THE ENDOCRINE CONTROL OF REPRODUCTION IN THE PLAICE, PLEURONECTES PLATESSA, L.

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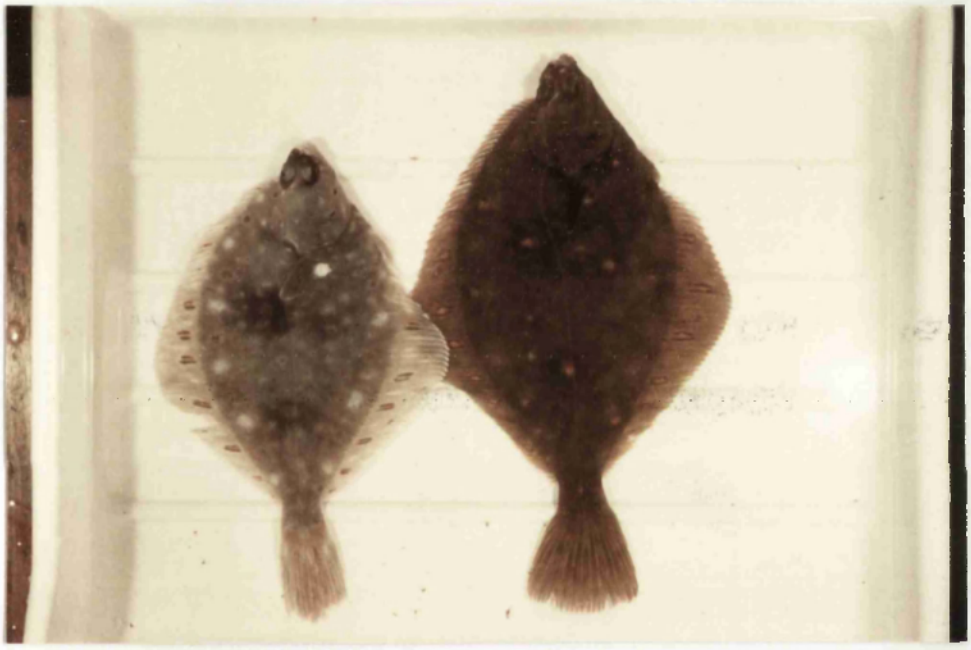
Frontispiece. The effect of hypophysectomy on the chromatophores.
Hypoyhysectomised fish on the left.



Frontispiece

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I wish to thank Professor C.M. Yonge and my supervisor Dr. J.D. Robertson for the encouragement which I have received from them during the course of this study. I would like to express my gratitude to Professor J.M. Dodd for allowing me to use the facilities of the Gatty Marine Laboratory, St. Andrews and for the practical interest which he has taken in my work. I wish to acknowledge my debt to the Director and staff of the Marine Station, Millport without whose assistance in providing the material, this work would not have been possible. Finally, I would like to thank Dr. B.M. Hobson, Director of the Pregnancy Diagnosis Laboratory, Edinburgh, for his help in the bioassay of the pituitary material and for much helpful discussion of my thesis.

Contents.

I. Introduction and significance of the work presented.

Although reproductive endocrinology is a well-established science in the higher vertebrate, its comparative aspects are still in a very early stage. Endocrine mechanisms are similar throughout the vertebrates and it is recognised that variations in hormone function are of a secondary nature. While this is true of the Teleostei, our knowledge is largely restricted to a few species which have proved amenable to laboratory conditions and experiment. It is difficult to make generalisations on the basis of this information since it is often inadequate and conflicting and any extrapolation to include the enormous number of species of fish would be unjustified.

Much of the work done has been carried out by pisciculturists whose immediate aim has been to discover means of manipulating the environment in such a way as to bring the production of new generations of fish under their control. Unfortunately, the planning and execution of many of the experiments has left much to be desired with the result that there is a great deal of information of a rather superficial kind; the basic studies necessary for a true understanding of reproductive physiology have yet to be undertaken.

Information on the reproductive endocrinology of flatfish is completely lacking and the present study, on the plaice, Pleuronectes platessa,L., a large marine flatfish of some commercial importance, was undertaken in an attempt to fill this gap. The concepts current in the reproductive endocrinology of teleosts have been built up largely from the

study of specific aspects of the problem in a variety of species and in the present work it is hoped to make a comprehensive study in a single species. By approaching the problem from a number of viewpoints, it may be possible to elucidate some of the basic principles underlying reproductive endocrinology. With this in mind, it was felt that detailed information on the morphology of the pituitary gland and gonads was essential in order to have a background of knowledge which could be used as a basis for comparison with the results of experimental studies.

Although there is a great deal of information on the morphology of the internal generative organs in viviparous and ovo-viparous teleosts, the complete cycle of changes has been investigated in only a few species of oviparous fishes, most of which have been fresh-water forms. Accordingly, samples of plaice were taken at frequent intervals over a period of some fifteen months and a detailed examination made of the histological changes in the reproductive organs.

Similarly, the structure of the pituitary gland has been described for a number of species of fish but in only a few cases are there any data on cyclical changes obtained by the application of modern tinctorial and histochemical techniques. The site of production of gonadotrophic hormones is generally agreed to be the basophil cells, but there is still some doubt and gonadotrophic functions have been attributed to the carminophil cells. A detailed histological study was therefore carried out on pituitary glands taken from plaice throughout the reproductive cycle.

The techniques of hypophysectomy and administration of endocrine material have contributed largely to the knowledge of the control of

reproduction in mammals. Some attempt has been made to apply these techniques to teleosts with varying degrees of success. Hypophysectomy is difficult to perform and post-operative mortality has been high. The effect of hypophysectomy on the gonad has been studied in only 7 species of fish and the results indicate that the pituitary gland is necessary for proper function of the gonads. There is, however, little information on the detailed effects of hypophysectomy and it is not known whether the effect of the operation varies in relation to the gonadal condition of the fish.

