the number of intracellular colloid droplets; the vascular supply of the gland may also be increased. These changes have been studied by Astwood et al. (1943), Salter (1950), Braunstein et al. (1953) and Gross (1957) and their work has shown most convincingly that the loss of colloid is not the direct effect of thiourea but the result of an increase in the rate of its reabsorption under the stimulation of T.S.H.: if mammals treated with thiourea or some other anti-thyroid drug and showing the typical hyperthyroid features are hypophysectomised, colloid accumulates and the thyroid assumes the histological appearance of a resting gland,

even when thiourea treatment continues. An interesting suggestion has been made by Albert et al. (1947) who consider that thiourea may enhance the activity of T.S.H. In addition to the hyperthyroid features already mentioned, several authors have noted that when embryos are subjected to treatment with anti-thyroid drugs, the follicle lumen may become enormously enlarged (Tixier-Vidal, 1958; Adams & Buss, 1952; Chick; Peterson & Young, 1952; Logothetopoulos & Scott, 1956 - Guinea-pig). Although these conclusions are based on the results obtained from experiments on mammals, the thyroid of fish treated with T.S.H. or anti-thyroid drugs for a sufficient length of time responds in an identical way, presenting the usual picture of cellular hypertrophy, hyperplasia and colloid loss (Pickford & Atz, 1957) and it is therefore assumed, in the absence of contradictory evidence, that the mechanism is the same as in mammals. The appearance of any of the typical hyperthyroid features may thus be taken to indicate the presence of T.S.H.

Considering now the effects of thiourea on thyroid development in Herichthys cyanoguttatus, the first difference between the experimental and control fish is seen on the 4th day after hatching (Experiment A). At this time colloid fails to accumulate in the thiourea-treated fish; the empty follicles enlarge and there is an increase in the amount of intracellular colloid droplets. By the end of the 2nd week the thyroid shows marked signs of thyrotrophic stimulation: the thyroid epithelium is slightly hyperplastic and the follicles have increased enormously in size and contain relatively

little colloid. This latter feature indicates a rapid reabsorption of colloid under the influence of T.S.H. and it may be concluded that this hormone is released by the 4th day after hatching, when the first signs of colloid loss and follicular enlargement are seen. Until the 4th day the thyroids of control and experimental fish closely resemble one another, including, in both groups of fish, empty follicles and follicles filled with a granular colloid. On the 4th day after hatching, changes occur in the thyroids of both control and experimental fish.

a) The follicles of control fish accumulate dense homogeneous colloid; these follicles increase rapidly in number and also slightly in size. Few empty follicles remain by the 5th day; those that do are also slightly enlarged.

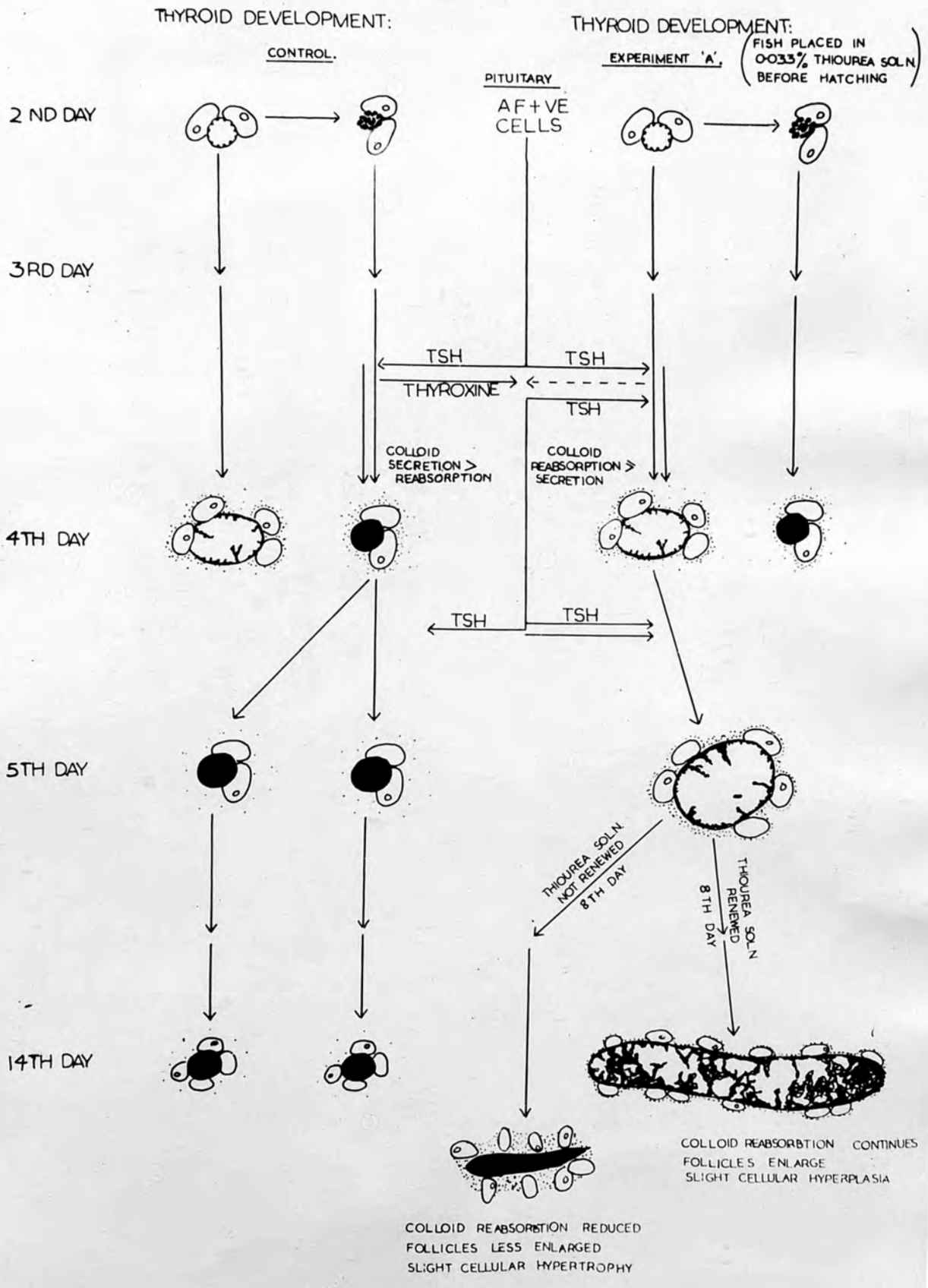
b) In the experimental fish, few follicles accumulate homogeneous colloid, but the empty follicles persist and become very considerably enlarged.

These observations suggest that significant quantities of T.S.H. are released between the 3rd and 4th day; in the control fish normal concentrations of this hormone promote the further production of colloid and the release of thyroxine and result in the formation of typical follicles with homogeneous colloid. In the thiourea-treated fish, on the other hand, the inability to produce thyroxine stimulates the release of increased quantites of T.S.H. with the result that the ratio of colloid reabsorption to colloid production is

increased and enlarged empty follicles develop. This scheme is illustrated in figure 28. In her paper on the development of the chick thyroid Tixier-Vidal (1958) includes photographs of thyroid follicles cultured in contact with pituitaries capable of releasing T.S.H. In these photographs it is possible to identify empty follicles similar to those found in Herichthys. Vivien & Gaiser (1952) have also noted such empty follicles in 50-day old Lebistes subjected to thiourea treatment.

While empty follicles are abundant after the 4th day in Herichthys treated with thiourea they are also found in both control and experimental fish from the earliest stages of colloid production. Similar empty follicles have been described by Stolk (1951) in the developing thyroid of Lebistes reticulatus and interpreted by him as follicles in the initial stages of secretion. Since intermediate stages occur between these empty follicles and those filled with granular collid, it seems probable that Stolk's interpretation is the correct one. On the other hand, some follicles seem to be formed by the accumulation of irregular granular colloid masses and it is possible to interpret these results by suggesting that T.S.H. is secreted by the beginning of the 2nd day after hatching and stimulates the reabsorption of colloid during the early stages of thyroid activity when relatively little thyroxine would be produced. In this case, the accumulation of homogeneous colloid in the control fish would result from a reduction in the release of T.S.H. in response to a rising concentration of thyroxine as more thyroid follicles were

FIGURE 28 SCHEME TO SHOW THE INTERACTION BETWEEN PITUITARY AND THYROID DURING DEVELOPMENT.



differentiated. On the other hand, were the thyroid subjected to stimulation from the thyrotrophic hormone from such early stages one would not expect any formation of typical follicles with homogeneous colloid in thiourea-treated fish on the 4th day but, rather, a steady increase in the size of empty follicles from the 2nd day onwards. This was not observed. For these reasons it would not appear justifiable to postulate the release of T.S.H. until just before the 4th day and it may be concluded that the thyroid of Herichthys is capable of a certain level of autonomous secretion. Since pale A.F.+ve cells can be detected in the pituitary before this period, release of the thyrotrophic hormone may be dependent either on the development of a more adequate blood supply to the pituitary than that which exists during the first days or on the production of a higher concentration of T.S.H.

It is valid, at this stage, to consider briefly the significance of the colloid globules which are found principally in the cephalic veins and heart of both control and experimental fish during the second and subsequent days and the smaller droplets in the capillaries around the pituitary (see Part I,§6). Similar droplets, staining with both A.F. and P.A.S., have been noted by Phillips and Schmidt (1959) in the fetal rat, where their appearance coincides with the sudden degranulation of the thyrotrophs and with the secretion of colloid by the thyroid. These authors suggest that this colloid may be the thyrotrophic hormone. If this interpretation is correct, their appearance in Herichthys on the 4th day would lend support to the

view that T.S.H. is first secreted at this time. Alternatively, it is possible that the larger colloid globules in the cephalic veins are droplets of thyroid secretion. This latter possibility could be tested by rearing the fish in water containing radioactive I^{131}. Such an experiment would also be of value in determining how long before the secretion of colloid the thyroid cells are capable of concentrating iodine.

The work of Iakovleva (1949) on Acipenser and that of Evropeitzeva (1949) on Coregonus led these authors to conclude that the thyroid in both these fish is capable of autonomous secretion for a considerable length of time during early development, before the thyrotrophs are either histologically or functionally differentiated. In Coregonus the thyroid follicles were both hypertrophied and emptied of colloid after 17 days of thiourea treatment and there thus seems to be little justification for her conclusion. No observations were made on the effect of thiourea during the early development of the thyroid so that it is impossible to know at what stage pituitary influence begins. That the thyroid of these fish was less strongly affected than that of Herichthys may be due partly to the fact that the experiment was not begun until after the thyroid had started to secrete colloid. Experiments B and C, described in § 3c and § 3d, show that the later thiourea treatment begins the longer is the interval before any stimulation by the pituitary can be detected, probably because a store of thyroxine is already available for release in these later stages. In the case of Acipenser thiourea treatment did not result in

cellular hypertrophy but inhibited the formation of new follicles and led to a loss of colloid. Both Iakovleva and Evropeitzeva attribute the loss of colloid to the direct inhibitory effect of thiourea on the secretory activity of the thyroid cells - an effect which has not been observed to occur in other animals. Neither of these workers gives measurements of the size of the pituitary in control and experimental fish. This is unfortunate, since in Herichthys, as in the developing guinea-pig (Peterson & Young, 1952) and chick (Tixier-Vidal, 1958) thiourea treatment leads to an enlargement of the pituitary, which has been shown to be due, in chick and Herichthys, to an increase in both size and number of thyrotrophs. In all these animals a considerable time-lag is involved before this glandular enlargement becomes noticeable.

Several workers have used the development of certain morphological characters as criteria for the presence of thyroxine (Iakovleva, 1949; Fortune, 1960). In Herichthys treatment with thiourea affects the development of the skin and mucus glands, melanophores, yolk sac and air bladder. It should be remembered, however, that thiourea may itself directly affect the development of certain structures, since in high doses it can inhibit the oxidase enzymes of systems other than the thyroid (Salter, 1950).

Fortune (1960) has noted in both Phoxinus and Salmo fry, reared from an early stage in thiourea solution, that the melanophores fail to concentrate at the normal age. The same effect is seen in Herichthys and it seems likely that this can be attributed to the

absence of thyroxine, rather than to the direct action of thiourea, since fish placed in this drug at a slightly later age (Experiment B) show normal melanophore concentration; in these cases a certain amount of thyroxine was probably formed before the inhibitory action of thiourea took effect. Fortune finds that thyroid extracts cause melanophore concentration when applied to strips of excised minnow skin and she therefore suggests that melanophore concentration in developing minnows indicates the onset of thyroxine release; thyroid follicles are differentiated at about this time. Such an explanation is not valid for H. cyanoguttatus in which thyroxine is released several days prior to melanophore concentration and in which the melanophores remain expanded in fish on a black background. Since these fish show complete melanophore adaptation in about 1½ hours when transferred from a black to a white background, or vice-versa, it is possible that melanophore concentration on the 8th day in fish on a white background reflects the establishment of nervous control.

It has been shown that thyroxine hastens the maturation of the nervous system (Kollross, 1943; Weiss & Rossetti, 1951; Gorbman, 1959); conversely thiourea treatment will retard neural development in mammals (Barnett, quoted by Salter, 1950) and it seems probable that the delay in melanophore concentration noted in thiourea-treated Herichthys reflects the effect of this drug on the development of the chromatophore nerves.

It is possible that the failure of the swimbladder to expand in thiourea-treated fish also reflects a disturbance of neural

development. Enami & Imai (1955, 1956a & b) have described an aggregation of neurosecretory cells in the caudal region of the spinal cord, which they term the urohypophysis. Working with adult goldfish, Enami showed that removal of the urohypophysis led to a loss of buoyancy while injections of crude extract of this region of the spinal cord resulted in a marked increase of buoyancy, forcing the fish to float at the surface of the water (Enami, 1959). He suggests that the urohypophysis influences the secretion of gases into the swimbladder. If the initial expansion of the swimbladder is dependent on stimulus from the urohypophysis, it is possible that the failure of the swimbladder to expand in thiourea-treated fish may result from an inhibitory effect of this drug on the development of the neurones composing the urohypophysis.

The thinning of the epidermis is also probably the result of thyroxine lack. Several authors have observed that the skin becomes markedly thicker after treatment with thyroxine or thyroid extract (Harms, 1935, Periophthalmus; La Roche & Leblond, 1952, Salmonids) while Sembrat (1954) noted that anti-thyroid drugs led to a decrease in thickness in Salmonids, as was observed in Herichthys (present investigation).

PART III

The Cellular Origin of Chromatophore-Regulating Hormones in the Pituitary

§ 1. INTRODUCTION.

Although it is well established that the teleost pituitary is concerned with melanophore regulation, there is a difference of opinion among current workers both about the number of hormones involved and their site of production within the pituitary.

The release of these hormones is under nervous control and influenced, via the eyes, by the background on which the fish is kept; thus a black background results in melanophore dispersion and a white background in melanophore concentration. Injections of teleost pituitary extract result in either the dispersion or the concentration of the melanophores, the result being constant for any given species regardless of which teleost is used as donor. Since one may assume that the fish will respond in like manner to the release of its own hormone, it follows that some species produce a melanophore-dispersing ("B", or blackening) hormone and others a melanophore-concentrating ("W", or whitening) hormone as judged by their effect on the donor. The observation that the same pituitary extract will elicit either response, depending on the fish, can be explained in two ways: either the "B" and "W" hormones of all fish are chemically similar but the melanophores of any teleost can only respond in one particular way, or the extract contains both "B" and "W" hormones (Odiorne, 1957).

That the pituitary produces two hormones with opposite effects has been suggested by Healey (1951) and Niell (1940) on other grounds. In the minnow which is known to produce a "W" hormone, melanophores which have been separated from central nervous control by spinal section appear to take longer to make the small chromatic adaptation required when the fish is transferred from darkness to an illuminated black background than they take to make the longer chromatic change when transferred from a white to a black background. This would seem inexplicable if adaptation to a black background required only the inhibition of "W" hormone release, but can be explained by assuming that a "B" hormone is also involved, whose production is inhibited in darkness. Such a view is in accordance with the bihumoral hypothesis proposed by Hogben and Slome (1931) to explain similar time intervals in Amphibia. Reference to Healey's figures (1951) shows however that the variation in time response between individual minnows is very considerable; also there are wide limits of error possible in determining the exact time when chromatic adaptation is complete. There is therefore some doubt that time differences are as significant as was first thought (Kent, 1960; Kent & Healey, personal communication).

Only Enami (1955) claims to have succeeded in separating both a "B" and "W" hormone from the same teleost pituitary, although subsequent attempts by other workers have proved unsuccessful (Kent, 1960). The properties of extracts from different regions of the hypothalamus and the pituitary led Enami to suggest that in Parasilurus asotus the "W" hormone is contained in the neuro-secretion, produced

in the nucleus lateralis tuberis of the hypothalamus and stored in the pituitary in the region of the meso-adenohypophysis.

These results introduce the problem of the site of chromatophore-regulating hormone production. In tetrapods it is now reasonably certain that the "B" hormone is produced by the cells of the pars intermedia (reviews by Parker, 1948; Waring & Landgrebe, 1950). This hormone is alternatively known as the melanocyte-stimulating hormone (M.S.H.), or intermedin. It has been assumed, by homology, that the meta-adenohypophysis in teleosts is responsible for the production of a chromatophorotropic hormone, and this seems likely in the case of Phoxinus phoxinus (Hewer, 1926; Kent, 1959), Gadus callarius (Hewer, 1926) and Cyprinus carpio (Kazskii & Persov, 1949), in which the posterior region of the pituitary contains the highest concentration of the hormone. Enami's work suggest, however, that the "W" hormone is not produced in the meta-adenohypophysis. The results of experiments made by Healey (1940, 1948) are also relevant. He found that when the posterior region of the pituitary is destroyed in Phoxinus the fish can concentrate its melanophores on a white background, but cannot adapt to a black background; but in spinal-sectioned fish in which the melanophores are no longer under nervous control complete hypophysectomy prevents adaptation to any background. It has therefore been suggested that the "W" hormone is produced in the pro-adenohypophysis (Pickford & Atz, 1957).