A successful technique of hypophysectomy has been devised for the plaice and the effects of pituitary removal have been studied at different times of the year and on fish at different stages in their reproductive cycle. Studies complementary to these have been made on the effect of administration of hormones into hypophysectomised animals.

The presence of pituitary hormones is necessary for the full development and functioning of the gonads in mammals and the experimental data are best explained by the presence of two different gonadotrophins. Follicle stimulating hormone (FSH) causes growth of the ovarian follicles in the female and the seminiferous tubules in the male, and is primarily responsible for the development of the eggs and for spermatogenesis. Luteinising hormone (LH) causes the secretion of androgen by the interstitial cells of the testis and the secretion of oestrogen by certain cells of the ovarian follicles. This hormone also produces pre-ovulatory follicular swelling, ovulation and conversion of the follicle into the corpus luteum. In the female, and probably in the male, an important synergistic relationship exists between LH and FSH, which involves the processes of

of follicular growth and ripening, the secretion of steroid hormones and ovulation. However, there are certain limitations in our knowledge concerning the fundamentals of pituitary gonadotrophins in mammals and these uncertainties are still more obvious in fishes where many of the basic data are still to be determined.

Although the results of work on the teleosts may all be fitted into a scheme involving FSH and LH, it is not at all certain whether more than one gonadotrophin is secreted by the fish pituitary, or whether the hormone or hormones in question can be considered identical in function to the gonadotrophin of other classes of vertebrates.

In the present study, an attempt has been made to answer some of these questions and much has been made of bio-assay techniques involving animals from other vertebrate classes. In the past a great deal of importance has been placed on the concept of species specificity of pituitary hormones, though there is little doubt that much of the evidence on which this concept was based is scanty and in many cases contradictory.

A major disadvantage in the use of teleosts (particularly marine species) for experimental work is the difficulty of husbandry. Most workers have reported survival periods of four to six weeks after hypophysectomy, (Matthews 1939, Buser-Lahaye 1953), although longer periods have been obtained by a few workers.

The difficulties involved in securing adequate survival were particularly evident in the early stages of the present investigation, at which time mortality was excessively high and a large number of fish did not survive hypophysectomy long enough to show a recognisable effect on the target organs. It proved necessary to devote much time and

attention to husbandry before these difficulties were surmounted. However, survival periods of more than a year were eventually achieved and the basis exists for a much more extensive series of experiments. The latter should determine the extent to which the endocrine organs control reproduction in teleosts and should yield further information on the interrelationships between the various endocrine glands of these fish.

II. Material and Methods.

A. Source of Fish

This work was carried out on the plaice, Pleuronectes platessa.L. The fish are trawled from the Firth of Clyde near Mount Stuart, by the vessels of the Marine Biological Station, Millport and are transported by boat to the mainland in open baths in which the water is changed frequently. They are then transferred to large metal tanks (4'x2'x1') fitted with canvas covers and equipped with an oxygen supply. The journey to St. Andrews by lorry takes about 6 hours and most of the fish arrive in good condition.

It is possible to transport as many as 80 fish by this method and casualties are either completely absent or amount at most to one or two. The mortality rate during the first few days after the journey is higher in summer; this point will be further discussed later. It is therefore, apparent that plaice travel well under conditions that must involve some stress. The success of this method is perhaps due to aeration, which, apart from providing an adequate supply of oxygen, removes some of the mucus that accumulates in the water; the mucus is brought to the surface by bubbles and tends to form a surface froth. The optimum number of fish in each tank is between 10 and 15.

B. Husbandry.

1. Aquarium facilities.

The fish are kept in large, indoor tanks, each with a capacity of 800 gallons. As many as 50 fish can be kept in each tank but the

usual number is between 10 and 30. For some experiments, 4 or 5 fish are kept in shallow porcelain sinks with a capacity of about 10 gallons. In both cases, the tanks are well aerated and there is a continuous circulation of fresh sea water. The water temperature varies from about 3.3'C in winter to about 17.2'C in summer (Fig. 1.).

In January 1959, some of the large tanks were provided with thermostated heaters which kept the water temperature constant at about 10'C. In the period before the installation of the heaters, the mortality rate due to the cold was very high; on one occasion, 40 fish dying in one day. It appears that plaice in captivity do not tolerate temperatures below 5'C.

2. Feeding problems encountered.

The natural diet of P. platessa is known (Todd 1914: Ritchie 1938), and the presence of various species of molluscs, echinoderms, polychaets and fish has been recorded from the stomach contents. The most frequent records are of species of Scrobicularia, Solen, Ophiura and Ammodytes. There are, however, few references to the feeding habits of plaice in captivity.

Dawes (1930a) kept plaice in cages suspended in the sea and fed them a diet of chopped mussel. He reports that "these male fishes generally displayed avidity for food ... even snapping at each other at feeding times". The fish were fed daily and the uneaten food removed from the tanks after feeding. In another series of experiments (1930b) the fish were presented with fragments of mussel liberally mixed with olive oil.

At the Marine Station, Millport, plaice kept in the public aquarium are fed once or twice weekly on a diet of boiled mussels and

cockles. Most of the fish take this food avidly but a few make no attempt to eat (personal observation).

This review of the literature indicated that Pleuronectes could be induced to take food. It was therefore, decided to offer a wide choice of food, including the items mentioned in the literature. Accordingly the fish were offered molluscs (mussel, cockle and clam), echinoderms (Ophiura), and polychaete worms (lugworm and ragworm). Some of the fish were seen to take the food, but it soon became apparent that the majority were not eating. It became evident that in order to maintain a healthy experimental population, a more successful method of feeding would have to be devised.