It is clear that there is still much uncertainty both about the number of hormones produced by any given species of teleost and about their site of production.

The cellular origins of several pituitary hormones have been determined by observing the cytological changes which accompany hormone release. No such observations have been made during melanophore changes in teleosts, although Ortman (1954, 1956) has shown for Amphibia that adaptation to a black or white background has different effects on the cytological appearance of the pars-intermedia cells. The meta-adenohypophysis of teleosts contains two types of cell, and Stahl (1958) has suggested that these are the site of "B" and "W" hormone production. In Part I, § 3, it was shown that in cichlids only one of these cells appears to respond to changes in background.

The work discussed in the following section was undertaken in order to investigate the effects of black and white backgrounds both on those teleosts which are known to produce a "W" hormone and those which produce a "B" hormone, and thus to determine:

(i) whether one or two types of cell respond to background changes, indicating the participation of one or two hormones in melanophore activity;

(ii) whether similar tinctorial cell types are involved in different fish on the same background, enabling one to identify a particular cell type with a specific hormone.

§ 2. MATERIAL AND METHODS

Four species of fish were used for these investigations. Specimens of Rana temporaria were also examined.

Fish of the same species were kept together in large white or black painted sinks (50 cm x 40 cm x 20 cm) with a constant flow of tap-water. The temperature throughout the year ranged from 7·0 to 20·5°C.

Fish were transferred to these sinks from river or pond conditions: the trout were transferred from large indoor tanks. All fish were kept under diurnal illumination. Specimens were killed and examined after the following periods.

Species		No. from white sink	No. from black sink	Length of experiment	Month of year when killed
Phoxinus phoxinus	Adult minnows	8	8	4 weeks to 1 yr.	-
Salmo trutta	Parr stage	-	3	2½ weeks	October
		5	5	6 weeks	November
Salmo gairdneri	Parr stage	1	1	6 weeks	November
Carassius auratus	Melanic goldfish ca 6 months	1	1	5 weeks	December
	Melanic goldfish ca 18 months	2	2	5 weeks	December
	Xanthic goldfish ca 2 years	4	4	10 months	September
Ameiurus melas	Adult catfish	5	5	5 weeks	February
Rana temporaria		2	2	2 weeks	May

In addition, minnows were examined after the following treatment:

After several months on a white background, three minnows

were transferred in August to a black background and examined after 5 days. Nine minnows which had been black-adapted for several months were transferred in October to a white background, six were examined after 8 days and the remaining three after 4 weeks.

Ten minnows were kept in a glass tank (20 cm x 20 cm x 30 cm) which was placed in a light-proof box. Five of these minnows had previously been kept on a white background and five on a black background for several months. The white-adapted fish were smaller than those from the black background; they were marked by cutting off part of the caudal fin. The light-proof box was kept in a dark room, so that the water could be changed, and the fish fed in complete darkness: this was done thrice weekly.

The water was kept at 10 °C. and continuously aerated. The fish were killed after 11 weeks.

All fish were killed by decapitation; the pituitary was exposed and the whole head fixed in Bouin. The remaining connective tissue around the brain and pituitary was removed when the specimen was in the clearing agent.

Sections were cut at 5 μ and stained with one of the stains listed in Part I, § 2.

Most of the minnows used during these investigations were probably more than two years old, and were about 7 - 8 cm. A few were younger, measuring about 5 - 6 cm.

§ 3. RESULTS.

A. The effect of black and white backgrounds on the minnow pituitary.

Three cell types occur in the meta-adenohypophysis:

(a) acidophils, which stain brightly with azocarmine after Azan and give a positive reaction with lead haematoxylin;

(b) acidophils, which stain with orange G after Azan but appear completely chromophobic with lead haematoxylin. These cells occur usually at the periphery of the gland;

(c) cyanophils, which stain with aniline blue and give a weak reaction with P.A.S.

(i) White-adapted minnows.

In most specimens lead haematoxylin+ve cells predominated (fig.29a). These cells had a characteristic club shape, the cytoplasm being prolonged into a broad stalk which extended between the neighbouring cells to the nervosa (fig.31a). The cytoplasm was non-granular and dense, with occasionally small clear vacuoles in the cell stalk, and stained extremely darkly with both lead haematoxylin and azocarmine. The nucleus of these cells was rounded, or a compressed U-shape. In one specimen, which had been kept on a white background for several months, there was a complete absence of typical lead haematoxylin+ve cells (fig.29b). These cells were round instead of club-shaped, and stained only faintly with lead haematoxylin.

The orange G acidophils were large and rounded, with slightly granular cytoplasm.

The effect of black and white backgrounds on the meta-adenohypophysis of the minnow (Phoxinus phoxinus).

Figure 29. a. L.S. of pituitary of long-term white-adapted minnow showing strongly stained lead-haematoxylin cells (lead haematoxylin; yellow filter; x 150).

b. L.S. of pituitary of long-term white-adapted minnow in which the lead-haematoxylin cells are partially degranulated (lead-haematoxylin; yellow filter; x 150).

Figure 30. a. L.S. of pituitary of long-term black-adapted minnow retaining some strongly stained lead-haematoxylin cells (lead haematoxylin; yellow filter; x 150).

b. L.S. of pituitary of long-term black-adapted minnow, showing degranulated cells and fringe of darkly stained cell 'stalks' bordering the neurohypophysis (lead haematoxylin; yellow filter; x 150).

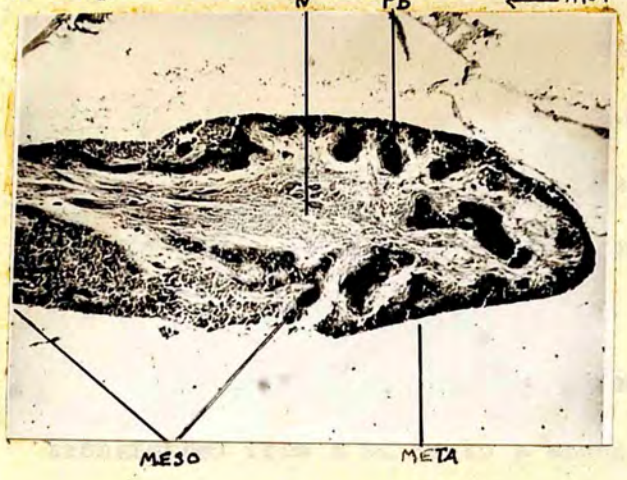
Figure 31. a. Diagram showing the appearance of meta-adenohypophysis cells of long-term white-adapted minnow.

b. Cells of the meta-adenohypophysis of long-term black-adapted minnow (Azan; green filter; x 1200).

Abbreviations:

BV : blood vessel
D Pb : degranulated lead-haematoxylin cells
F : fringe of darkly stained cell stalks
MESO : meso-adenohypophysis
META : meta-adenohypophysis
N : neurohypophysis with neurosecretion
PAS : P.A.S.+ve cyanophil
Pb : densely stained lead-haematoxylin cells
PbS : long cell stalks of lead-haematoxylin cells

FIGURE 29a



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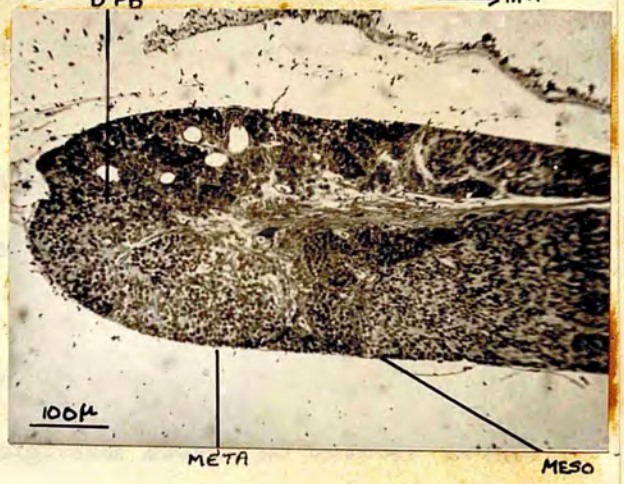
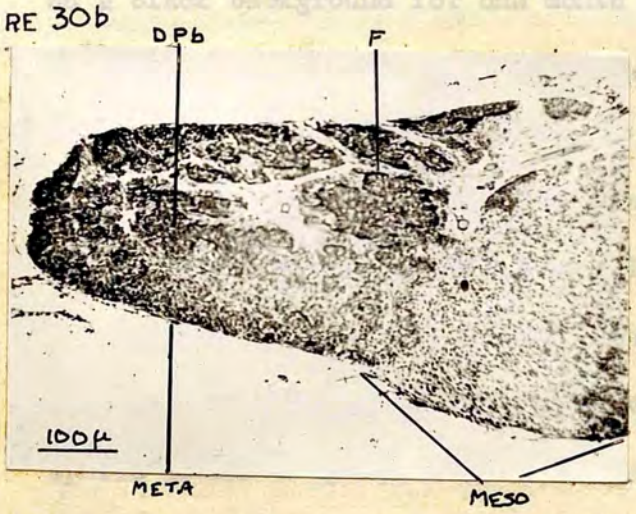


FIGURE 30b



30a

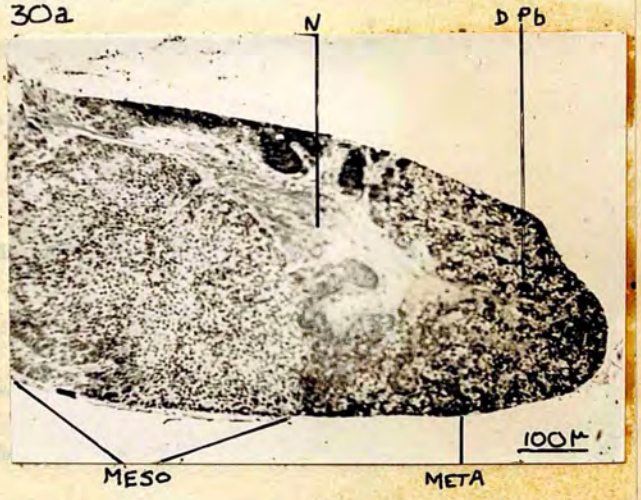
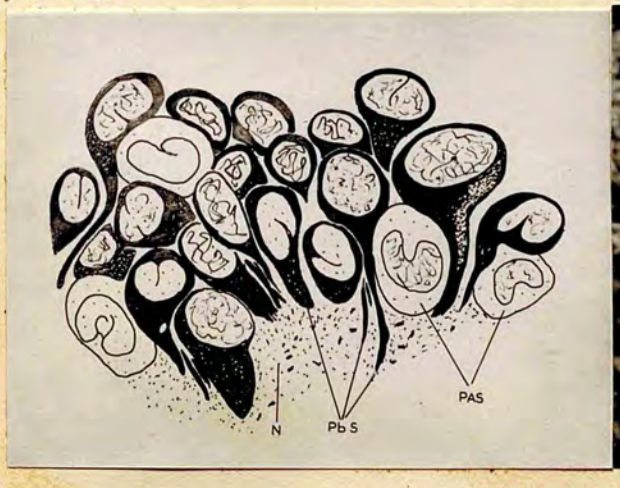
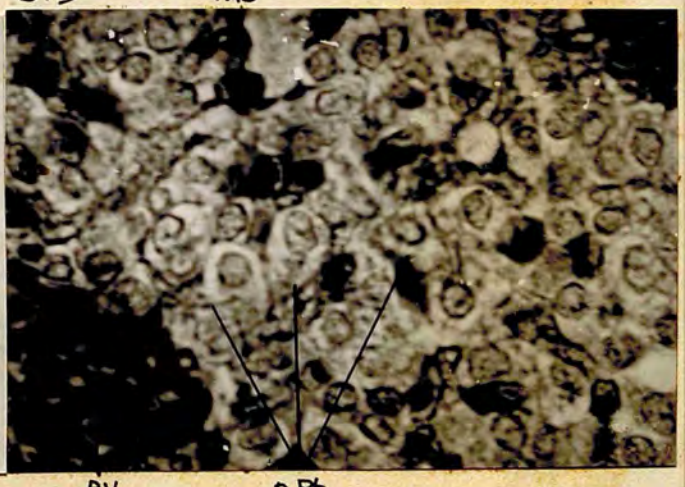


FIGURE 31a



31b



The cyanophils could not be easily detected after Azan, but in some specimens they stained a dull mauve-blue. They gave either a weak or negative reaction with P.A.S.

All the cells of the meta-adenohypophysis were packed closely together and surrounded an extensive neurohypophysis.

(ii) Black-adapted minnows.

After 5 days the meta-adenohypophysis of fish which had been transferred from a white to a black background showed no notable change from that of white-adapted controls (fig.32), but in fish maintained on a black background for one month or longer the lead haematoxylin acidophils were profoundly affected, while the cyanophils were also, but less strikingly changed (fig.30).

Although in some fish a few of the acidophils still gave a strong reaction with lead haematoxylin, the majority of the cells had become almost completely degranulated and appeared chromophobic with lead haematoxylin or Azan. Other cells less strongly affected had sparse granular cytoplasm, which stained grey with lead haematoxylin and purple with Azan. Most of the cells, as well as being degranulated, had lost their typical "club" shape and were rounded, and slightly enlarged (fig.31b). A curious feature in some pituitaries was the presence of what appeared to be darkly staining cell stalks forming a short dark fringe between the gland zone and the neuro-hypophysis (fig.30b). These stalks could not be traced back to any particular cell.

The cyanophils usually stained brightly with aniline blue

and in most cases gave a stronger reaction with P.A.S. than in white-adapted fish. In some fish these cells only occurred in a compact zone in the dorsal region of the meta-adenohypophysis; in other cases they were scattered through this region.

The orange G cells did not appear to differ from those of white-adapted fish.

The meta-adenohypophysis as a whole appeared hypertrophied, while the nervosa was in most cases somewhat reduced in area and sometimes highly vascular. This hypertrophy may have been partly due to an increase in cell number, since mitotic figures were occasionally seen, but it is possible that another causative factor was the rounding-off and enlargement of the existing lead haematoxylin+ve cells.

There was a certain amount of variation between different specimens in the extent to which the cells were affected by a black background. This appeared to be an individual variation rather than a reflexion of the length of time the fish had been kept on a black background: almost all the lead haematoxylin cells were degranulated in some fish after about a month, while other fish retained some very dark staining cells even after a year on a black background.

(iii) Fish transferred from a black to a white background.

The variation observed in black-adapted fish makes it difficult to determine the extent to which the lead haematoxylin cells are affected by transfer to a white background. Only in two of the six fish examined after 8 days was there a distinct increase in granulation of the lead haematoxylin cells (fig.33a). The short

Figure 32. L.S. of pituitary of long-term white-adapted minnow after adaptation to a black background for 5 days (lead-haematoxylin & phloxin; yellow filter; x 150).

Figure 33. L.S. of pituitary of long-term black-adapted minnow after adaptation to a white background for 8 days.

a. Pituitary in which the cells seem to have recovered some lead-haematoxylin granulation (lead-haematoxylin; x 500).

b. Pituitary in which lead-haematoxylin remains degranulated (lead-haematoxylin; x 150).

Figure 34. L.S. of pituitary of minnow maintained in total darkness for 11 weeks.

a. Minnow transferred to darkness after long-term adaptation to a white background.

b. Minnow transferred to darkness after long-term adaptation to a black background.