3. Diet.

In deciding on the diet, it is necessary to consider whether the proposed food is acceptable to the fish, whether it forms part of the natural diet and whether it is readily available. None of the foods mentioned in the review is available in sufficient quantity at St. Andrews, but finely minced raw herring has been successfully used in feeding dogfish at the Gatty Marine Laboratory. This was tried and found to be equally successful for feeding plaice, although it is not part of their natural diet. It has been noticed, however, that some of the fish will eat herring that has been regurgitated by other fish, indicating that it is not unacceptable as a food. Herring is available in sufficient quantity at St. Andrews throughout the year and was therefore adopted as the article of diet.

4. Force-feeding.

The technique employed is an adaptation of one of the methods used at the Gatty Marine Laboratory for feeding dogfish. The apparatus consists

of a perspex prophylactic syringe of about 1 cm. diameter and 14 cm. long. These syringes are produced by Ortho Pharmaceuticals Ltd.; the barrel is of uniform bore throughout its length and a fitted piston traverses its entire length. The capacity is 6 g. minced herring.

A stomach tube is made consisting of a rubber tube of about ¾ cm. external diameter and about 8 cm. in length. A piece of glass tubing about 1 cm. external diameter and 3 cm. long projects from one end of it. This glass connecting piece can be inserted into a rubber sleeve fitted to the end of the "ortho syringe" (Plate 1, Fig. 1.). This allows easy interchange of syringes during feeding.

The fish is placed on a table and the rubber tube, and attached "ortho syringe", both full of herring, are inserted into its mouth. The tube is manoeuvred gently past the cardiac sphincter and the contents of the syringe ejected by means of the piston (Plate1, Fig, 2.). The empty syringe is thendetached and replaced by a full one, the process being repeated until the desired amount of food is delivered. The food material must be placed in the stomach; if placed in the oesophagus it is regurgitated. The "ortho syringe" is filled using a confectioner's icing syringe. The latter is fitted with a round nozzle with an aperture of about ¾ cm. in diameter. It is charged with finely minced food material; the nozzle is held against the rubber sleeve of the "ortho syringe" and a jet of food material is ejected into the latter.

5. Amount and frequency of feeding.

Dawes (1930a) in a series of experiments on growth and maintenance of plaice showed that approximately 6g. food per day was necessary for growth in female plaice approximately 150g. in weight. The fish were

presented with a known quantity of food each day and were weighed at fortnightly intervals over a period of 6 months. Dawes does not say whether all the food presented was eaten. The same author (1930b) established the fact that food takes approximately 60 hours to pass through the alimentary tract. From this it appeared that the fish should be fed every 3 days and that they should receive an average of at least 6g. of food per day.

However, the adverse effects of handling have to be considered. Plaice struggle violently when handled, although they will lie quietly when placed on a flat surface. Dermal scales are reduced in these fish and the skin is covered by a mucus coating. The fish must be held firmly while the stomach tube is being inserted and the mucus coating is likely to be destroyed and the epithelium damaged, producing conditions favourable to bacterial infections. It was therefore decided to force-feed once weekly. It soon became apparent that this was not sufficient as the fish were losing weight or, at best, merely maintaining their body weight. The frequency of feeding was consequently raised to twice weekly. Mature plaice vary greatly in size, females ranging from about 30 to 50 cm. in length and males from about 18 to 35 cm. in length. The amount of food which the stomach can hold also varies considerably, being about 6g. for a small (18 cm.) fish to 36g. for a large (50 cm) one. This means that the fish were receiving between 12 and 72g. of herring per week.

6. Adequacy of diet.

Experience has shown that twice weekly feeding, i.e. 12-72g. of food, will maintain an adult plaice in good condition. A number of

experimental fish showed a loss of weight but this may have been due to spawning, although this was not seen. In most fish, however, the body weight is maintained and in some a gain has been recorded. It seems reasonable to conclude that the diet is adequate for maintenance and growth. The original food material was minced, filleted herring but this was later discarded in favour of minced, whole herring. It would appear that this is a more satisfactory food for plaice (and dogfish which were also kept at the Catty). Certain control fish which received mainly filleted herring showed signs of a much delayed vitellogenesis and it is possible that this was due to lack of some important nutrient which is present in whole herring but not in fillets - the most obvious suggestion being calcium or phosphate. In certain other controls, which received whole herring, vitellogenesis was normal and delayed by only a few weeks.

Insufficient food can have an adverse effect on the growth and normal development of the gonads. It is well known that inanition in toads has the same effect on the gonads as hypophysectomy and has often been called "pseudo-hypophysectomy". In work investigating the effects of hypophysectomy, controls and operated animals must therefore receive adequate amounts of food and a method of force-feeding is justified in spite of the disadvantage of handling the fish. It could be expected that inanition effects - if any - could be expected to appear in controls as well as operated fish.

C. Hypophysectomy.

Hypophysectomy has not previously been performed in flatfish. The operations performed were either total hypophysectomies or control operations: partial hypophysectomy is impossible owing to the compact nature of the pituitary. In the control operations, the procedure was identical with that used for hypophysectomy, but the pituitary was left in situ.

Over the period covered by this research a total of 400 operations were carried out. The results reported here were obtained from 198 fish, of which 45 were dissected post mortem. The latter were dissected very shortly after death and in no case did the gonads show any histological characteristics which could be attributed to moribundity or histolysis. However, it was considered advisable to use such material with caution. The conclusions reached in this thesis are therefore based on those fish which were alive at dissection, with supporting evidence provided by the post mortem material.

1. Technique of Hypophysectomy in Plaice.

a. Anaesthesia.