Abbreviations:

DPb : degranulated lead-haematoxylin cells
MESO : meso-adenohypophysis
META : meta-adenohypophysis
N : neurohypophysis
Pb : darkly stained lead-haematoxylin cells

FIGURE 32

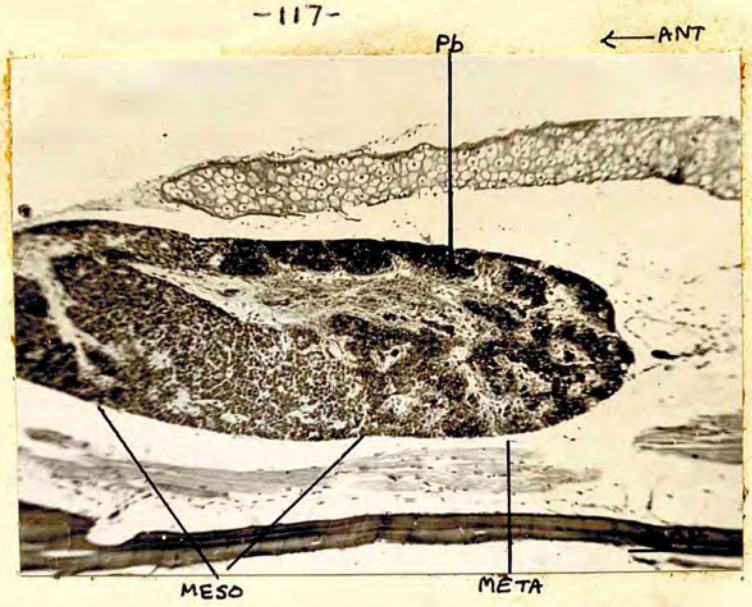
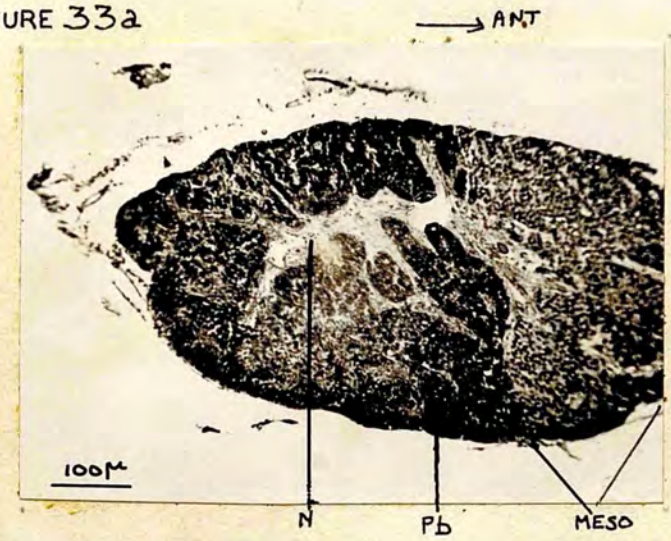


FIGURE 33a



33b

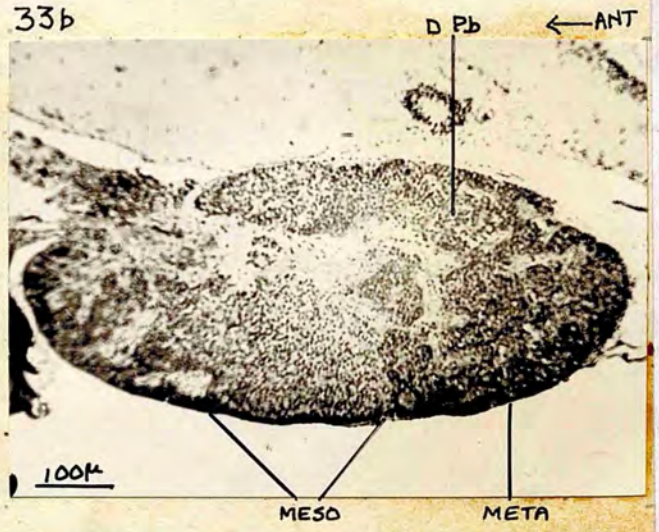
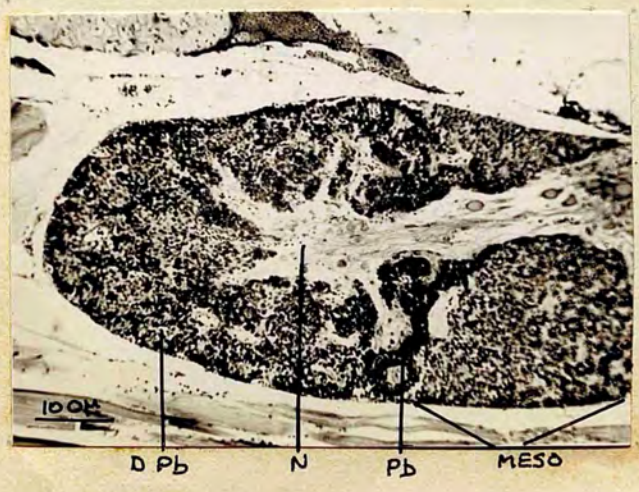
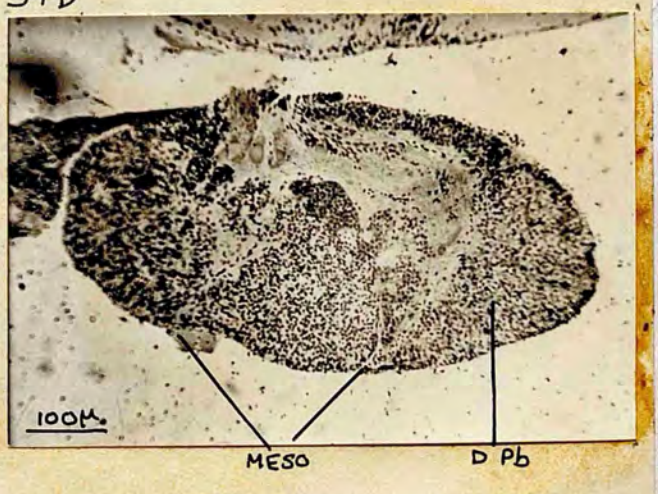


FIGURE 34a



34b



fringe of cell stalks seen between the cells and the nervosa in black-adapted minnows was not seen in any of these fish, but in four specimens a notable feature was the appearance of darkly staining cells with extremely long cell stalks extending to the nervosa. The nucleus of these cells was surrounded by only a sparse amount of dark cytoplasm.

After 4 weeks on a white background the cells had still not regained the characteristic "club" shape seen in white-adapted fish (i). In two cases the lead haematoxylin cells were more strongly stained than was usually observed in black-adapted fish, in the third case nearly all the cells were completely chromophobic (fig.33b).

(iv) The effect of continuous darkness.

The pituitary of minnows kept in continuous darkness for 11 weeks resembled that of black-adapted fish. In minnows which were white-adapted before being placed in the dark, the lead haematoxylin cells were almost completely degranulated (fig.34b) and the cyanophils stained distinctly with P.A.S. and aniline blue. The lead haematoxylin cells of previously black-adapted fish were surprisingly far less degranulated. This observation is inexplicable from the present knowledge of factors affecting these cells. These fish were larger, and probably a year older than those transferred from a white background. It may be significant that in these larger fish the P.A.S.+ve cyanophils of the meso-adenohypophysis (presumably gonadotrophs) were extremely vacuolated although a correlation between gonadotroph activity and staining intensity of haematoxylin+ve cells was not apparent in illuminated fish.

B. The effect of black and white backgrounds on the trout pituitary.

The meta-adenohypophysis is similar in Salmo trutta and S. gairdneri, and seems to be comparable with that of S. salar described by Olivereau (1954). This region of the pituitary encloses a large neurohypophysis into which it projects, forming finger-like cords of cells. Two types of cell can be distinguished: one which gives a strong positive reaction with lead haematoxylin and a second type which remains chromophobic. None of the cells is stained by P.A.S., nor is the distinction between the two types obvious with Azan after which most of the cells are stained a pale grey-brown.

White-adapted trout.

In white-adapted trout the majority of cells were stained strongly with lead haematoxylin (fig.35a,36a). Both the cells and nuclei were very variable in size, the cytoplasm was granular and extended to the nervosa, the nucleus was frequently polymorphic with a distinct nucleolus. The chromophobic cells were rounded with non-granular cytoplasm which stained only a very faint grey.

Black-adapted trout.

In all fish adapted to a black background for 6 weeks and even after 18 days there was a considerable degranulation in the lead haematoxylin+ve cells (fig.35b,36b). Although some of the cells at the periphery of the gland remained darkly stained, those at the centre of the gland were almost entirely unstained; they could only be distinguished from the original chromophobes seen in white-adapted fish by their characteristic shape which is not lost as it is in

The effect of black and white backgrounds on the meta-adenohypophysis of the trout (Salmo spp.).

Figure 35. a. L.S. of pituitary of trout adapted to a white background for 6 weeks.(lead-haematoxylin; x 75.)

b. L.S. of pituitary of trout adapted to a black background for 6 weeks (lead haematoxylin; x 75).

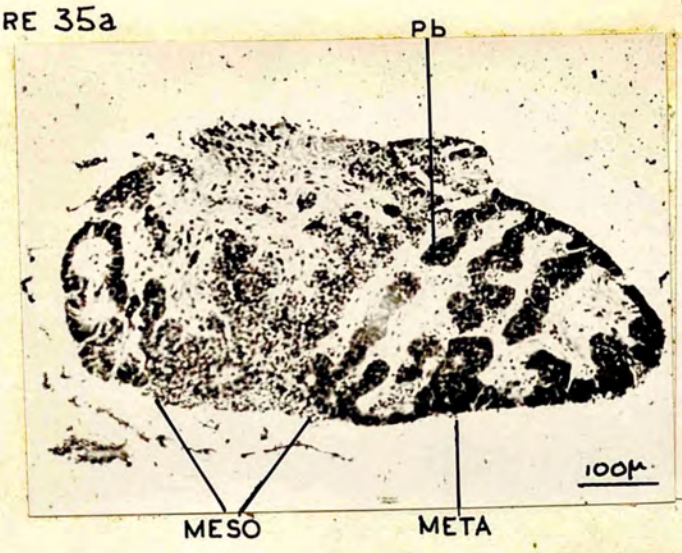
Figure 36. a. Cells from the meta-adenohypophysis of 35a (yellow filter; x 1500).

b. Cells from the meta-adenohypophysis of 35b (yellow filter; x 1500).

Abbreviations:

C : chromophobe
DPb : degranulated lead-haematoxylin cells
MESO : meso-adenohypophysis
META : meta-adenohypophysis
Pb : lead-haematoxylin cells
PRO : pro-adenohypophysis with enlarged follicle

FIGURE 35a



35b

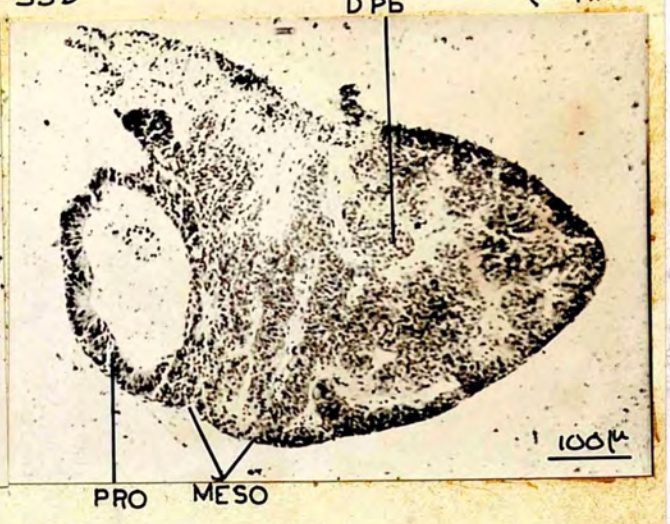
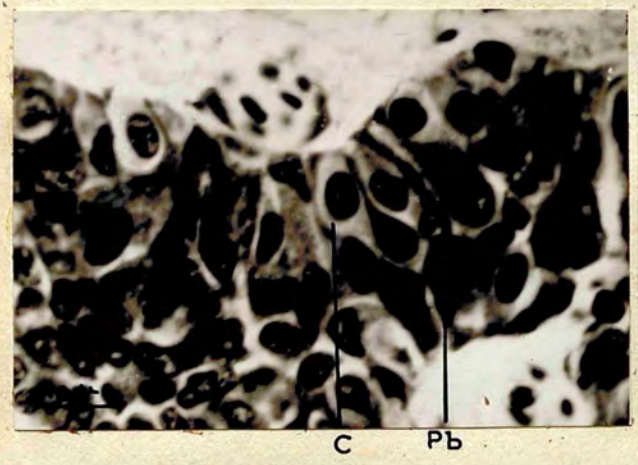
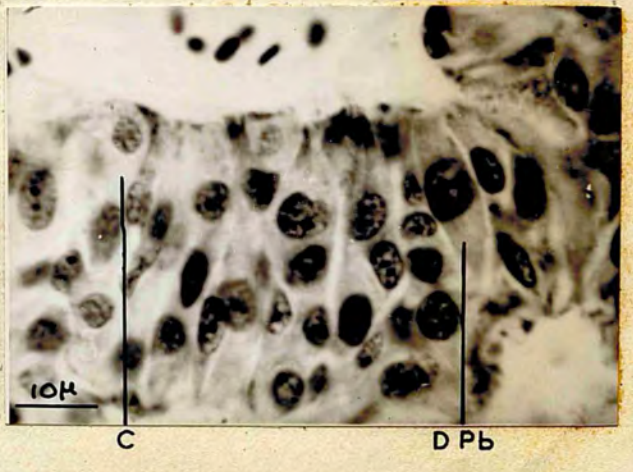


FIGURE 36a



36b



minnow, and because the stalks of the cells often still contained lead haematoxylin+ve granules. The cytoplasm which was devoid of granules, was stained a very faint grey like that of the chromophobes: this made it possible to see that many of the cells contained a clear spherical vacuole.

In all black-adapted fish the meta-adenohypophysis was relatively extensive and mitotic figures were frequent. It is uncertain, however, if this can be related to the effects of a black background since this region was also fairly extensive in three of the white-adapted fish.

An interesting variation was seen in the follicles of the pro-adenohypophysis. In five of the black-adapted fish, one of the follicles was enormously enlarged, the maximum diameter of the lumen ranging from 220 μ to 500 μ (fig.35b). This may be compared with the maximum follicular lumen diameter of other black-adapted fish and of fish from a white background, which varied from 9 μ to 36 μ. Such enlarged follicles are usually only seen in mature adult salmonids and although male fish may occasionally become sexually mature at the parr stage this is apparently not accompanied by follicular enlargement (Olivereau, 1954). The sex and state of gonad development was unfortunately not noted for Salmo trutta used for these experiments. The specimens of S. gairdneri included an immature parr on the black background and a mature male parr on the white background. In both fish the follicles were all small and normal.

C. The effect of black and white backgrounds on the pituitary of Carassius auratus.

The meta-adenohypophysis was examined in goldfish at three different developmental stages: melanic fish of about 6 months, melanic fish of about 18 months and xanthic fish about 2 years old. The cytological appearance of the cells differed in fish at these three stages, but did not appear to be affected by changes of background.

(i) The meta-adenohypophysis of 6-month old goldfish.

Two cell types could be distinguished. The majority of cells were club-shaped and stained brightly with azocarmine. After lead haematoxylin the entire cell, or in some cases only the cell stalk, was very strongly stained. The cytoplasm frequently included a large clear vacuole. A few cells were stained blue after Azan. Some cells, probably the cyanophils, gave a very weak positive reaction with P.A.S.

(ii) The meta-adenohypophysis of 18-month old melanic goldfish.

All these fish, examined in December, were immature. The meta-adenohypophysis included two types of cell, both of which were usually fairly large and rounded, with an oval or polymorphic nucleus and gramular cytoplasm. Just over half the cells stained grey with lead haematoxlin and pale purple with Azan. The other cells stained with orange G after Azan. None of the cells gave a positive reaction with P.A.S., nor did they show any orientation with respect to the nervosa. (Figure 37a.)

Figure 37. a. Cells of the meta-adenohypophysis of unripe, 18 month old melanic goldfich examined in winter (lead-haematoxylin; yellow filter; x 1500).

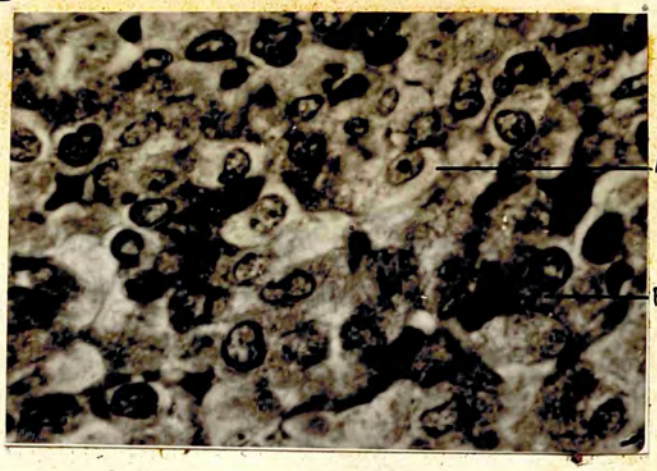
b. Detail of cells of the meta-adenohypophysis of unripe, 2-year old goldfish examined in summer (Azan; yellow filter; x 1500).

c. Cells of the meta-adenohypophysis of ripe, 2-year old goldfish examined in summer; note organisation of cells around nervosa (Azan; yellow filter; x 600). Compare magnifications.

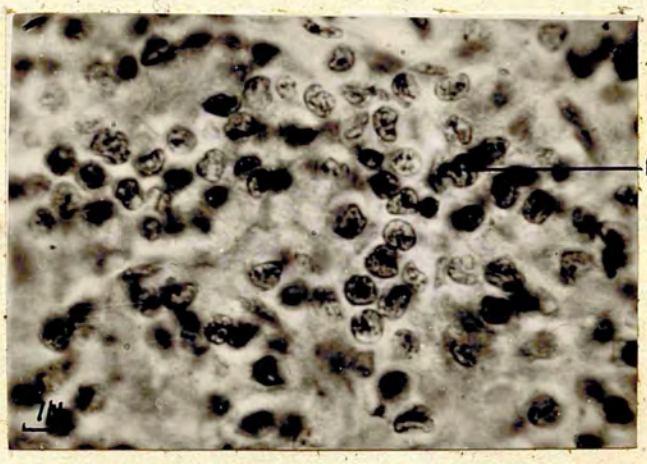
Abbreviations:

A : orange-G acidophil
BV : blood vessel
N : neurohypophysis
Pb : lead-haematoxylin+ve cyanophils
POL : POLYMORPHIC NUCLEUS

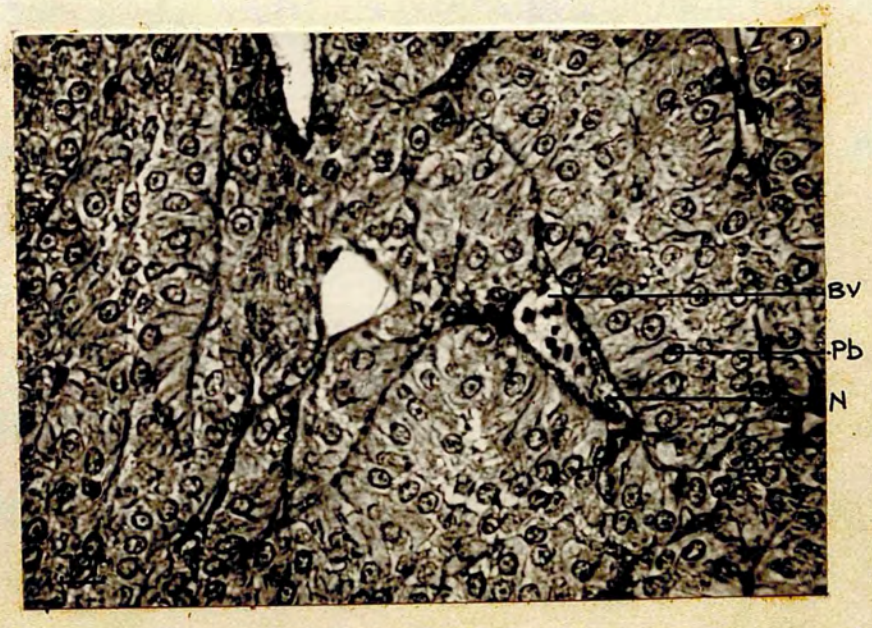
FIGURE 37a



37b



37c



(iii) The meta-adenohypophysis of 2-year old xanthic goldfish.

These fish were examined in September after 10 months on either a black or white background. Two of those from a black background had very large testes; two others, one from a white, the other from a black background, had smaller but still well developed testes; the gonads of the remaining four fish were extremely small.

The cells of the meta-adenohypophysis showed no changes that could be correlated with background adaptation, but showed distinct changes that appeared to be associated with the degree of maturity.

The cells were smaller and more crowded in the immature summer fish than in the immature winter fish, and the nuclei were horseshoe-shaped or polymorphic (fig.37b). Most of the cells were very weak cyanophils, staining pale blue after Azan and pale grey with lead haematoxylin; other cells were pale acidophils and stained with orange G.

In the mature fish the meta-adenohypophysis was much enlarged, forming more than two-thirds of the entire gland. This was probably due to the increase in size of the cyanophils, which were columnar and showed a definite orientation towards the neurohypophysis. The nucleus of these cells was rarely polymorphic but rounded, and situated at the cell pole away from the nervosa (fig.37c). In this region of the cell the cytoplasm was vesicular and chromophobic, while the rest of the cytoplasm stained pale blue with Azan and grey with lead haematoxylin. The orange-G acidophils, much fewer in number than the cyanophils, were also arranged along the branches of the nervosa.

These appeared similar to those of immature fish. The nervosa contained abundant blue staining colloid. In all fish a few of the cells in the dorsal region of the meta-adenohypophysis bordering the meso-adenohypophysis gave a strong reaction with lead haematoxylin and stained dark blue or mauve with Azan.

D. The effect of black and white backgrounds on the pituitary of Ameiurus melas.

The cells of the meta-adenohypophysis appeared similar in black- and white-adapted fish after 5 weeks adaptation. Two cell types could be distinguished: one which stained mauve or pale blue with Azan and dark or pale grey with lead haematoxylin; and a second type which stained weakly with orange G (fig.38). Both types of cell were usually rounded, although the lead haematoxylin+ve cells were occasionally club-shaped. Neither type of cell gave a positive reaction with P.A.S.

E. The effect of black and white backgrounds on the pars intermedia of Rana temporaria.

The effect of adaptation to black and white background was investigated by Ortman (1954, 1956) working on Rana pipiens. He showed that in white-adapted frogs the cells stained more strongly with P.A.S. but less strongly with Baker's acid haematin than in frogs adapted to a black background for 9 to 22 days.

Specimens of Rana temporaria were examined during the present study to determine whether the staining intensity of these cells with lead haematoxylin was parallel to their reaction with P.A.S. or with acid haematin.

Figure 38. The meta-adenohypophysis of catfish (Ameiurus melas) (lead-haematoxylin; yellow filter; x 400).

Figure 39. a. L.S. of pituitary of frog adapted to a white background for 2 weeks (lead-haematoxylin & phloxin; yellow filter; x 480).

b. Detail of cells of intermediate lobe of frog adapted to a black background for 2 weeks (Azan; x 1000).

Abbreviations:

ANT : anterior lobe of pituitary
BV : blood vessel
D Pb : degranulated lead-haematoxylin-ve cyanophils
N : neurohypophysis
Pb : darkly stained lead-haematoxylin-ve cells

FIGURE 38

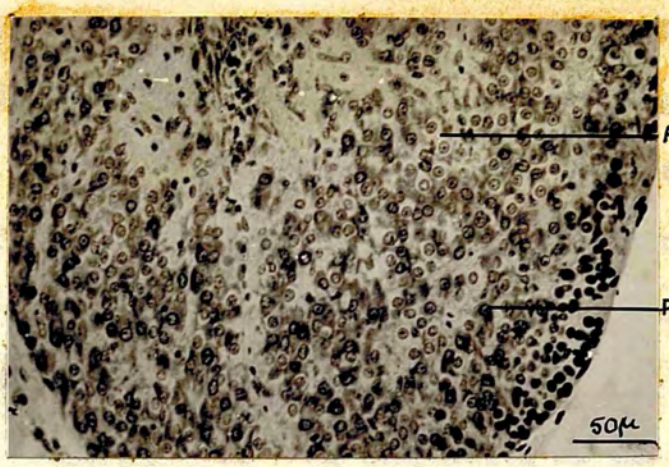
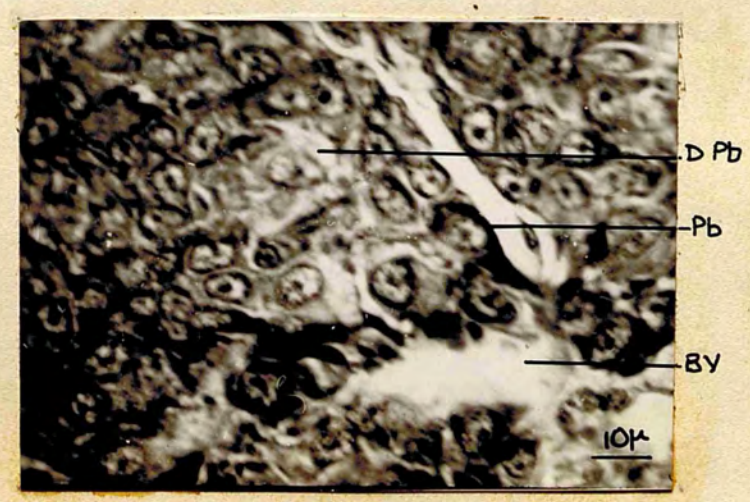


FIGURE 39a

39b



Only one distinct cell type occurred. In white-adapted frogs the cells stained brightly with aniline blue and gave a strong reaction with P.A.S. and lead haematoxylin. In frogs adapted to a black background for 2 weeks most of the cells were degranulated and chromophobic. A few cells around the blood vessels stained dark mauve with Azan, and also gave a positive reaction with P.A.S. and lead haematoxylin (figs.39a&b). A comparison of the cell types occurring in the meta-adenohypophysis of teleosts described in this section is given in Table 4.

Table 4. The cells of the meta-adenohypophysis of teleosts and the pars intermedia of Rana.

Species	Lead-haematoxylin cells.	P.A.S.+ve and other cells.
Minnow.	Azocarmine acidophils.	Cyanophils.
Trout.	Grey-brown or chromophobic with Azan.	No P.A.S.+ve cells. Chromophobic with Azan.
Cichlasoma.	Cyanophils.	Orange-G acidophils.
Ameiurus.	Pale cyanophils.	No P.A.S.+ve cells. Orange-G acidophils.
Carassius.	Pale cyanophils.	No P.A.S.+ve cells. Orange-G acidophils.
Rana.	Cyanophils.	

Words which are underlined indicate those cells which undergo cytological changes during chromatic adaptation to either a black or a white background.

§ 4. INTERPRETATION.

The results described in the preceding section and in Part I, § 3, show that adaptation to black and white backgrounds is accompanied by cytological changes in the meta-adenohypophysis of Phoxinus phoxinus, two species of Salmo, Cichlasoma nigrofasciatum and in the cells of the pars intermedia of Rana temporaria, but has no apparent effect on the pituitary cells of either Ameiurus nebulosus or Carassius auratus. The cytological changes have been most fully studied in Phoxinus, and these will be considered in some detail before attempting to interpret the effects of black and white backgrounds on the pituitary of other species.

(i) Minnow (Phoxinus phoxinus).

In this species it was found that the cytology of both the lead haematoxylin acidophils and the P.A.S.+ve cyanophils in the meta-adenohypophysis of the black-adapted fish differed from that seen in the white-adapted fish. The lead haematoxylin cells in most white-adapted minnows gave an intense staining reaction and were club-shaped, the cell stalks extending to the neurohypophysis. In the black-adapted fish, on the other hand, the majority of these cells were degranulated, giving only a weak staining reaction; they were no longer club-shaped but had become more rounded, and in many cases seemed to have lost contact with the neurohypophysis. Even in those specimens in which degranulation was least extensive the proportion of darkly stained cells never approached the condition seen in most

white-adapted fish. However, one specimen from a white background was examined in which the lead haematoxylin cells were completely degranulated. The P.A.S.+ve cells appeared most densely stained in black-adapted fish.

It was suggested in the introduction that both 'B' and 'W' hormones may be produced in the minnow pituitary. While the identification of a 'B' hormone rests on inconclusive evidence, there is good evidence of the presence of a hormone causing melanophore concentration, at least in the minnow itself, whatever its effect may be on other species. Hypophysectomy in spinal-sectioned minnows abolishes the white background response, while injections of minnow pituitary extract result in melanophore concentration (Healey, 1951; Kent, 1960).

The effect of partial hypophysectomy (Healey, 1948) shows that some 'W' hormone is produced in the anterior region of the pituitary, while Kent (1959, 1960) estimated that the concentration of this hormone in the posterior region of the pituitary is four to five times as high as that in the anterior half. Reference to figure 29a shows that the distribution of the lead haematoxylin acidophils fulfils these requirements, and the fact that these cells are cytologically different in black- and white-adapted fish suggests that they are concerned with the production of a chromatophore-regulating hormone. The cytological appearance of these cells, however, may be interpreted in two ways. It might be suggested that the degranulated condition observed in black-adapted fish results from an intensive

release of 'B' hormone. Thus thyrotrophs became degranulated during an increased rate of release of thyrotrophic hormone following thyroidectomy (Purves & Griesbach, 1951; Farquar & Rinehart, 1954). On the other hand the characteristic club shape of the lead haematoxylin cells in white-adapted fish and their close association with the neurohypophysis is also in keeping with a state of cellular activity and hormone release. In view of the correlation already noted between the distribution of the lead haematoxylin cells and the 'W' hormone, it would seem most likely that it is this hormone which is produced by the lead haematoxylin cells; the degranulation of these cells in black-adapted fish may reflect an absence of hormone because it is no longer being produced. If this identification of the lead haematoxylin cells is correct there is no longer any reason to suppose as Pickford and Atz (1957) have done that the pro-adenohypophysis is concerned with the production of a 'W' hormone.

The staining intensity of the P.A.S.+ve cyanophils also seemed to be different in fish from white and black backgrounds, although the differences were less definite than those between the lead haematoxylin cells. In some specimens, these cyanophils extended, with the lead haematoxylin cells, into the anterior region of the gland but in other specimens they seemed to be confined to the posterior region. They therefore seem less likely to be the site of 'W' hormone production than do the lead haematoxylin cells.

Assuming that the intensity of staining reaction is proportional to the amount of 'W' hormone present, then the presence

of very strongly stained cells in white-adapted fish suggests that the amount of hormone produced exceeds that required for complete melanophore concentration, and that the excess hormone is being stored. Injection experiments show that the degree of melanophore concentration depends on the level of 'W' hormone in the blood (Kent, 1960); yet chromatic adaptation to a white background can be maintained after the number of cells has been reduced by partial hypophysectomy (Healey, 1948); even in intact fish it is hardly likely that the number of active cells remains constant throughout the life cycle. This suggests that a feed-back mechanism must exist to regulate the rate at which hormone is released from the pituitary, maintaining the correct level of hormone in the blood for any given background colour.

There are several possible ways in which such a feed-back mechanism could operate. Vilter (1946) found that the distribution of retinal pigment in several lower vertebrates (eel, carp, triton) is influenced by the level of intermedin in the blood. It may be this effect of the hormone which modifies the intensity of nerve impulses from the eye to the pituitary. Alternatively, the hormone may affect the nerve impulses at the hypothalamic level.

An interpretation of the cytological picture in terms of hormone production and release is suggested in Table 5. Some points require further amplification.

Healey (1951) and Kent (1960) have estimated the average times required by a spinal-sectioned minnow to re-adapt its

Table 5. Cytological changes in the lead haematoxylin cells of minnow associated with the release of the melanophore concentrating hormone.

Background	Histology of lead-haematoxylin cells	Denervated melanophores	Interpretation
White	Many cells darkly stained and club-shaped.	Concentrated M.I. 1.	Production of 'W' hormone. Release of 'W' hormone. Production > release
White ↓ Black	After 5 days: Cells still darkly stained.	Dispersed in 2 days M.I. 5.	Inhibition of 'W' hormone release.
Black	After 4 weeks-1 year: Most cells degran-ulated and rounded-off. Cellular hypertrophy.	Dispersed. M.I. 5.	No production of 'W' hormone.
Black ↓ White	After 8 days: Slight increase of granulation in some fish. A few darkly stained cells with long stalks. After 4 weeks: as above.	Concentrated in 5 - 6 days M.I. 1.	Resumption of 'W' hormone production by some cells. Release of 'W' hormone.
Darkness	Pituitary assumes the appearance of black-adapted fish.	Dispersed M.I. 4.5.	No production or release of 'W' hormone.

M.I. = Melanophore Index, a scale for expressing the degree of dispersion of the melanophores.

M.I. 1 = fully concentrated melanophores.

M.I. 5 = fully dispersed melanophores.

melanophores after transfer from one background to another. Adaptation to a black from a white background is completed in about 2 days. Since after 5 days lead haematoxylin cells are still abundant in the pituitary, reduction of 'W' hormone in the blood must reflect not its depletion in the pituitary but the prevention of further release. When spinal-sectioned minnows are transferred in the opposite direction, that is, from a black to a white background, about 5 to 6 days are needed for complete melanophore concentration. This prolonged period probably reflects the time which the degranulated lead haematoxylin cells require to resume cellular activity. The appearance at this time of cells with very long cell stalks in some specimens may indicate a renewed connexion with the neurohypophysis; alternatively it is possible that the connexion was never lost, but only becomes obvious with the production of lead haematoxylin+ve material. It is significant that in minnows which have been adapted to a black background for only a brief time melanophore concentration is achieved relatively rapidly (Healey, personal communication). In these cases little or no re-organisation of the cells would be involved.

By no means all the cells regain their granulation during re-adaptation to a white bakcground; indeed, one specimen was observed in which almost no strongly stained lead haematoxylin cells were present - even after 4 weeks on a white background. It is possible that once a cell has reached a certain level of degranulation, or has lost all contact with the neurohypophysis, it is no longer able to recover. Should only a limited number of cells recover, then even

those which are actively secreting hormone will probably not appear very densely stained; it was suggested earlier than an intense staining reaction resulted from hormone storage, and this is unlikely to occur if a feed-back mechanism demands a high rate of hormone secretion from a few cells.

In black-adapted fish the proportion of degranulated cells was rather variable. A similar variation was observed in white-adapted fish, and one specimen from a white background was examined in which all the lead haematoxylin cells were degranulated. If, however, one is correct in assuming that these cells are the site of 'W' hormone production, such variation might be anticipated from observations made by Healey (1951). He found that the rate at which spinal-sectioned fish could equilibrate to black or white backgrounds, and the extent of dispersion or concentration achieved by the melanophores, was extremely variable. Using the melanophore index to express the degree of melanophore concentration or dispersal (fully concentrated melanophores have a melanophore index of about 1.0, while fully dispersed melanophores have a melanophore index of nearly 5), Healey found that some fish on a white background never achieved a melanophore index of less than 3.6, while on a black background other fish retained a melanophore index of 3 after 9 days adaptation. The cause of this variation is not clear. The problem is discussed further in § 5. These observations suggest a convenient way in which the cellular origin of the 'W' hormone could be confirmed. According to the identification suggested in this thesis, fish which were unable to adapt to a white

background would lack lead haematoxylin cells; conversely one might expect to find a relatively high proportion of densely staining lead haematoxylin cells in fish remaining pale after a month or more on a black background.

In white-adapted fish the eye is stimulated by reflected light from the white background, and in turn exerts a nervous control over the pituitary (Butcher, 1938; Hogben & Landgrebe, 1940; Parker, 1948). It is reasonable to suppose that the loss of lead haematoxylin granules in fish maintained on a black background or in darkness results from the absence of nervous stimulation. A parallel example of degranulation and cellular hypertrophy has been observed in the pars intermedia cells of several mammals after pituitary stalk section, by which the nervous control over these cells is destroyed (rabbit, Brooks, 1938; rat, Desclin, 1942; Bogdanove et al., 1955; monkey, Holmes et al., 1959). After such treatment in rats the hormone content is reported either to fall (Hamori, 1960) or to remain the same (Fisher, 1937) but it is not known whether this reflects a decrease in cellular activity or an increase in the rate of hormone secretion. It would be wrong, however, to assume that the loss of granulation necessarily indicates complete cellular inactivity. In Amphibia, Etkin (1941) has shown that the cells of the pars intermedia possess an inherent ability to produce and release hormone, and the possibility that this may also be so for the lead haematoxylin cells of minnow should not be overlooked. In this case, a 'W' hormone precursor may be produced on

a black background or in darkness and transformed to the active hormone on a white background. This would explain why the cells do not become atrophied but remain apparently healthy in conditions not requiring the release of 'W' hormone.