The fish are anaesthetised in a glass tank containing 5 litres of 1.5% ethyl carbamate in sea water, under continuous aeration. Fish anaesthetised sufficiently for hypophysectomy do not respond to slight pressure on the pectoral fin; the time necessary to induce this degree of anaesthesia varies with size between 10 and 20 minutes. Respiratory movements recommence shortly after the fish are returned to sea water.

There have been recent reports that urethane is a carcinogen and skin cancers have been seen in adult specimens of Xenopus laevis, anaesthetised with urethane (Dr. B.M. Hobson, personal communication). However, no ill effects have been observed during the present work and there has been no mortality in unoperated fish which have been anaesthetised.

Tricane methane sulphonate is now widely used as an anaesthetic for aquatic animals and this has been tried on plaice with good results. At a concentration of 1 in 10,000, the required degree of anaesthesia was induced in about 15 minutes.

b. Preliminary procedure.

Since the normal operating time is less than 10 minutes, it is unnecessary either to perform the operation under water or to maintain a constant flow of water across the gills.

After being removed from the anaesthetic bath the fish is placed, right side uppermost, on a wooden board and a cork wedge inserted under the head so that the roof of the mouth faced obliquely upwards. The small gape of the jaws and the fact that the pituitary gland lies fairly far back makes it imperative to use an opercular approach. The operculum is therefore lifted by means of retractors and the gills retracted in the opposite direction. The retractors consist of broad pieces of metal shaped to fit over the edges of the operculum and gills. They are attached to rubber bands and tension is achieved by pinning the stretched rubber bands to cork blocks. This procedure exposes a wide natural cavity in the floor of which the pituitary gland is situated. (Plate 2, Fig. 1.).

The anatomy of the cavity will be described in some detail as it affords a number of landmarks which are useful in determining the site of the incision. On either side of the keel-shaped parasphenoid bone lies a band of strong transverse muscles forming the roof of the pharynx. Posteriorly these terminate abruptly in a distinct transverse ridge and the right pseudobranch can be seen lying more or less vertically in a small concavity behind the transverse ridge. The blood supply to the pseudobranch - the afferent pseudobranchial - can be seen running upwards, underneath the mucous membrane.

An oblique incision is made in the mucous membrane slightly posterior to the transverse ridge on the right of the mid line and terminating immediately anterior to the right pseudobranch. The sheet of muscle thus exposed is cut with a scalpel and parts of the pro-otic and alisphenoid bones exposed, together with the posterior portion of the parasphenoid. The length of the incision is approximately 5 mm. Owing to the pull of retractors, the incision tends to gape and it is unnecessary to retract the mucous membrane. (Plate 2, Fig. 1 and 2.).

The braincase is then drilled using a small hand trephine. These trephines are made of hardened silver steel and are 7.5 cm. in length. The external diameter is 2 mm. and the trephines are hollow and of uniform bore with 6 teeth set in the perimeter. Since the bone surface is not flat, the hollow trephine tends to slip and it is necessary to start the operation with a similar trephine which has a locating spike projecting from its centre.

This trephine is inserted vertically into the incision close to the pseudobranchial artery. Gentle pressure is applied until the spike penetrates the surface of the bone; the trephine is then rotated until the teeth begin to cut. At this point a plain trephine is substituted and the rotation continued until the drill penetrates through the skull. When the disc of bone is removed a small amount of fluid wells up and on removing this by suction, the pituitary gland can be seen lying 'above' the optic nerve and immediately in front of the saccus vasculosus. The gland is sucked out with a pipette attached to a vacuum pump. The disc of bone is then replaced and the retractors removed. No attempt is made to suture the mucous membrane.

Since the incision is rather deep and shadowed by the operculum and gills, it is difficult to illuminate the site adequately. The most satisfactory solution is to use a surgeon's head-lamp which leaves most of the field in darkness but concentrates a narrow beam of bright light which can be directed by moving the head.

c. Effectiveness of the operation.

The pituitary body is attached to the brain by a slender stalk, and slight suction is sufficient to detach the entire gland.

In most cases the gland was removed from the pipette and checked for completeness under a binocular microscope. The effectiveness of the operation was also checked by serial sections of the pituitary region when this seemed necessary.

d. Complications encountered during the operation.

The initial incision through the mucous membrane and muscle of necessity lies close to the pseudobranch. In making this incision, care must be taken not to damage the afferent pseudobranchial artery which supplies the pseudobranch, since, if this vessel is perforated, blood obscures the field and adds to the difficulties of the operation. The pro-otic and alisphenoid bones are fairly thick, but rather brittle. This must be borne in mind when using the trephine and care must be taken to use only a slight pressure so that the drill does not break through the bone and damage the brain or dislodge the pituitary.

The landmarks described earlier serve to define the region of the pro-otic and alisphenoid bones, and the curve of these bones forms a slight 'V' with the keel of the parasphenoid. This natural depression acts as a groove into which the trephine tends to slip. The major complication in drilling the bone lies in the fact that the pro-otic bone carries two foramina, the carotid foramen, at the junction of the pro-otic and parasphenoid bones which carries the right internal carotid artery into the brain case, and the jugular foramen which carries the superior jugular vein back towards the heart and the ophthalmic artery forward from the pseudobranch. These two foramina and the trigemino-facial foramen form the apices of a triangle inside which the trephine must be positioned. Most of the difficulty in performing the operation is due to the consequence of damaging one or other of these blood vessels. In most cases the vessels have been ruptured inside the brain case and it is consequently impossible to stop the profuse bleeding which occurs.

About 80% of the hypophysectomies performed have been successful and the remaining 20% have failed owing to one or other of the above complications.

e. Post operative history.