If the two-hormone hypothesis outlined in the introduction is valid one might expect two cytological conditions to be fulfilled: (a) two cell types from the meta-adenohypophysis should undergo cytological changes during adaptation to a black or a white background. This in fact occurs; the P.A.S.+ve cells seem to give a stronger staining reaction in black-adapted fish;
(b) the cytological appearance of these cells should be different in black-adapted fish from those maintained in darkness, since the hypothesis requires that the 'B' hormone can only be produced under illuminated conditions. This second requirement is not fulfilled, the cytological appearance of the pituitary being the same in both groups of fish.

The function of the P.A.S.+ve cyanophils is not clear nor is it easy to determine from their cytological appearance whether these cells are active in fish from a white or a black background. It is not at all certain that the 'W' hormone of minnow, which in this species causes melanophore concentration, is identical with the erithrophore dispersing hormone. Although teleost pituitary extracts cause both melanophore concentration and erithrophore dispersion, not all substances which bring about erithrophore dispersion will cause melanophore concentration; thus mammalian intermedin will disperse

both types of chromatophores (Pickford & Atz, 1957). Moreover erithrophore dispersion is never seen in white-adapted minnows when the 'W' hormone is released, but is not uncommon in black-adapted fish. It therefore seems possible that a second hormone causing erithrophore dispersion is produced by the P.A.S.+ve cyanophils. If this is so, one could expect to find signs of active secretion by these cells during the breeding season when the erithrophores become fully dispersed. (Experiments by Giersberg (1934) on the development of breeding colour in intact and hypophysectomised minnows indicate that erithrophore dispersion is dependent on pituitary secretion and not the direct effect of reproductive hormones as in some other teleosts). Minnows in full breeding colour were not examined during this investigation.

(ii) Trout.

In both Salmo trutta and S. gairdneri the meta-adenohypophysis contains lead haematoxylin cells which as in minnow give a strong staining reaction in white-adapted fish and become degranulated in black-adapted fish. With the stain employed the second type of cell occurring in this region appears chromophobic in both black- and white-adapted fish, and it is therefore impossible to determine whether or not its activity is changed during background adaptation.

The little data available suggest that Salmo species may produce a 'W' hormone. Injections of fish pituitary extracts cause melanophore concentration in Salmo gairdneri (Robertson and Rinfret, quoted in Pickford & Atz, 1957), but the effects of hypophysectomy have not been investigated. Although it is tempting to suppose that,

as in minnow, the lead haematoxylin cells may produce a 'W' hormone, there are insufficient data available to permit such an interpretation, and the cytological changes may equally well be interpreted in terms of the release of a 'B', or a melanogenetic hormone on a black background.

Olivereau (1954) has described fluctuations in the activity of the meta-adenohypophysis during the life cycle of Salmo salar. Judging by the condition of the cell chondriome, and by the frequency of mitotic figures, she considers this region to be most active during smoltification and in young smolts. Prior to the seaward migration many of the cells in the meta-adenohypophysis degenerate and are only partially replaced by the mitotic activity of some remaining cells. Mitotic activity is frequent also in adult fish which have returned to fresh water to breed, and in splayed fish this region is relatively extensive composing, together with the nervosa, about three-fifths of the entire gland. It is difficult to correlate these cytological changes with the changes in pigmentation. Activity of the meta-adenohypophysis during smoltification accompanies the degeneration of melanophores and the deposition of guanine crystals. On the other hand, Olivereau (1954) has suggested that mitotic activity may be associated with the deposition of melanin in the skin: mitotic activity during the spawning migration accompanies a loss of guanine and the appearance of black and red pigmentation (Menzies, 1931). Melanogenesis appears to be promoted in fish maintained on a black illuminated background (Odiorne, 1937; Osborne, 1941; Fortune, 1960), and thus if Olivereau's suggestion that the cells of the meta-adenohypophysis are

concerned with melanogenesis is correct, it is possible that the degranulation of the lead haematoxylin cells in black-adapted parr reflects the release of a hormone stimulating melanin production, while the more intense staining reaction in white-adapted trout may indicate a storage of this hormone.

(iii) Catfish (Ameiurus melas), goldfish (Carassius auratus), Cichlasoma nigrofasciatum and Rana temporaria.

The chromatic responses of amphibia to injections of pituitary extracts and to partial or total hypophysectomy have been studied in considerable detail. Relevant data are given by Hogben & Slome (1931), Etkin (1941), Parker (1948) and Pickford & Atz (1957), and leave little doubt that a 'B' hormone is produced in the pars intermedia.

Similarly, the effects of hypophysectomy and of pituitary extract injections indicate the presence of a 'B' hormone in both Ameiurus and Carassius (Osborne, 1938; Chavin, 1956; Pickford & Atz, 1957).

The chromatic responses of Cichlasoma nigrofasciatum have not been studied. Stolk (1960) states that in the cichlid Pterophyllum, pituitary extracts induced melanophore concentration, and he found that this hormone occurred in greater concentrations in the posterior region of the gland, including the meta-adenohypophysis, than in the anterior region of the gland.

The factors controlling the production and release of a 'B' hormone are clearly not the same as for a 'W' hormone. In white-adapted fish, light is reflected from the background and stimulates

the dorsal retina of the eye; nerve impulses from this region will in turn influence the pituitary (Butcher, 1938; Hogben & Landgrebe, 1940; Parker, 1948). It was suggested earlier than in the minnow both production and release of 'W' hormone are dependent on such a stimulus; consequently, on a black background from which no light is reflected, both production and release of hormone are halted. This cannot be the case where a melanophore-dispersing hormone is involved in chromatic adaptation; the potentials for the production and release of a 'B' hormone must exist in black-adapted specimens, and will thus be present in white-adapted specimens also, although in this latter case stimulus from the white background presumably prevents the potential for either the production or release of 'B' hormone from expressing itself.

It is uncertain whether the production of a 'B' hormone is in some cases dependent on the stimulus of overhead illumination, or whether both hormone production and secretion are an inherent property of the cells requiring no stimulus. Amphibia and teleosts become paler in the dark than on an illuminated black background (Hogben & Slome, 1936; Osborne, 1938; Neil, 1940; Bagnara, 1960), but it is possible that this pallor is due to the activity of the pineal rather than an inability to produce and release 'B' hormone. A chemical, melatonin, can be extracted from the pineal, which induces melanophore concentration in Fundulus larvae (Wyman, 1924) and Amphibia (Lerner & Case, 1954; Bagnara, 1960), and lipophore concentration in Phoxinus (Supriewski et al., 1960). Bagnara (1960) showed that in Amphibia the melanophores did not become concentrated in the dark after epiphysectomy and he

suggests that the release of melatonin is inhibited in the light, but occurs in the dark, causing pallor. Hoar (1955) found that blinded salmon smolts, in which the melanophores were partially dispersed, became very dark when the pineal was destroyed.

Experiments made by Osborne (1938) on Ameiurus nebulosus also suggest that 'B' hormone may be released in the dark. He found that the melanophores of intact catfish kept in the dark were more dispersed than those of hypophysectomised catfish in the dark. It is interesting that the melanophores of nearly all teleosts become dispersed after blinding (Parker, 1948).

The experiments of Etkin (1941) on Rana pipiens show that in this species the cells of the pars intermedia possess an inherent ability to produce and release 'B' hormone. This investigator found that after infundibular lesions the pars intermedia became enormously enlarged, up to as much as twelve times the normal size: the cells gave a strong staining reaction, and the melanophores became fully expanded after 2 days, indicating the release of intermedin. Similar changes were observed when the adenohypophysis was grafted on to the choroid plexus, or into the optic vesicle of a previously hypophysectomised host of the same species. Etkin concluded that on a white background intermedin release is actively inhibited. This conclusion is supported by the observations of several other workers. The relevant data are given in Table 6.

The pars intermedia cells of Rana pipiens and R. temporaria maintained on a white background stain strongly with P.A.S. and lead

Table 6. The effect of different illuminations and nerve lesions on the intermediate lobe and the secretion of intermedin in Amphibia.

Species	Treatment	Response	Reference
Rana pipiens	Destruction of infundibulum.	Hypertrophy of intermediate lobe. Cells increase in staining reaction. Melanophores fully dispersed (= release of 'B' hormone).	Etkin, 1941.
Amphibia generally	Blinding	Melanophores partially expanded. More expanded in illuminated conditions than in dark.	Parker, 1948.
Bufo arenarum	Blinding	Increased concentration of 'B' hormone in pituitary.	Masselin, 1939b.
Bufo arenarum	Continuous darkness 1 week - 2 months	Increased concentrationof 'B' hormone in pituitary.	Masselin, 1939b.
Bufo arenarum	Continous darkness	Cells more chromophilic than illuminated toads.	Masselin, 1939a.
Rana temporaria	Continous darkness	Pars intermedia cells enlarge and vacuolise.	Florentin & Stutinsky, 1936.
Rana temporaria	Continous darkness	Intermediate lobe increases in volume.	Cehovic, 1956.
Bufo arenarum	2 weeks Continuous illumination on white background - Natural increase in illumination during Spring - Electrical stimulation of optic nerve in blinded toads.	Decreased concentration of 'B' hormone in pituitary.	Masselin, 1936b.

continued.

Rana esculanta	Continuous illumination for 1 week.	Intermediate lobe cells become small and chromophobic.	Stutinsky, 1936.
Rana pipiens	White background Diurnal conditions. 12-21 days.	Intermediate lobe cells stain strongly with P.A.S, but not with Baker's acid hematin.	
	Black background Diurnal conditions. 9-22 days.	Intermediate lobe cells lose affinity for P.A.S. but stain with Baker's acid hematin.	
	Black & white backgrounds.	No difference in intermedin content of pituitaries of frogs from different backgrounds.	Ortman, 1954, 1956.

haematoxylin (Ortman, 1954, 1956; present investigation). If one may make the assumption that the affinity for these stains is proportional to the concentration of hormone present, then this histological picture indicates hormone storage. Conversely, the loss of granules from these cells in black-adapted frogs suggests that the release of hormone under illuminated conditions reduces the intermedin content of the pituitary. The observations of Etkin (1941) and Masselin (1939a & b) show that in blinded and dark-maintained frogs hormone release does not involve a depletion of hormone, and a consideration of these results, together with those of Stutinsky (1936) and Masselin (1939b) on the effect of constant illumination on the pars intermedia suggest that under illuminated conditions production itself may be inhibited. Ortman's observations, and those on R. temporaria during the present work, were made on frogs with diurnal illumination; under these conditions hormone could be produced during the periods of darkness and stored during the day in white-adapted frogs, but released in black-adapted frogs. Theoretically if this conclusion were correct, the intermedin content in the pituitary of black-adapted frogs under continuous illumination should be significantly reduced. This has never been tested; but Ortman (1954) was unable to detect any difference between the intermedin content in the pituitary of white- and black-adapted frogs.

In Cichlasoma the P.A.S.+ve acidophils in the meta-adeno-hypophysis of black-adapted specimens were large and darkly stained, while both their size and staining reaction was significantly reduced in white-adapted specimens (I, § 3).

It was suggested (I, § 4) that these cytological conditions reflect the active production and release of hormone in fish on a black background but that, as in amphibia, these activities are inhibited in white-adapted specimens. Such an interpretation indicates that the hormone involved may be either a 'B' hormone, or one stimulating melanogenesis.

In both Carassius and Ameiurus, chromatic adaptation did not appear to be accompanied by any cytological changes in the meta-adenohypophysis. This does not necessarily contradict the view that the chromatophore-regulating hormone is produced in this region of the pituitary.

In minnow, trout and Cichlasoma, it is suggested that the cytological changes result from a change in the level of stored hormone; it has also been suggested above that where a 'B' hormone is involved this may theoretically be present in white-adapted specimens. Thus it is possible that in Ameiurus and Carassius, both of which appear to produce a 'B' hormone, the hormone is present but not released in fish on a white background, while on a black background the rate of hormone production equals that of hormone release; the release of hormone therefore need not be accompanied by any marked cytological changes.

In conclusion, the observation that in some teleosts, chromatic adaptation involves cytological changes in the meta-adenohypophysis supports the view that this region of the pituitary is responsible for the production of a chromatophore-regulating hormone. These investigations have also made it clear that a particular cell

type, as identified by staining with lead haematoxylin or P.A.S., is not involved in chromatic changes in different species, nor can it be associated in all teleosts with a specific hormone. Thus the lead haematoxylin cells undergo degranulation in black-adapted minnows, but show no cytological changes related to background adaptation in Cichlasoma.

The chemical basis for a staining reaction with lead haematoxylin is not know, although it is obviously not based on the pH of the cell cytoplasm. Intermedin from several mammals has been analysed and shown to be a polypeptide including, among others, the amino-acids histidine, tyrosine and tryptophane (Harris & Roos, 1956; Jutisz & de la Llase, 1961). These have all been identified by histochemical methods in the P.A.S.+ve cyanophils of the meta-adenohypophysis of minnow (Matty and Matty, 1960). Kent (1960) has found that the hormone or hormones causing melanophore concentration and erythrophore dispersion in minnow are polypeptides, and if one may assume that they contain the same amino acids as mammalian intermedin then the observations of Matty & Matty suggest that a chromatophore hormone is produced by the P.A.S.+ve cyanophils but appears to contradict the identification of the lead haematoxylin acidophils as the site of 'W' hormone.

Although an attempt has been made to interpret the cytological appearance of the meta-adenohypophysis, it is clear the explanations offered are far from conclusive. Many of the results may be interpreted in more than one way, and alternative views have been indicated

although emphasis has naturally been placed on the view which seems most reasonable in the light of available data. It is desirable to emphasise however that very little is known about the conditions affecting the production and release of the chromatophore-regulating hormones, and the interpretations proposed above should be regarded primarily as a basis for further experiments, and not conclusive in themselves.

§ 5. DISCUSSION.

In the preceding section the chromatophore-regulating hormone has been considered as an agent causing either pigment concentration or dispersion, resulting in chromatic adaptation to the background, and it is usually supposed that these changes serve to camouflage the animal and thus have a protective value.

In teleosts it is generally supposed that the chromatophore hormone is produced in the meta-adenohypophysis, and evidence supporting this view has been given in III, § 1 and 4. Yet it was observed in some species that the cells in this region show changes in activity which are not explicable in terms of background adaptation. Pickford & Atz (1957) make the comment, "To the best of our knowledge no-one has studied the possibility that in fishes intermedin is concerned with functions other than pigment dispersion, maintenance of melanophores and melanogenesis." It is the purpose of the following discussion to consider whether there is any evidence for believing that the chromatophore-regulating hormone in lower vertebrates may have another function, which might explain those cytological variations which were not caused by a change of background.

A chromatophore-regulating hormone - intermedin - is also produced by higher vertebrates, in the pars intermedia of most mammals and in the pars distalis of birds and certain mammals which lack a pars intermedia (Kleinholz & Rahn, 1939; Waring & Landgrebe, 1950). Although this hormone causes physiological colour change when injected

into lower vertebrates, whose melanophores never exhibit pigment dispersion or concentration. It is therefore assumed that in these higher vertebrates the hormone has some other function.

There is considerable evidence that in mammals intermedin is released and presumably functionally concerned during several different physiological conditions. The relevant data are most conveniently presented in tabular form (Table 7). Some of the data are apparently contradictory, for instance that of Giroud et al. (1944) and Freiden (1951) concerning the effect of thyroidectomy on intermedin release in rats. Again, Ivy & Albert (1957), also working on rats, found that the intermedin content in the pituitary remained normal after adrenalectomy and was reduced by 40% after injections of ACTH, but that the two procedures together resulted in an increase in the intermedin content. It is not clear whether these changes reflect an alteration in the rate of hormone production or the rate of its release from the pituitary. Other experiments suggest that the release of intermedin is inhibited by cortisone, while conversely a lack of cortisone, following adrenalectomy or in Addison's disease, is accompanied by an increase in the intermedin content of the blood (Johnsson & Högberg, 1953; Lerner, 1954). During pregnancy, however, in spite of an increase in serum cortisone, there is also an increased release of intermedin (Lerner, 1954; Snell & Bischitz, 1960). In these circumstances it would appear that the influence of some other hormone, possibly progesterone or estrogen, over-rides the effect of cortisone. The data suggests that the release of intermedin is affected by adrenal,

Table 7. The effect of hormonal changes on the intermediate lobe and the secretion of intermedin in mammals.

Species	Treatment	Response	Reference
Rat	Thyroidectomy	Slight increase in intermedin content of pituitary.	Giroud et al.,[1] 1946.
Rat	Thyroidectomy Thiouracil.	50% decrease in intermedin content of pituitary.	Freiden, 1951.[2]
	Thyroxine powder added to food.	Slight increase in intermedin content of pituitary.	
Rat	Injection of intermedin.	Reduced I^{131} uptake by thyroid.	Courrier & Cehovic, 1960.
Hamster	Pellets of diethystilbestrol implanted under skin.	Slight hypertrophy and enormous hyperplasia of the intermediate lobe cells. After $3\frac{1}{2}$ months Golgi apparatus enlarged; and increased basophilia.	Koneff et al., 1946.
Hamster	Estrogen pellets implanted under skin, monthly for 1 year.	Hypertrophy of intermediate lobe due to increase in cell number and size.	Racadot, 1959.
Rat	Pregnancy.	Pars intermedia cells proliferate into neural process.	Stutinsky, 1954.
Rat	Estrogen injections.	No change in intermedin content of pituitary.	Ivy & Albert, 1957.[3]
Man	Pregnancy. Progesterone injections.	Increase in melanisation of skin.	Lerner et al., 1954.
Guinea pig	Oestrogen & Progesterone	Increased concentration of melanin in skin and melanophores. No change in number of melanophores.	Bischitz & Snell, 1960.
	Ovarectomy.	Reduction of melanin concentration within melanophores. Reduction in number of melanophores.	

continued.

Man	ACTH injections.	Darkening of skin.	Lerner et al., 1954.
Cat	ACTH injections.	Hypertrophy of intermediate lobe due to increase in cell size.	Karkun et al., 1954.
Rat	ACTH	40% reduction of pituitary intermedin content.	Ivy & Albert, 1957.
	ACTH injections after adrenalectomy.	Increase in pituitary intermedin content.	
	Adrenalectomy.	No effect on pituitary intermedin content.	
Rats	Adremalectomy Addisons disease.	Increase in intermedin content of blood.	Johnsson & Högberg, 1953.[4] Lerner et al., 1954.
Adrenalectomised dogs. Man, with Addison's disease.	Cortisone	Pigmentation in man, and intermedin content of blood in dogs returned to normal.	Lerner et al., 1954.
Rat	Stress (continuous sound) for 4 weeks.	Intermediate lobe cells increase in number, and staining reaction. Increase of extracellular colloid. Hyperactivity of adrenal cortex.	Werner, 1959.
Rodent - Meriones crassus.	Dessication	Increased weight of pars	Legait, 1960.
	Dextrose solutions	No increased weight of pars intermedia. Increase of intranucleolar vacuoles.	

1. Intermedin tested on isolated carp scales. Only a few specimens investigated. Due to the variation of intermedin content between individual rats noted by Freiden (1951) these results may not be statistically significant.
2. Intermedin tested on amphibia.
3. Presence of intermedin tested on strips of isolated frog skin.
4. ACTH extracted from serum before testing on amphibia.

thyroid, reproductive and possibly other pituitary hormones, and conflicting observations probably result because intermedin release is subject to more than one influence at any one time. In mammals an increase of this hormone in the blood is accompanied by an increase in melanogenesis (Lerner & Case, 1959; Bischitz & Snell, 1960; Snell & Bischitz, 1960), but this cannot be seriously considered as more than a secondary effect of the hormone.

The question arises whether, with the loss of physiological colour change in higher vertebrates, intermedin has acquired new functions and new target organs, or if in lower vertebrates also the hormone possesses some function in addition to chromatic control. If a second function did exist, one might expect the cells of the meta-adenohypophysis to show changes of activity unrelated to background adaptation, but possibly as in higher vertebrates related to changes in the level of thyroid, adrenal or reproductive hormones.

Cytological changes have been observed in the meta-adenohypophysis of Xiphias (Lee, 1942), Cyprinus sp. (Parhon et al., 1959) and Carassius auratus (Scruggs, 1951; and present investigation) and appear to accompany changes in reproductive activity. Thus in Carassius the enlargement of the lead haematoxylin+ve cyanophils and the change in shape of their nuclei suggest an increase in activity accompanying maturation of the gonads, while the acidophils undergo a numerical increase immediately after the reproductive season (Scruggs, 1951). Chavin (1956) found that the acidophils are affected by stress conditions. In Salmo salar, Olivereau (1954) records an increase in

activity of the meta-adenohypophysis during smoltification, at which time there is also an increase in thyroid activity (Fontaine, Leloup & Olivereau, 1952; Olivereau, 1954), and a rise of serum cortisone (Fontaine & Hatey, 1954). In Cichlasoma nigrofasciatum cytological variations occur in the lead haematoxylin+ve cells, which are not related to background adaptation (present investigation, I,§ 3). It has not been proved that all the cells of the meta-adenohypophysis are concerned with the production of a chromatophore-regulating hormone, and thus the cytological changes cited above may not reflect the release of this hormone. It will be noted, however, that in Carassius both types of cell are affected by conditions other than a change in background colour.

If one is correct in suggesting that in teleosts the release of chromatophore hormone can be influenced by factors other than the background colour, then at such times the hormone must abandon its function of maintaining background adaptation. It may be no coincidence that under the conditions in which cytological changes were observed in the meta-adenohypophysis, the melanophores do not show background adaptation but appear in some cases to be influenced by other hormones rather than the chromatophore hormone. Thus, during the reproductive period, many teleosts exhibit breeding colours. This type of colouration can be induced by injections of androgens or estrogens, and may occur even after hypophysectomy, indicating the direct effect of the reproductive hormones on the chromatophores (Giersberg, 1934; Burger, 1942; Pickford & Atz, 1957). In Phoxinus on the other hand

Giersberg (1934) found that androgens failed to induce nuptial colouration after hypophysectomy, and he suggested that in this species a pituitary hormone stimulating erythrophore dispersion is involved. Kent (1960) has presented evidence to show that the melanophore-concentrating and erythrophore-dispersing hormones have the same general distribution in the pituitary and suggests the possibility that the two hormones are identical. However, both melanophores and erythrophores are fully dispersed during the breeding season and thus if the 'W' hormone is indeed responsible for erythrophore dispersion, some other factor must, at this time, induce the dispersion of the melanophores. It is significant that very large doses of pituitary extract are required to bring about melanophore concentration in minnows which are in breeding colour (Kent, personal communication). Kent (1960) has also noted that minnows maintained on a black background may develop fully dispersed erythrophores. These observations may explain why, in those minnows transferred from a black, illuminated background to darkness the lead haematoxylin cells were more densely granulated than expected. In these fish, also, the cyanophils of the meso-adenohypophysis (presumably gonadotrophs) were highly vacuolated (see however §4, page 140).

Godet (1959) found that in Protopterus the melanophores became fully dispersed during aestivation. Melanophore changes occur only slowly in this fish, and appear to be dependent on hormonal influence: spinal section had no effect on the melanophores, but hypophysectomy of aestivating fish resulted in melanophore concentration; the dispersed condition was restored by intermedin injections. Godet concludes that

melanophore dispersion during the aerial phase is due to the release of a 'B' hormone, and he states that at this time, in contrast to the aquatic phase, the fish is unable to adapt to a white background. This suggests that hormone release is stimulated by some factor other than the level of illumination. No investigations appear to have been made on the state of cortical or thyroid activity during aestivation.

The adoption of some other function by the chromatophore-regulating hormone does not necessarily imply that background adaptation is lost. In many teleosts, the melanophores are under central nervous control, and the fact that innervated melanophores may undergo considerable chromatic changes in a few minutes indicates that the influence of nervous control can over-ride the effect of physiological doses of hormone. In some teleosts, such as Fundulus, the response to even high doses of chromatophore-regulating hormone is only revealed after the central nervous control has been destroyed (Pickford & Atz, 1951). If it is assumed that chromatic control is the only function of the chromatophore-regulating hormone, it is paradoxical that this hormone should occur where chromatic effectors capable of achieving background adaptation are absent. Thus, in both Salmo and Carassius the melanophores degenerate relatively early in life. Macropodus opercularis, on the other hand, possesses melanophores which are under nervous control, but which even after denervation do not respond to injections of chromatophore-regulating hormone (Pickford & Atz, 1957).

It has been shown that changes may occur in the meta-adenohypophysis irrespective of the background but which in some cases

accompany changes in the activity of either the reproductive organs, thyroid or adrenals. There is no evidence, admittedly, that these changes are correlated. The suggestion that the chromatophore-regulating hormone influences other target organs besides the chromatophores implies that these target organs may be affected under conditions, such as a white or black background, which stimulate the release of this hormone. Few investigations have been directed towards this aspect of the problem in fish. An interesting observation was made by Vilter (quoted by Callamand, 1943) who found that swordtails (Xiphophorus helleri) became precociously sexually mature when they were reared on a black background. Unfortunately Callamand gives no details about this experiment, nor a reference to any written report.

The glandular disturbances resulting from constant illumination or total darkness will not be considered in this context. Although these conditions may affect the release of chromatophore hormone (see Table 6) overhead illumination appears to have a direct effect on the activity of other pituitary hormones - an effect which in some cases seems to be mediated through the pineal (Atz, 1953; Pfugfelder, 1953; 1956; Rasquin & Rosenbloom, 1954; Kitay, 1957; Tixier-Vidal, 1959).

In Amphibia intermedin not only controls background adaptation but appears to have a marked effect on thyroid activity. Cehovic (1956; 1957; 1960) showed that in Rana esculenta and R. pipiens injections of intermedin caused a 50% decrease in the fixation of iodine^{131} by the thyroid. The same effect was observed in darkness, a condition

promoting the release of intermedin. The same author found that in Amphibia, iodine^{131}, presumably incorporated in thyroxine, accumulates in higher concentrations in the pars intermedia than in any other region of the pituitary (Cehovic, 1956). With a decrease in the activity of the thyroid, caused by intermedin, the level of iodine in the pituitary is also reduced. These results are comparable with those of Courrier & Cehovic (1960) on the rabbit, in which intermedin was also found to reduce iodine fixation and thyroid activity. In rats, thyroidectomy appears to have an effect on the intermedin content of the pituitary (Table 7). Ortman (1956) found that in Rana pipiens the intermedin content of the pituitary is not affected by thyroidectomy. Thyroxine or thyroid extracts cause melanophore concentration in Amphibia (Warren, 1940), but without parallel experiments on hypophysectomised frogs it is impossible to say whether this indicates a reciprocal effect of thyroxine on intermedin release. In teleosts, thyroxine may induce either melanophore dispersal or melanophore concentration, prolonged treatment in both cases resulting ultimately in melanophore degeneration. Again, it has not been shown that the chromatophore-regulating hormone is involved. Thyroxine will itself induce melanophore concentration on isolated strips of Phoxinus or Salmo skin (Fortune, 1960).

Various functions of intermedin have been suggested. Evidence for the role of this hormone in carbohydrate metabolism is reviewed by Waring & Landgrebe (1950). The work of Hanaoka (1953a & b) and others suggests that the hormone may influence the synthesis of visual

purple,while in lower vertebrates (Triton, Anguilla, Carp) Vilter (1946) found that the hormone regulated the dispersion of retinal pigment. The most recent investigations in this field are those of Krivoy & Guillemin (1961; Guillemin & Krivoy, 19 ;), who found that intermedin had a facilitating effect on impulses across a spinal reflex arc; the occurrence of an enzyme distributed throughout the cerebral tissue, which causes the breakdown of intermedin, suggests that this hormone may exert its influence throughout the central nervous system.

The term intermedin in mammals includes a mixture of two similar but distinct polypeptide hormones: α & β intermedin have been identified in several mammals, while different forms of β intermedin occur in different species and even within a single species (reviews by Geschwind, 1959; Jutsz & da la Llaso, 1961). These hormones differ in their physiological properties; thus α intermedin has a more marked effect on iodine uptake by the rabbit thyroid than β intermedin (Courrier & Cehovic, 1960) while conversely only β intermedin was found to cause nerve facilitation in the cat (Krivoy & Guillemin, 1961). A further difference between the actions of these two hormones has been reported by Lerner & McGuire (1961) who find that while α intermedin will consistently stimulate melanin production, the effects of β intermedin are variable.

Among teleosts, the variations in chromatic response to pituitary injections, and the differences between species in the staining reaction of the meta-adenohypophysial cells, suggests that more than one form of chromatophore-regulating hormone also occurs in this class of vertebrates, and probably even within one species. So far,

however, no satisfactory means has been found by which to distinguish these different hormones. The data considered above indicate that while the chromatophore-regulating hormone is recognised by its obvious effect on the chromatophores, it may have other less obvious but equally important effects on other target organs. It therefore seems possible that a study and comparison of these effects may not only explain the cause of variation in the meta-adenohypophysial cells between members of one species kept on identical backgrounds, but also prove a means of distinguishing between the different types of chromatophore hormones in teleosts.

SUMMARY OF THE MAIN CONTRIBUTIONS OF THIS THESIS TO THE KNOWLEDGE OF THE STRUCTURE AND DEVELOPMENT OF THE TELEOST PITUITARY GLAND AND THE FUNCTIONS OF SOME OF ITS CELLS.

1. The histology of the pituitary gland of two species of cichlid fish - Herichthys cyanoguttatus and Cichlasoma nigrofasciatum has been described both from adult specimens and during the early stages of development (pp13-30&45-58) The blood supply of the pituitary gland of fish of different ages has also been examined (pp.39-44).

2. The pituitary receives arterial blood from the internal carotid and venous blood from the median hypocranial vein and from the meninges (pp.39&42-43)

   The neurohypophysis and adenohypophysis do not have an independent blood supply as occurs in tetrapods (p.42).

   Capillaries from the hypothalamus drain into the meningeal vessels so that a vascular connection exists between the hypothalamus and the pituitary (p.43).

3. Cells including granules, which stain with the neurosecretory stains A.F. and Gomori's C.A.H., occur in the ventral hypothalamus of young cichlids. These cells border small blood capillaries which drain either into the meningeal vessels or end in the wall of the 3rd ventricle.(p 43-44.)

   Similar cells were noted in the hypothalamus of Carassius auratus, Phoxinus phoxinus and Barbus partipentazona, but were not found in adult cichlids.

The possibility that these are secretory cells whose secretion influences the pituitary is considered (p.57 , & pp.43-44).

4. The organisation of the pituitary is similar to that of other teleosts: three distinct regions can be recognised in the adenohypophysis, all of which are penetrated by branches of the neurohypophysis.

Seven cell types can be distinguished:

Pro-adenohypophysis - Azocarmine acidophils
Lead-haematoxylin+ve amphiphils.

Meso-adenohypophysis - Azocarmine acidophils.
Two groups of P.A.S.+ve and A.F.+ve cyanophils (groups A & B).

Meta-adenohypophysis - P.A.S.+ve, orange-G acidophils.
Lead-haematoxylin+ve cyanophils.

The neurohypophysis includes nerve fibres, some containing neurosecretion, and two types of pituitcytes (pp.13-30).

5a. During the latter stages of oöcyte vitellogenesis some of the group B cyanophils of the meso-adenohypophysis reach their peak in size, vacuolisation and staining intensity. It is suggested that the cells of this group are gonadotrophs (p.24 & p.34).

b. Cells with this distribution in the pituitary are differentiated before the end of the second week of development in Herichthys but few cells with this distribution can be recognised in the developing pituitary of Cichlasoma even by the 5th week. A difference also exists between these two species in the rate at which the primary oöcytes are differentiated during the first few weeks of development. These two differences may or may not be connected (pp.51-53 & 61-62).

6. The effect of thiourea treatment on the adult thyroid and pituitary has been examined in a limited number of specimens of Cichlasoma (p25-26). None of the pituitary cells in the adult gland are strongly affected by this treatment but reasons are advanced for believing the group A cyanophils of the meso-adenohypophysis to be the thyrotrophs (p.36-37).

7. In Herichthys A.F.+ve cells, presumably thyrotrophs, are the first cells to become histologically differentiated during development; they can be detected by the 2nd day after hatching (4th day after oocyte deposition and fertilisation).
In Cichlasoma even at 5 weeks nearly all the A.F.+ve cells occur in the position of the group A cyanophils, lending support to the view that the cells of this group are thyrotrophs (pp.52 & 60).

8. Specimens of Herichthys cyanoguttatus were reared in 0.033% thiourea solution from the 1st day of development (day of fertilisation). The effect of such treatment on the development of the thyroid, pituitary and other structures, compared with their development in control fish, has been examined.

a. Colloid secretion begins in the thyroid on the 2nd day after hatching. Differences between the control and experimental fish, in the number of thyroid follicles, their size and colloid content, become apparent on the 4th day after hatching. These differences become more marked with prolonged treatment with thiourea (pp.75-85).

8b. The differences between the thyroid of control and experimental fish on the 4th day are suggestive of excessive thyrotrophic stimulation in the latter case.

It is suggested that thyrotrophic hormone is released immediately before this time (pp.97-98). An alternative interpretation of the observations, that the thyroid is under the influence of the pituitary from the first stages of colloid secretion, is considered (p.98).

c. Treatment with thiourea results in an increase in both the number and size of the thyrotrophs by the end of the 2nd week. There is no apparent difference in the appearance of the thyrotrophs of control and experimental fish at this stage (pp.88-89).

d. The dermal melanophores of white-adapted, thiourea-treated fish fail to show pigment concentration on the 8th day after hatching. A possible reason for this has been suggested (p.103).

e. The swim-bladder of thiourea-treated fish fails to dilate on the 7th day after hatching. A possible explanation for this is considered (pp 103-104.)

f. The skin becomes much thinner and the mucus glands degenerate in thiourea-treated fish.(p.93).

9. Specimens of Herichthys cyanoguttatus were maintained in thiourea solution from the 4th day, and from one week after hatching. There is a latent period before the thyroid follicles show signs of thyrotrophic stimulation; this becomes more extended the older the fish is when thiourea treatment begins.

Treatment with thiourea at these later stages does not prevent the subsequent concentration of the melanophores and the dilation of the swim-bladder, but does cause a thinning of the skin (pp.85-88 & 92-93).

10. The P.A.S.+ve acidophils of the meta-adenohypophysis usually become differentiated on the 9th day after hatching in Cichlasoma and on the 14th day after hatching in Herichthys.
These cells can only be detected at this stage in fish reared on a black background and not in fish reared on a white background (p.56.).

11. The effect on the meta-adenohypophysis of prolonged adaptation to black and white backgrounds has been examined in adult Cichlasoma, adult minnows (Phoxinus phoxinus), parr stage of trout (Salmo sp.), Goldfish (Carassius auratus) of different ages and adult but unripe catfish (Ameiurus). Previous observations by other workers on the effect of similar long-term adaptation of the intermediate lobe of the frog have been confirmed (pp.29-30 & 111-131.)

a. The meta-adenohypophysis, or intermediate lobe of all these species contains lead haematoxlin+ve cells; in Phoxinus and Cichlasoma a second cell type in this region is stained by P.A.S. In frog, a single cell type is stained with lead haematoxylin and P.A.S.

b. The cell types in the meta-adenohypophysis and the intermediate lobe of different species are not histologically comparable when examined with lead haematoxylin, P.A.S. and Azan (p.131).