After operation the fish are marked for identification by small holes punched in the fins. The incision in the roof of the mouth takes as much as 12 weeks to heal and in many cases the disc of bone is resorbed and replaced by connective tissue.

As already stated, all experimental fish are fed twice weekly. The insertion of a stomach tube for this purpose does not damage the wound as this is situated in a small concavity to the right of the mid-line.

Those fish in which the jugular vein or carotid artery were perforated during the operation generally died within 3 days of the operation. A few, however, survived and lived for several months.

f. Post operative complications.

Fin-rot. Some fish developed a disease of the tail and fins. This disease, commonly known as fin-rot, appears to be caused by a combination of a fungal and a bacterial infection. No effective treatment was found although several methods were tried. In most cases the infection subsided naturally and the damaged areas healed. In a few fish, however, the tissue of the tail was completely destroyed and the bones of the tail exposed. The exposed area bled persistently and the fish were destroyed.

Paralysis. In a few cases the operation was followed by partial paralysis of the fish. Such paralysis occurred both in hypophysectomies and in control operations and was generally characterised by the inability of the fish to right itself. When disturbed, the fish swam in a circle for long periods before settling on the bottom of the tank. Most of these fish died, but a few eventually recovered and regained normal muscular control.

The cause of the paralysis is not apparent. An obvious suggestion is damage to the brain during operation, but if this were valid, one would expect the paralysis to appear immediately after operation. Such was not the case, onset of the paralysis occurring at any time up to 6 weeks after the operation. A similar phenomenon was recorded by Dawes (1930a) in plaice which had not been operated upon.

It was hoped to study the effect of hypophysectomy on fish at different times of the year. This hope was not realised as extremely high mortality rates were experienced in fish collected between July and November. In each of these months, more than 20% of the fish died in the first few days after arrival at the laboratory, without any operation being performed. None of the experimental fish survived more than 3 weeks. Many fish, both operated and intact were autopsied, but in no case was the cause of death apparent.

The most likely explanation is that the fish were in poor condition after the spawning season, which ends in April. It is well known that fish use much of their reserves of food in the manufacture of the reproductive products and in plaice, where the ripe ovary may account

for a quarter of the total weight, a great deal of energy must be expended in the act of spawning. Deaths after spawning are common in mature fish and it is likely that the fish are physically incapable of withstanding any unusual degree of stress. The strain of being transported from Millport and the handling which they received in the course of being force-fed are likely to have initiated a condition of shock which led to the death of the weaker fish, while the shock of the operation caused the death of the remainder. Some 250 fish were lost in this way.

2. Technique of hypophysectomy in Xenopus laevis.

a. Introduction.

In most of the bio-assays of plaice pituitary material, intact male Xenopus laevis were used as test animals. There is some indication that such injected material may stimulate the production of gonadotrophin from the pituitary of the test animal, thus reducing the reliability of the assay. This interference can be avoided by the use of hypophysectomised test animals. Hypophysectomy is a relatively easy operation in Anura and has been successfully performed by several workers (eg. van Oordt 1951 for Rana temporaria and Hansen 1954 for Rana pipiens). The technique described below, designed by the present author, is similar to the procedure used by other workers.

b. Procedure.

The toads are placed in a jar containing M.S. 222 (Sandoz) at a concentration of 1: 20,000 and fifteen to twenty minutes are necessary for complete anaesthesia. The toads are then placed ventral side uppermost

on a cork operating board, the upper jaw is held in position with a hooked retractor placed in the internal nares and held in place with an elastic band. The lower jaw is then retracted backwards to expose the roof of the mouth. At this point, a cup-shaped concavity can be seen in the roof of the mouth just posterior to the dagger-shaped parasphenoid bone. The skin and muscle anterior to this depression is incised and the cut continued backwards for about ½ cm. Posterior to the parasphenoid, the bone is extremely thin and the pinkish pituitary can be seen through it. At this stage, a small wedge of cardboard is inserted into the mouth to keep the tongue clear of the operating area. The posterior portion of the parasphenoid is then drilled with a fine dental drill and the hole widened until all the bone is cleared from the area immediately above the pituitary. On incising the dura mater, a small amount of fluid wells up and the pituitary protrudes through the incision. It can then easily be removed either by suction or with fine forceps.

In most cases, only the anterior lobe was removed, the neuro-intermedia lobe being left <u>in situ</u>.

The relation of the pituitary lobes to one another is such that the anterior lobe can be removed without the other lobes and as its attachment to the brain and to the other lobes is rather fragile, total removal of this lobe is usually achieved. The effectiveness of the operation was checked at dissection in all cases and no remnants were observed.

D. Treatment of Material.

1. Gravimetric data.

Samples of mature and immature male and female fish were collected at monthly intervals for 15 months. Overall length was measured to the nearest 0.5 cm. and total weight and gonad weight were recorded to the nearest 0.5 gm. Similar data were also recorded at the time of operation and autopsy for fish used in experimental studies. Gonad weights were expressed as a percentage of the total body weight.

2. Histological and histochemical techniques.

Samples of mature and immature fish were also taken over the same period for routine histological study.

a. Pituitary.

The pituitary region was removed as follows: an incision was made in the head in the region of the medulla oblongata and the roof of the skull anterior to this carefully cut away. The entire brain with the pituitary gland *in situ* was then carefully dissected out. This technique was also employed for fish which had been hypophysectomised. On opening the brain, a careful examination of the pituitary region was made under a binocular microscope. In no case were remnants of the gland observed, but the procedure described above was carried through and the results checked histologically.

The brain, with attached pituitary gland, was fixed in Halmi's modification of Bouin's fluid or in Helly's fluid and embedded in paraffin wax (M.P. 56°C). Serial sections were cut at 4 μ. Most

of the glands were cut on the longitudinal plane, but transverse sections were also made. Sections close to the sagittal plane of the glands were mounted singly or in groups of two or three to enable a variety of staining procedures to be used on sections close to the median plane. Every third slide was stained with Heidenhain's Azan technique. Histochemical techniques were used on the other slides. The periodic acid Schiff (PAS) stain was used according to the procedure of Purves and Griesbach (1951) and Wilson and Emrin (1954) and the aldehyde fuchsin (AF) stain according to the technique of Halmi (1950).

b. Testis.

Testes and testis ducts were dissected in toto and parts of them fixed in Bouin's and Helly's fluids and embedded in paraffin wax. Sections were cut at 3 μ and 7 μ and Mayer's haemalum, Heidenhain's haematoxylin and Heidenhain's Azan were used as stains. Several testes were fixed in Baker's formaldehyde calcium and embedded in gelatine. Frozen sections were cut at 10 μ and stained in Sudan black (Pantin 1960).

c. Ovary.

Teleost ovaries contain large yolk-filled eggs and are extremely difficult to cut. Consequently, special methods of preparation had to be used and ovaries containing ripening or ripe ova were fixed in Smith's formol-bichromate (Pantin 1960) and embedded in polyester wax (Steedman 1957). Using this combination of fixative and wax, sections were cut at 4 μ and 7 μ and stained as described above for testes. Ovaries from immature or spent fish were treated in the same way as testes.

3. Cell counts.

a. Pituitary.

In order to determine whether any cyclical variation was present in the meso-adenohypophysis, cell counts were made in the pituitaries of fish collected at monthly intervals. Sections at or near the median sagittal plane and 150 μ on either side of it were stained with Azan and all the cells in one field of the high power objective (X 2400) were counted. The numbers of acidophils, basophils and chromophobes present were expressed as a percentage of the total. The area occupied by the meso-adenohypophysis is fairly small and only 3 fields were counted. The number of cells in any one field varied between 150 and 300.

Since there was no apparent variation of pituitary size from one month to the next, it was assumed that the numbers of cells counted gave a true indication of the variation in the proportion of cell types present, i.e. the area counted was assumed to be constant and could be used as a fixed quantity.

b. Ovary.

In order to study the effects of experimental procedures on the different stages of oogenesis, it was essential to have an estimate of the variation of the proportions of these stages throughout the year. Here, the difficulty of obtaining a fixed base line became evident. Since the volume of the ovary varies considerably, the method of counting the number of oocytes in a fixed area could not be used. Variations in the size and shape of the ovigerous lamellae precluded their use as a reference point.

It was therefore decided to count the number of oocytes of each stage present in a large number (400-500) of oocytes along one or more lamellae. The numbers of oocytes of each stage were expressed as a percentage of the total. Several such counts were made for each ovary and the proportions of each stage averaged. Since the size and shape of the lamellae were so variable, the length of lamella used in each count was not constant, but subjective attempts were made to keep the lamellae lengths comparable for each count.

4. Collection of pituitary material for bio-assay.

Large numbers of fish were trawled at Millport at monthly intervals and brought alive to the laboratory in water-filled baths. They were then transferred to tanks with running water and used within 3-4 hours of being trawled. The fish were killed by decapitation immediately before the pituitary gland was removed as follows:- a cut is made in the region of the cerebellum and the roof of the skull anterior to this cut away thus exposing the anterior portion of the brain with the pituitary gland attached. The stalk attaching the gland to the brain is very fragile and the pituitary is removed by passing the jaws of forceps between it and the brain and lifting it away from its attachment. The gland is then placed in a petri dish containing anhydrous acetone while the sex and maturity of the fish are determined. The gland is then transferred to a bottle containing a large volume of anhydrous acetone, which is changed frequently until the glands are thought to be completely free from water. The acetone is then pipetted off over a hot-plate and the glands transferred to glass vials which are sealed after re-drying for several hours in a vacuum desiccator.

Strict precautions are taken to ensure that the glands are free from moisture before the vials are sealed and it has been found that material stored in this way will remain active for long periods. Assays have been carried out on material which had been stored for more than 18 months and no evidence of deterioration has been found and other workers have reported that pituitary material is still active after several years.

As described earlier, the plaice pituitary is divided by a transverse groove into 2 regions. In some cases the glands were divided into 2 parts along this groove before drying. Here the procedure is to place the gland on a dry microscope slide under the low power of a binocular microscope. Under these conditions the division can be clearly seen and the gland is bisected using a sharp scalpel.

E. Bio-assay.

1. Materials and Methods.

Males of the South African clawed toad Xenopus laevis have been used as a test animal by a number of workers notably, Hobson (1952), and Hobson and Landgrebe (1954). In the present study, the animals are kept in tanks at 18° - 24°C. The water is changed frequently and the animals are fed at least once a week on fresh chopped liver. The material under test is always contained in 1ml. liquid and is injected into the dorsal lymph sac. The toads are then placed in separate jars. Eighteen hours later, a few drops of urine are collected with a pipette by slight irritation of the cloacal folds with the tip of the pipette, the urine placed on a slide and examined microscopically. A clean

pipette is used for each animal to avoid contamination. The appearance of sperm constitutes a positive reaction. (A small air bubble in the field makes focusing easier). Animals under test which are negative at the 18-hour stage are returned to their jars and re-examined 24 hours after injection.

Schofield albino mice (9-10 g., 19 day old immature females) were used in the mouse uterus assay (Levin and Tyndale, 1937). The material under examination is given in 3 subcutaneous injections 24 hours apart. The mice are killed in ether vapour 24 hours after the last injection and the uteri dissected out, freed from extraneous tissue and weighed after excess moisture has been removed by pressing lightly on blotting paper. In all tests, the material is given in 0.5 ml. normal saline and the uteri weighed on a torsion balance.