11c. The cells which respond to changes of background are not the same in different species.

i) In both Phoxinus and Salmo the lead haematoxylin cells become degranulated after prolonged adaptation to a black background (pp.114 & 119).

Reasons are advanced for believing that in Phoxinus these cells are the site of production of the hormone stimulating melanophore concentration (pp.133-134). No such conclusion can be reached about these cells in Salmo (pp.141-143).

ii) In Phoxinus the P.A.S.+ve cyanophils of the meta-adenohypophysis also appear to show cytological differences in specimens from white and black backgrounds. The possible function of the hormone produced by these cells is considered (p.140-141).

iii) In Cichlasoma the P.A.S.+ve acidophils but not the lead haematoxylin cyanophils of the meta-adenohypophysis show cytological differences from long-term white- and black-adapted specimens. These changes are interpreted in terms of hormone release. Possible functions of the hormone are considered (pp.37-38 & 148-149).

iv) In both Carassius and Ameiurus, long-term adaptation to white and black backgrounds is not accompanied by any cytological changes in the meta-adenohypophysis (pp.123-127).

v) In Rana, the degranulation of the P.A.S.+ve, lead haematoxylin cyanophils in black-adapted specimens is confirmed (p.127-130)

12. The nervous control of the production and release of both chromatophore-concentrating and chromatophore-dispersing hormones is discussed and forms the basis for theoretical interpretations of the cytological picture of the pituitary of white- and black-adapted teleosts and frog (pp132-151).

13. Attention is drawn to the fact that cytological changes may occur in the meta-adenohypophysis which are not related to background adaptation. The possibility that the hormones produced in this region may have some function besides chromatophore control is discussed (pp152-163).

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BIBLIOGRAPHY

Adams, A.E. & J.A.M. Buss. 1952. The effect of a single injection of an anti-thyroid drug on hyperplasia in the thyroid of the chick embryo. Endocrinology, 50, 234-253.

Adler, L. 1914. Metamorphosestudien an Batrachierlarven. 1. Extirpation endokriner Drüsen. A. Extirpation der Hypophyse. Arch. EntwMech. Org., 39, 21-45.

Albert, A., R.W. Rawson, P. Merrill, B. Lennon & C.B. Riddell. 1946. The effect of goitrogenic and other reducing agents on inactivated thyrotropic hormone extract. Endocrinology, 40, 299-307.

Albert, A., R.W. Rawson, P. Merrill, B. Lennon & C.B. Riddell. 1946. In vitro augmentation of the thyrotropic hormone and partial reactivation of iodinated thyrotropic (inactive) thyrotropic hormone extract by goitrogens. Endocrinology, 40, 361-369.

Allen, B.M. 1918. The result of thyroid removal on the larvae of Rana pipiens. J. exp. Zool., 24, 499-520.

Allen, B.M. 1938. The endocrine control of amphibian metamorphosis. Biol. Rev., 13, 1-19.

D'Angelo, S.A. 1955. Pituitary regulation of the thyroid gland function. Brookhaven Symp. Biol., 1, 9-21.

Aron, M. 1931. Récherches histophysiologiques sur le fonctionment et les corrélations des glandes endocrines embryonnaires chez les vertébrés. Bull. Biol., Fr., 65, 438-521.

Astwood, E.B. 1955. Mechanism of action of antithyroid compounds. Brookhaven Symp. Biol., 7, 61-73.

Astwood, E.B., J. Sullivan, A. Bissel & T. Tyslowitz. 1943. Action of certain sulphonamides and of thiourea upon the function of the thyroid gland of the rat. Endocrinology, 32, 210-225.

Atz, A.H. 1953. Experimental differentiation of basophil cell types in the transitional lobe of the pituitary of a teleost fish, Astyanax mexicanus. Bull. Bingham Oceanogr. Coll., 14 (2), 94-116.

Atz, J.W. & G.E. Pickford, 1959. The use of pituitary hormones in fish culture. Endeavour, 43, 125-129.

Bagnara, J.T. 1960. Pineal regulation of the body lightening reaction in amphibian larvae. Science, 132, 1481-1483.

Ball, J.N. 1960. Reproduction in female bony fishes. Pp. 105-134 of "Hormones in fishes", Symposium No. I of The Zoological Society of London.

Balon, E.K. 1959. Die Entwicklung der Texas-Cichlide (Herichthys cyanoguttatus Baird et Ginard) nach dem Schlüpfen. Zool. Anz., 162, 339-355.

Balon, E.K. 1960. Vývoj Cichlasoma nigrofasciatum (Guenther) v embryonálnej perióde života. Vestn. cs. spol. zool., 24, 199-214.

Bargmann, W. & W. Hild. 1949. Über die Morphologie der neurosekretorischen Verknüpfung von Hypothalamus und Neurohypophyse. Acv. Anat., 8, 264.

Barrington, E.J.W. 1960. Some features of the vascularisation of the hypothalamus and pituitary stalk in the minnow Phoxinus phoxinus L. Proc. zool. Soc., 135, 551-558.

Barrington, E.J.W. & J. Matty. 1955. The identification of thyrotrophin-secreting cells in the pituitary gland of the minnow (Phoxinus phoxinus). Quart. J. micr. Sci., 96, 193-201.

Bischitz, P.G. & R.S. Snell. 1960. A study of the effect of ovariectomy, oestrogen and progesterone on the melanocytes and melanin in the skin of the female guinea pig. J. Endocrin., 26, 312-320.

Bogdanove, E., B.N. Spirtos & N.S. Halmi. 1955. Further observations on the pituitary structure and function on rats bearing hypothalamic lesions. Endocrinology, 57, 302-315.

Braunsteiner, H., K. Fellinger & F. Pakesch. 1953. Electron microscope observations on the thyroid. Endocrinology, 53, 123-133.

Bretschneider, L.H. & J.J.D. de Wit. 1947. "Sexual endocrinology of non-mammalian vertebrates." Monogr. Prog. Res. Holland during the war, (11). Elsevier Pub. Co. Inc., Amsterdam.

Brewer, D.B. 1957. Congenital absence of the pituitary gland and its consequences. J. Path. Bact., 73, 59-67.

Brooks, C.M. 1938. A study of the mechanism whereby coitus excites the ovulation-producing activity of the rabbit pituitary. Am. J. Physiol., 121, 157-177.

Buchmann, H. 1940. Hypophyse und Thyroidea im Individualzyklus des Herings. Zool. Jb., Abt. 2, Anat. Ontog., 66, 191-262.

Burger, J.W. 1942. Some effects of androgens on the adult male Fundulus. Biol. Bull, Wood's Hole, 82, 233-242.

Butcher, E.O. 1938. The structure of the retina of Fundulus heteroclitus and the regions of the retina associated with the different chromatophoric responses. J. exp. Zool., 79, 275-293.

Callamand, O. 1943. L'anguille européene (Anguilla anguilla L.). Les bases physiologiques de sa migration. Ann. Inst. oceanogr. Monaco, 21, 361-441.

Cehovic, M.G. 1956. Sur la pénétration de l'iode radioactif (I^{131}) dans l'hypophyse de grenouille (Rana temporaria). C.R. Acad. Sci., Paris, 243, 1450-1452.

Cehovic, M.G. 1957. Effet de l'hormone mélanophorique (intermédine) sur la fixation d'I^{131} dans la thyroide et la peau des grenouille (Rana esc. et Rana temp. L.). C.R. Acad. Sci., Paris, 245, 740-743.

Cehovic, M.G. 1960. Contribution à l'étude de l'action de l'hormone mélanophorique (intermédine) sur la fonction thyroidienne de la grenouille (Rana esculanta). C.R. Acad. Sci., Paris, 250, 1114-1116.

Chavin, W. 1956. Pituitary-adrenal control of melanization on xanthic goldfish Carassius auratus L. J. exp. Zool., 133, 1-45.

Cordier, R. 1953-1954. Cytologie hypophysaire et sa signification functionelle chez l'amphibien Xenopus. C.R. Ass. Anat., __, 484-490.

Courrier, R. & M.G. Cehovic. 1960. Action de l'hormone mélanophorotrope purifiée (α-MSH) sur le fonction thyroidienne chez le Lapin. C. R. Acad. Sci., Paris, 251, 832-834.

Desclin, L. 1942. Influence de la section de la tige hypophyse sur le lobe moyen de l'hypophyse. Acta biol. belg., 2, 376-380.

Eakin, R.M. 1950. Developmental failure of the pituitary in amphibian embryos treated with sugar. Science, 111, 281.

Enami, M. 1955. Melanophore contracting hormone (MCH) of possible hypothalamic origin in the catfish Parasilurus. Science, 121, 36-37.

Enami, M. 1959. The morphology and functional significance of the caudal neurosecretory system of fishes. Pp. 697-727 of "Comparative Endocrinology", edited by A. Gorbman. John Wiley & Sons Inc., New York.

Enami, M. & K. Imai. 1955. Studies in neurosecretion.V. Caudal neurosecretory system in several freshwater teleosts. Endocrinol. Japon., 2, 107-116.

1956a. Studies in neurosecretion VI. Neurohypophysis-like organisation near the caudal extremity of the spinal cord in several estuarine teleosts. Proc. imp. Acad. Japan, 32, 197-200.

1956b. Studies in neurosecretion VII. Further observations on the caudal neurosecretory system and neurohypophysis spinalis (urohypophysis) in marine teleosts. Proc. imp. Acad. Japan, 32, 633-638.

Etkin, W. 1939. A thyrotropic field effect in the tadpole. J. exp. Zool., 82, 469-495.

Etkin, W. 1941. On the control of growth and activity of the pars intermedia of the pituitary by the hypothalamus of the tadpole. J. exp. Zool., 86, 113-139.

Evropeitzeva, N.V. 1949. Influence of thiourea on development of the thyroid gland in sigludova (Coregonus lavaretus ludoga. Pol.) Dolk. Akad. Nauk. USSR., 68, 977-980. (In Russian).

Farquar, M.G. & J.F. Rinehart. 1954. Cytological alterations in the anterior pituitary gland following thyroidectomy: an electron microscope study. Endocrinology, 55, 857-876.

Fisher, C. 1937. The site of formation of the posterior lobe hormones. Endocrinology, 21, 19-29.

Florentin, P. 1936. Sur la vascularisation des noyaux végétatifs du diencéphale chez les téléostéens. C.R. Soc. Biol., Paris, 122, 190-192.

Florentin, P. & F. Stutinsky. 1936. Modifications cytologiques de la glande pituitaire des grenouilles maintenues a l'obscurité. C.R. Soc. Biol., Paris, 122, 674-676.

Fontaine, M. & J. Hatey. 1954. Sur la teneur en 17-hydroxycorticosteriodes du plasma de saumon (Salmo salar L.). C.R. Acad. Sci., Paris, 239, 319-321.

Fontaine, M., J. Leloup & M. Olivereau. 1952. La fonction thyroidienne du jeune saumon Salmo salar L. (parr et smolt) et son intervention possible dans la migration d'avalaison. Arch. Sci. physiol., 6, 83-104.

Fontaine, M. & M. Olivereau. 1949. L'hypophyse du saumon a diverses étapes de sa migration. C.R. Acad. Sci., Paris, 228, 772-774.

Fortune, P.Y. 1955. Comparative studies of the thyroid function in teleosts of tropical and temperate habits. J. exp. Biol., 32, 504-513.

Fortune, P.Y. 1960. The effect of induced hypothyroidism of the dermal melanophores of teleosts. Proc. Zool. soc. London., 135, 55-64.

Frieden, E.H. 1951. Experimentally induced changes in the melanophore hormone content of the rat pituitary. Endocrinology, 49, 557-564.

Fugo, N.W. 1940. Effects of hypophysectomy in the chick embryo. J. exp. Zool., 85, 271-297.

Geschwind, I.I. 1959. Species variation in protein and polypeptide hormones. Pp. 421-443 in "Comparative Endocrinology", edited by A. Gorbman. John Wiley & Sons Inc., New York.

Giersberg, H. 1934. Physiologie des Farbwechsels bei Tieren. Zool. Anz., suppl. 7, 96-126.

Giroud, A., P. Kfouri & M. Martinet. 1944. Relation entre la thyroide et la fonction mélanophorique hypophysaire. C.R. Soc. Biol., Paris, 138, 74-75.

Glegg, R.E., Y. Clement & C.P. Leblond. 1952. The use of lead tetraacetate, benzidine, o-dianisidine and a "film-test" in investigating the periodic-acid-Schiff technic. Stain tech., 27, 277.

Godet, R. 1959. Hypophyse et pigmentation chez Protoptère (poisson Dipneuste). C.R. Soc. Biol., Paris, 153, 1432-1434.

Gorbman, A. 1959. Problems in the comparative morphology and physiology of the vertebrate thyroid gland. Pp. 266-282 in "Comparative Endocrinology", edited by A. Gorbman. John Wiley & Sons Inc., New York.

Grassé, P.P. 1958. "Traité de Zoologie", Vol. XIII No. 2. edited by P.P. Grassé. Masson et Cie, Paris.

Green, J.D. 1951. The comparative anatomy of the hypophysis with special reference to its blood supply and innervation. Amer. J. Anat., 88, 225-311.

Grignon, G. 1957. Aspects histophysiologiques du développement du complexe hypothalamo-hypophysaire chez l'embron de poulet. Bull. Soc. Sci. Nancy, 16, 86-100.

Gross, J. 1957. The dynamic cytology of the thyroid gland. Pp. 265-288 of "International review of cytology", Vol. 6, edited by G.H. Bourne & J.F. Danielli. Academic Press, New York.

Guillemin, R. & W.A. Krivoy. 1960. L'hormone mélanophoretique β MSH joue-t-elle un rôle dans les fonctions du système nerveux centrale chez les Mammifères supérieurs? C.R. Acad. Sci., Paris, 250, 1117-1118.

Halmi, N.S. 1952. Differentiation of two types of basophils in the adenohypophysis of the rat and the mouse. Stain tech., 27, 61.

Hamori, J. 1960. Gewebsreaktionen und Functionsanderungen des Hyophysenmittellappens der Albinoratten nach Hypothalamus und Hypophysenstiellasion. Acta Morphol. Acad. Sci. hungar., 9, 155-160.

Hanaoka, T. 1953a. Potency of melanophore hormone towards the regeneration of visual purple. Jap. J. Physiol., 3, 219-230.

1953b. Effect of melanophore hormone towards the regeneration of visual purple in solution. Nature, Lond., 172, 866.

Harms, J.W. 1935. Die Realisation von Genen und die consecutive Adaption. IV. Mitteilung. Experimentell hervorgerufener Medienwechsel: wasser zu Feuschluft b.z.w. zu Trochenluft bei Gobiiformen (Gobius, Boleophthalmus und Periophthalmus). Z. wiss. Zool., 146, 417-462.

Healey, E.G. 1948. The colour change of the minnow. Bull. Anim. Behav., 1(6), 5-15.

Healey, E.G. 1951. The colour change of the minnow (Phoxinus laevis Ag.). J. exp. Biol., 28, 298-319.

Herlant, M. 1954. Anatomie et physiologie comparées de l'hypophyse dans la série des vertébrés. Bull. Soc. zool. Fr., 79, 256-281.

Hewer, H.R. 1926. Studies in the colour changes of fish. 1. The action of certain endocrine secretions on the minnow. Brit. J. exp. Biol., 3, 123-140.

Hoar, W.S. 1955. Phototactic and pigmentary responses of sockeye salmon smolts following injury to the pineal organ. J. Fish. Res. Bd. Can., 12, 178-185.

Hogben, L.T. & F.W. Landgrebe. 1940. The pigmentary effector system. IX. The receptor fields of the teleostean visual response. Proc. roy. Soc., 128 B, 317-342.

Hogben, L.T. & D. Slome. 1936. The pigmentary effector system. VIII. The dual receptive mechanism of the amphibian background response. Proc. roy. Soc., 120 B, 158-173.

Holmes, R.L., E.B. Hughes & S. Zuckerman. 1959. Section of the pituitary stalk in monkeys. J. Endocrin., 18, 305-318.

Iakovleva, I.V. 1949. The independence of the thyroid gland from the thyrotropic function of the hypophysis in the post-embryonic development of acipenserines. (Quoted by Pickford and Atz, 1957, who give an incorrect journal reference. Correct reference not known).

Irikhimovich, A.I. 1948. Comparative investigation of larval development of fishes and Amphibia in connexion with development and function of the hypophysis-thyroid complex. C.R. Acad. Sci. Moscow, 60, 1101-1104. (In Russian).

Ivy, H.K. & A. Albert. 1957. Attempts to alter the concentration of melanophore hormone in the pituitary gland of the rat. Endocrinology, 61, 667-675.

Johnsson, S. & B. Högberg. 1953. The occurrence of melanophore hormone in serum from human subjects in different pathological conditions and its relation to corticotropin. Acta endocr. (Kbh.), 13, 325-342.

Jost, A. 1957. The secretory activities of fetal endocrine glands and their effect upon target organs. In Trans. Third Conf. Gestation, edited by C.A. Villee, New York, Josiah Macy, Jr., Foundation.

Jost, A. & P. Damysz. 1952. Récherches histochemiques (reaction de McManus) et expérimentales (decapitation) sur la physiologie de l'hypophyse foetale du lapin. C.R. Soc. Biol., Paris, 143, 1062-1066.

Jost, A. & P. Gouse. 1953. Application de la methode à l'acide periodique Schiff a l'étude histiphysiologique de l'hypophyse foetale du lapin. Arch. Anat. micr. Morph. exp., 42, 245-273.

Jost, A., F.F. Morel & M. Marois. 1952. Nouvelles récherches a l'aide du radio-iode I^{131} sur la fonction thyroidienne du foetus de Lapin décapité. C.R. Soc. Biol., Paris, 146, 1066-10 .