2. Treatment of pituitary glands and other gonadotrophic preparations.

The acetone-dried glands were subjected to a number of treatments before injection and except where stated were whole pituitary glands. The glands were macerated in o.9% saline in a tissue mortar and the material injected as a suspension, or the macerated material was centrifuged and the clear supernatant fluid injected. Materials treated in this way included plaice and toad (Xenopus laevis) glands, international preparation ALP (ox) and chorionic gonadotrophin ('Pregnyl', Organon and Laboratory Standard preparation).

Robertson and Rinfret (1957) have described a method of preparation of several fractions of salmon pituitaries and in many of the experiments, this technique was used. The method is as follows:-

Stage 1. The glands are macerated in a mixture of glacial acetic acid and acetone (16 ml. acid and 2.5 ml. acetone per gm.) in a tissue mortar and left overnight in a refrigerator.

Stage 2. 1 vol. of distilled water is added, the solution mixed and centrifuged. The insoluble precipitate was kept for some tests.

Stage 3. The supernatant is decanted and acetone added, a little at a time, until the solution becomes cloudy. Further small quantities of acetone are then added cautiously until a flocculant precipitate appears. The total volume of acetone required varies considerably, but is roughly twice the volume of the supernatant. The precipitate is centrifuged and dried by washing twice with 1 ml. of diethyl ether. Fraction 1.

Stage 4. One volume of acetone is added to the supernatant from stage 3, and further quantities added until a flocculant precipitate forms. This is centrifuged and dried as above. Fraction 2.

Stage 5. In some cases the insoluble material from stage 2 was neutralised, taken up in saline and tested for gonadotrophic activity.

Stage 6. In the majority of assays, fractions 1 and 2 were added together, neutralised and taken up in saline before injection.

III. Review of the Literature.

In studying the reproductive endocrinology of a single species, the structure of the endocrine tissues and their target organs must be considered in the normal animal in order to have a basis for comparison with the results of experimental studies. The following review of the literature is an attempt to summarise the existing knowledge of the pituitary-gonad relationships in teleosts and to indicate those areas where the precise significance of particular aspects of the problem is still in doubt.

A. The ovary.

Although a great deal of information is available concerning the ovarian structure and cyclical variation in viviparous and ovo-viviparous fish, the complete cycle of changes has been investigated in only a few species of oviparous fish. In these the basic pattern of oogenesis is similar and will be described briefly. The ovary is smallest in summer, just after the spawning season. From then on there is a gradual increase in its weight throughout the autumn and winter, paralleled by an increase in the size of the follicles and their contained oocytes and in the number of yolk vesicles present. The zona radiata appears as early as October and there is a rapid increase in the size of the follicles and the amount of yolk present during the spring. The maximum percentage of the body weight formed by the ovary is found in spring and early summer at the beginning of the spawning season.

After the spawning season, any mature oocytes become atretic

and the ovary collapses. In ovaries in this condition, small cells, each with a large vesicular nucleus containing a prominent nucleolus, make their appearance in the ovigerous lamellae. (see page 30). These cells are the young primary oocytes and are destined to be spawned in the next year. Nucleus and cytoplasm enlarge and several nucleoli appear. Yolk vesicles are formed and during the summer the follicle wall, which at first consisted of a few flattened cells, is composed of a large number of cells beneath which the zona radiata makes its appearance. These changes are characteristic of species which shed their eggs during the spring and the descriptions of oogenesis given for Pleuronectes platessa (von Frans, 1909), Pleuronectes limanda, (Wheeler, 1924) and Liopsetta obscura (Yamamoto, 1956) are very similar and vary only slightly from the basic pattern. A similar sequence of events occurs in autumn spawning species, but at the opposite season.

Despite this basic similarity, there are several aspects of oogenesis where the available information is contradictory. One fundamental question to be answered is whether the definitive germ cells in teleosts and in vertebrates generally are derived from undifferentiated cells which are set aside early in development or whether they are merely transformed soma cells which originate in the gonad from time to time even in the adult. (Brambell, 1930). Three hypotheses have been proposed to answer this question. The first postulates that the primordial germ cells segregate early in the development of the embryo and migrate from the entoderm, where they are first seen, into the gonad

where they form the only source of definitive germ cells. (Dodds,1911, Okelberg,1921, Hann,1927, Stenger,1959).

Supporters of the second hypothesis, (Essenberg,1923, Foley,1927) recognise an early differentiation of germ cells but suggest that new germ cells are also produced from certain cells of the soma. Finally a minority of workers (Waldeyer,1870, Calderwood,1892) have denied that there is an early segregation of the germ cells. According to these investigators, primordial germ cells do not exist and the definitive germ cells are later formed by a transformation of somatic cells contributed by the germinal epithelium which is present in the gonad.

The earlier literature is reviewed by Hayes (1931), and Everett (1945) and Johnston (1951) have reviewed the subject in the light of recent investigations. Everett maintains that the first theory is correct and suggests that in mammals at least, the apparent formation of sex cells from the germinal epithelium is due to the presence in it of primordial cells which segregated early and which have been stored there.

Closely related and almost as controversial is the question of the origin of new oocytes in the mature ovary after spawning. Those authors (Hann,1927 and Stenger,1959) who subscribe to the theory of early segregation of germ cells, believe that new oocytes are derived solely by mitotic division of residual oogonia already present in the ovary and that the epithelium lining the ovary plays no part in their production. This view is supported by the work of Matthews (1938) who describes oogonia lying underneath the germinal epithelium in

Fundulus heteroclitus. These cells may be found at any time of the year, but the large number of mitoses found in them after the spawning season indicates that this is a period of active proliferation.