Jost, A. & R. Taverier. 1956. Application de la méthode a l'acide periodique Schiff (McManus) a l'étude de l'hypophyse foetale du rat. C.R. Acad. Sci., Paris, 243, 1353-1356.

Jutisz, M. & P. de la Llaso. 1961. Relations entre la structure chimique et l'activité biologique des hormones hypophysaires. Année biol., 37, 81-105.

Karkun, J.N., B.K. Amiya & B. Kukerji. 1954. Response of the pars intermedia of the cat's hypophysis to adrenocorticotrophic hormone. J. Endocrin., 10, 124-128.

Kauffman, G., V. Hurst & C.W. Turner. 1948. Histological effect of thiouracil on the fetal thyroid of the mouse. Endocrinology, 43, 187-189.

Kazanskii, B.N. & G.M. Persov. 1949. Location of the antagonistic factor of melanotropic hormone in hypophysis of bony fishes. Dokl. Akad. Nauk., USSR, 65, 593-596.

Kent, A.K. 1959. Distribution of melanophore-aggregation hormone in the pituitary of the minnow. Nature, Lond., 183, 544-545.

Kent, A.K. 1960. Thesis for the degree of Doctor of Philosophy, London University.

Kerr, T. 1933. On the pituitary in Lepidosiren and its development. Proc. roy. Soc. Edinb., 53, 147-150.

Kerr, T. 1939. On the histology of the developing pituitary in the frog (Rana temporaria) and in the toad (Bufo bufo). Proc. zool. Soc. Lond., 109 B, 167-180.

Kerr, T. 1940. On the histology of some teleost pituitaries. Proc. roy. Soc. Edinb., 60, 224-244.

Kerr, T. 1942a. A comparative study of some teleost pituitaries. Proc. zool. Soc. Lond., 112 A, 37-56.

Kerr, T. 1942b. The pituitary of the perch Perca fluviatilis. Quart. J. micr. Sci., 83, 299-316.

Kerr, T. 1943. The evolution of the pituitary with special reference to the teleosts. Proc. Leeds phil. lit. Soc., 4, 75-83.

Kerr, T. 1948. The pituitary in normal and parasitésed roach, (Leuciscus rutilis Flem.). Quart. J. micr. Sci., 89, 129-137.

Kitay, J. 1957. The pineal gland and its relation. Bucarest Acad. RPR, scl. 24-5 Congr. nation. sci. med. Bucarest, pp. 395-401.

Kleinholz, L.H. & H. Rahn. 1939. The distribution of intermedin in the pars anterior of the chicken pituitary. Proc. nat. Acad. Sci. Wash., 25, 145.

Kollross, J.J. 1943. Experimental studies on the development of the corneal reflex in Amphibia. II. Localised maturation of the reflex mechanism effected by thyroxin-agar implants into the hind brain. Physiol. Zool., 16, 269-279.

Kollross, J.J. 1950. The growth of mesencephalic V nucleus cells as a metamorphic event in anurans. Anat. Rec., 108, 565.

Koneff, A.A., M.E. Simpson & H.M. Evans. 1946. Effects of chronic administration of diethylstilbestrol on the pituitary and endocrine organs of hamsters. Anat. Rec., 94, 169-195.

Krivoy, W.A. & R. Guillemin. 1961. On a possible role of β-melanocyte stimulating hormone (β-MSH) in the central nervous system of the mammalia: an effect of β-MSH in the spinal cord of the cat. Endocrinology, 69, 170-181.

La Roche, G. & C.P. Leblond. 1942. Effects of thyroid preparations and iodine on Salmonidae. Endocrinology, 57, 524-545.

Lee, R.E. 1942. The hypophysis of the broad billed swordfish, Xiphias gladius L. Biol. Bull., Wood's Hole, 82, 401-411.

Legait, H. 1958. La vascularisation du lobe distal de l'hypophyse des téléostéens comparée a celles des autres vertébrés. C.R. Soc. Biol., Paris, 151, 1940-1943.

Legait, H. 1960. Modifications de la pars intermedia chez Meriones crassus au cours d'epreuves de deshydration ou apres ingestion de solutions de dextrose. C.R. Soc. Biol., Paris, 158, 663-665.

Leloup, J. 1952. Fixation du radioiode dans la thyroide de deux téléostéens marins: le congre et le muge. C.R. Soc. Biol., Paris, 146, 1014-1016.

Lenys, D. 1959-1960. Récherches sur les relations vasculaires hypothalamo-préhypophysaires chez Tinca vulgaris et Emis leprosa. Bull. Soc. Hist. nat. Doubs, 62, 37-40.

Lerner, A.B., K. Shizume & I. Bundig. 1954. The mechanism of endocrine control of melanin pigmentation. J. clin. Endocrin., 14, 1463-1490.

Lerner, A.B. & J.D. Case. 1959. Pigment cell regulatory factors. J. invest. Derm., 32, 211-

Lerner, A.B. & J.S. McGuire. 1961. Effects of alpha and beta melanocyte stimulating hormones on the skin colour of man. Nature, Lond., 189, 176-179.

Li, C.H. & H.M. Evans. 1948. Chemistry of anterior pituitary hormones. Pp. 631-693, vol. I of "The Hormones", edited by G. Pincus & K.V. Thimann. Academic Press, New York.

Logothetopoulos, J. & R.F. Scott. 1956. Histology and function of the developing fetal thyroid in normal and goitrous guinea-pigs. J. Endocrin., 14, 217-227.

Masselin, J.N. 1939a. Histologie de l'hypophyse de crapauds soumis a l'obscurité et à la lumière. C.R. Soc. Biol., Paris, 132, 510.

Masselin, J.N. 1939b. Influence de la lumière et de l'obscurité sur l'action mélanophore dilatrice de l'hypophyse. C.R. Soc. Biol., Paris, 132, 511.

Matthews, S.A. 1937. The development of the pituitary gland in Fundulus. Biol. Bull., Wood's Hole, 73, 93-98.

Matty, A.J. & J.M. Matty. 1959. A histochemical investigation of the pituitary glands of some teleost fish. Quart. J. micr. Sci., 100, 257-267.

McConaill, . 1947. J. Anat., 81, 371.

Menzies, W.J.M. 1931. "The Salmon". W. Blackwood & Sons, Ltd., London.

Miller, R.N. 1944. The hypophysis of the teleost Corydora paliatus (sic). J. Morph., 74, 331-345.

Niell, R.M. 1940. On the existence of two types of chromatic behaviour in teleostean fishes. J. exp. Biol., 17, 74-95.

Odiorne, J.M. 1937. Morphological color change in fishes. J. exp. Zool,, 76, 441-465.

Odiorne, J.M. 1957. Color changes. Pp. 307-402 of "The physiology of fishes", Vol.II, edited by M. Brown. Academic Press, New York.

Olifan, V.I. 1945. Periodicity in the development and critical stages of the early postembryonic ontogeny of Acipenser stellatus. Bull. Acad. Sci., USSR. ser Biol., 1, 56-78.

Olivereau, M. 1954. Hypophyse et glande thyroide chez les poissons. Étude histophysiologique de quelques corrélations endocriniennes, en particulier chez Salmo salar L. Ann. Inst. oceanogr. Monaco, 29, 95-296.

Ortman, R. 1954. Cytochemical study of the physiological activities in the pars intermedia of Rana pipiens. Anat. Rec., 119, 1-10.

Ortman, R. 1956. A study of the effect of several experimental conditions on the intermedin content and cytochemical reactions of the intermediate lobe of the frog (Rana pipiens). Acta endocr., Copenhagen, 23, 437-447.

Osborne, C.M. 1938. The role of the melanophore-dispersing principal of the pituitary in the color change of the catfish. J. exp. Zool., 79, 309-330.

Osborne, C.M. 1948. Factors influencing the growth of integumentary pigment in fish. Proc. Soc. ex. Biol., 67, 440-445.

Parhon, C.I., M. Pitis & M. Dancasiu. 1959. Les variations saisonnières des glandes endocrines chez les poissons. C.R. Ass. Anat., 103, 624-626.

Parker, G.H. 1948. "Animal colour changes and their neurohumours". University Press, Cambridge.

Pasteels, J.L. 1954. Supression des connexions hypothalamo-hypophysaires chez la larve de pleurodèle (Pleurodeles waltii). Son retentissement sur la différenciation de l'hypophyse et son activité thyreotrope. C.R. Soc. Biol., Paris, 148, 939-940.

Pasteels, J.L.jr. 1960. Étude experimental des différentes categories d'elements chromophiles de l'hypophyse adult de Pleurodeles waltii, de leur fonction et de leur contrôle par l'hypothalamus. Arch. Biol.,Belg., 71, 409-471.

Peterson, R.R. & W.C. Young. 1952. The problem of placental permeability for thyrotrophin, propylthiouracil and thyroxine in the guinea pig. Endocrinology, 50, 218-225.

Petrović, A. & M. Aron. 1955. Récherches, par la méthode des greffes sur le fonctionnement de la préhypophyse foetal du corbaye. C.R. Ass. Anat., 92, 1159-1164.

Pflugfelder, O. 1953. Wirkung den Epiphysectomie auf die Postembryonalentwicklung von Lebistes reticulatus, Peters. Arch, EntwMech. Org., 147, 42-60.

Pflugfelder, O. 1959. Beeinflussung der Thyreoidea und anderer Organe der Haushuhnes durch Kaliumperchlorat, mit vergleichenden Untersuchungen an niederen wirbeltieren. Arch. EntwMech. Org., 151, 78-112.

Phillips, J.B. & B. Schmidt. 1958. A comparison of pituitary and thyroid gland development in the fetal rat. Anat. Rec., 131, 588.

Phillips, J.B. & B. Schmidt. 1959. A comparative study of the developing pituitary and thyroid glands of the fetal rat. J. exp. Zool., 141, 499-517.

Pickford, G.E. & J.W. Atz. 1957. "The physiology of the pituitary gland of fishes". New York Zoological Society, New York.

Pitt-Rivers, R. 1950. Mode of action of antithyroid compounds. Physiol. Rev., 30, 194-205.

Purves, H.D. & W.E. Griesbach. 1951. The site of thyrotrophin and gonadotrophin production in the rat pituitary studied by McManus-Hotchkiss staining for glycoproteins. Endocrinology, 49, 244-264.

Racadot, J. 1959. L'hypophyse du hamster après traitment prolongé par les oestrogènes. C.R. Ass. Anat., 104, 682-687.

Randall, R.V. & A. Albert. 1951. The effect of hypophysectomy on the uptake of radioactive iodine by the thyroid of the rat. Endocrinology, 48, 327-333.

Rasquin, P. 1955. Observations on the metamorphosis of the bonefish (Albula vulpes Linneus). J. Morph., 97, 77-109.

Rasquin, P. & L. Rosenbloom. 1954. Endocrine imbalance and tissue hyperplasia in teleosts maintained in darkness. Bull. Amer. Mus. nat. Hist., 104, 359-426.

Raynaud, A. 1959. Effects of destruction of the fetal hypophysis by X-rays upon sexual development of the mouse. Pp. 452-478 of "Comparative Endocrinology", edited by A. Gorbman. John Wiley & Sons, Inc., New York.

Raynaud, A. & M. Frilley. 1948. Procédé d'irradiation localisée, au moyens des rayons X, des embryons de souris dans l'utérus maternel, et principaux résultats obtenue. Bull. soc. Zool. Fr., 73, 186-188.

Rebel, A. & J. Marescaux. 1960. Localisation de la fonction thyreostimulante de la glande pituitaire: modifications cytologiques de la pars distalis chez Rana temporaria au cours de la metamorphose. C.R. Soc. Biol., Paris, 154, 190-192.

Rumph, P. & P.E. Smith. 1926. The first occurrence of secretory products and a specific structural difference in the thyroid and anterior pituitary during the development of the foetus. Anat. Rec., 33, 289-298.

Salter, W.T. 1950. The chemistry and physiology of the thyroid hormone. Pp. 181-300, and: The control of thyroid activity. Pp. 301-350 of Vol. II of "The Hormones", edited by G. Pincus & K.V. Thimann. Academic Press, New York.

Saxen, L. 1958. The onset of thyroid activity in relation to the cyto-differentiation of the anterior pituitary. Histochemical investigation using amphibian embryos. Acta Anat., 32, 87-100.

Saxen, L., E. Saxen, S. Toivonen & K. Salimaki. 1957. Quantitative investigation of the anterior pituitary-thyroid mechanism during frog metamorphosis. Endocrinology, 61, 35-44.

Scruggs, W.M. 1939. The epithelial components of the teleost pituitary gland, as identified by a standardised method of selective staining. J. Morph., 65, 187-213.

Scruggs, W.M. 1951. The epithelial components and their seasonal changes in the pituitary gland of carp (Cyprinus carpio, L.) and the goldfish (Carassius auratus, L.). J. Morph., 88, 441-469.

Sembrat, K. 1954. The effect of the thyroid gland on the skin of Teleostei. Acta physiol. Polonica, 5, 647-648.

Smith, P.E. 1916. Experimental ablation of the hypophysis in the frog embryo. Science, 44, 280-282.

Snell, R.S. & P.G. Bischitz. 1960. A study of the melanocytes and melanin in the skin of the immature, mature and pregnant female guinea pig. Z. Zellforsch., 51, 225-242.

Sokol, H.W. 1955. Experimental demonstration of thyrotropic and gonadotropic activity in the adenohypophysis of the guppy, Lebistes reticulatus (Peters). Anat. Rec., 122, 451.

Stahl, A. 1958. Sur la présence d'une dualité cellulaire au niveau du lobe intermédiaire de certains poissons. C.R. Soc. Biol., Paris, 152, 1562-1565.

Stolk, A. 1951. Activity of the embryonic thyroid gland in the viviparous cyprinodont Lebistes reticulatus Peters. Proc. Acad. Sci. Amst., 54C, 579-584.

Stolk, A. 1956. Changes in the pituitary gland of the cichlid Cichlasoma biocellatum (Regan) with thyroidal tumour. Proc. Acad. Sci. Amst., 59C, 494-505.

Stolk, A. 1959. Melanophore-aggregating hormone in the pituitary gland of Pterophyllum scalare. Naturwissenschaften, 46, 494-495.

*Stutinsky, F. 1954. La neurohypophyse du rat au cours de la gestation. C.R. Ass. Anat., 74, 296-300.

Supriewski, J., T. Marczynski & S. Misztal. 1960. Biological properties of melatonin (5-methoxy-N-acetyl-tryptamine). Bull. Acad. polon. Sci., Ser. Sci. biol., 8, 483, 487.

Tampi, P.R.S. 1953. On the structure of the pituitary and thyroid of Chanos chanos. Proc. nat. Inst. Sci. India, 19, 247-256.

Tixier-Vidal, A. 1958. Étude histophysiologique des relations hypophysaire et thyroide chez l'embryon de poulet. Arch. Anat. micr. Morph. exp., 47, 235-340.

Tixier-Vidal, A. 1959. Données actuelles sur l'influence de la lumière sur l'activité thyroidienne chez les vertébrés. Ann. Endocr., Paris, 20. 708-

Vilter, V. 1946. Déterminisme intrinsèque de migrations pigmentaires dans la rétine. C.R. Soc. Biol., Paris, 140, 277-278.

Vivien, J.H. 1941. Contribution a l'étude de la physiologie hypophysaire dans ses relations avec l'appareil génital, la thyroide et les corps suprarenaux chez les poisson selaciens et téléostéens. Bull. Biol., 75, 257-309.

Vivien, J.H. & M.L. Gaiser. 1952. Action d'un traitment de longue durée par la thiourée sur la structure et la fonctionnement de la thyroide et de l'hypophyse chez le Lebistes reticulatus, modification du rhythme de la croissance staturale et des processes de la différenciation génitals pubérale. C.R. Acad. Sci., Paris, 234, 1643-1645.

Waring, H. & F.W. Landgrebe. 1950. Hormones of the posterior pituitary. Pp. 427-514 of Vol.II of "The Hormones", edited by G. Pincus and K.V. Thimann. Academic Press, New York.

Warren, M.R. 1940. Studies on the effect of experimental hyper-thyroidism on the adult frog Rana pipiens. Schreber. J. exp. Zool., 83, 127-159.

Waterman, A.J. 1959. Development of the thyroid-pituitary system in warm-blooded amniotes. Pp. 351-367 of "Comparitive Endocrinology", edited by A. Gorbman. John Wiley & Sons Inc., New York.

Waterman, A.J. & A. Gorbman. 1956. Development of the thyroid gland of the rabbit. J. exp. Zool, 132, 509-538.

Weiss, P. & F. Rossetti. 1951. Growth responses of opposite sign among different neurone types to thyroid hormone. Proc. Nat. Acad. Sci. Wash., 37, 540-556.

Werner, E. 1959. Influence du son sur le lobe intermédiaire de l'hypophyse de rat. C.R. Ass. Anat., 103, 783-788.

Willier, B.H. 1955. Ontogeny of endocrine correlation. Pp. 574-619 of "Analysis of development", edited by B.H. Willier, P.A. Weiss & V. Hamburger. W.B. Saunders Company, Philadelphia & London.

Witschi, E. & C.Y. Chang. 1959. Amphibian ovulation and spermiogenesis. Pp. 149-160 of "Comparative Endocrinology", edited by A. Gorbman. John Wiley & Sons, Inc., New York.

Wyman, L. 1924. The reactions of the melanophores of embryonic and larval Fundulus to certain chemical substances. J. exp. Zool., 40, 161-180.

* Stutinsky, F. 1936. Effects de l'éclairement continu sur la structure de la glande pituitaire de la grenouille. C.R.Soc. Biol., Paris, 123, 421.