Calderwood (1892), however, describes three types of ova- "great, small and minute" in the ovary of Pleuronectes limanda. He states that the inner boundary of the ovigerous lamella is composed of germinal epithelium and that new ('minute') ova are formed from single epithelial cells; new ova are also formed from nests of epithelial cells. The number of eggs is said to increase also by direct division of young oocytes.

Wheeler (1924) also studying Pleuronectes limanda, was unable to observe the formation of new oocytes from the germinal epithelium either singly or in nests. He admits uncertainty as to the origin of the new oocytes but suggests that they are produced by some of the cells of the empty follicle after spawning. He uses this theory to account for the rapid disappearance of the follicle and for the absence of mitotic division in the ovary of the mature fish.

Bullough (1939) states that the origin of new oocytes in Phoxinus laevis is uncertain. In this species, however, mitotic divisions are common in the masses of cells which have been produced from the empty follicles and Bullough suggests that although most of these divisions produce new follicle cells, some may produce new oogonia. As evidence for this he states that oogonia, and intermediate stages, are found in close proximity to these dividing cells. Craig-Bennett (1931) maintains that new oocytes are produced by direct growth from cells of

the empty follicle in Gasterosteus aculeatus. This view is also held by Yamamoto (1956) for Liopsetta obscura.

The significance of follicular atresia and corpus luteum formation has been of some importance in mammalian endocrinology and the comparative aspects of the problem have been investigated by a number of workers. The mammalian oocyte is surrounded by three membranes, the granulosa, the theca interna and the theca externa. (Hisaw,1947, Brambell,1956). The mature oocyte lies in an antrum filled with follicular fluid and is connected to the follicle by a thin column of granulosa cells.

The follicles in all other vertebrates differ from those of mammals in that the growing oocytes fill them completely at all stages and no fluid-filled antra are developed. In teleosts, the follicular epithelium consists of a single layer of flattened cells which may become cuboidal in form as growth proceeds but which reverts to a squamous layer one cell thick in the mature oocyte. This follicular layer surrounds the oocytes of all teleosts described. (Brock,1878). Little information is available on the presence of thecal layers in teleosts and many authors fail to distinguish between granulosa and theca. (Matthews,1938, Stenger,1959). Buhler (1902) describes a theca interna and externa in Coregonus, but von Frans (1909) concludes that two layers cannot be distinguished in the theca. According to Hoar (1957) it is probable that an inner granulosa and an outer theca are potentially present whether or not they are clearly marked.

Atresia of developing oocytes is common among mammals. Here the oocyte degenerates and is invaded by cells from the theca interna after the degeneration of the granulosa cells. The resulting body resembles a corpus luteum in histological character, but is somewhat smaller. It is often called a corpus luteum atreticum.

Atretic follicles are present in all vertebrate groups and have been described in teleosts by a number of workers (Buhler,1902, Franz,1909, Matthews,1938, Stenger,1959) but little is known of the origin of the cells of which they are composed. Buhler (1902), states that follicular epithelium play a principal part on the removal of the oocyte.

The mammalian corpus luteum is developed from cells associated with the empty follicle after the oocyte has been discharged. There has been a considerable controversy concerning the origin of these cells. Recent investigation indicates that they are derived from the follicular epithelium or granulosa: but some workers maintain that thecal cells are involved. (see reviews by Asdell,1928, Harrison,1948 and Brambell,1956). The essential feature of the mammalian corpus luteum is its conversion to an endocrine gland responsible for the secretion of progesterone. Its formation appears to be dependent on the secretion of LH by the pituitary and luteotrophic hormone is probably necessary to make it functional (Evans and Simpson,1950, Cowie and Folley,1955).

The literature on the formation of corpora lutea-like bodies in teleosts is not extensive and there appears to be no correlation between the occurrence of these bodies and viviparity. There is no resemblance to mammalian corpora lutea in the ruptured follicles of Neotoca bilineata, and Zoarces viviparus, both viviparous species

(Wallace,1903, Mendoza,1943). Bailey (1933) on the other hand, reported signs of secretion in the hypertrophied follicles of Xiphophorus helleri, an ovo-viviparous species, while Matthews (1938) reported the presence of hypertrophied cells in the empty follicles of the oviparous Fundulus heteroclitus.

Bretschneider and de Wit (1947) state that corpora atretica and post-ovulatory corpora lutea, homologous to those of mammals, are not found in the teleost ovary. The follicle membranes left after the discharge of the ovum at spawning produce a "calyx". The granulosa degenerates and the ovulation wound is closed up by the theca cells.

These authors and Hoar (1955) suggest that the pre-ovulatory corpus luteum forms the main endocrine tissue of the teleost ovary and hypertrophy of the granulosa is a constant feature and is responsible for the secretion of ovarian hormone.

There is a considerable body of opinion (Dodd,1955, Pickford and Ats,1957 Dodd,1960) which holds that the term corpus luteum should not be used for these structures found in teleosts as the secretion of progesterone has not been demonstrated in them. Certainly there is no justification for the term "pre-ovulatory corpus luteum" as one of the main characteristics of the mammalian corpus luteum is the discharge of the oocyte before its formation.

The histological evidence for the secretory activity of the "pre-ovulatory corpus luteum" in teleosts is equivocal and descriptions of its structure do not rule out the possibility that it is only a matter of the degeneration and destruction of the unovulated egg. C.L. Smith

(1955) concluded that the pre-ovulatory corpora lutea found by Bretschneider and de Wit (1947) in amphibia are identical to the corpora atretrica described by earlier workers and held that they were unlikely to be endocrine structures.

B. The effect of hypophysectomy on the ovary.

Retrogression and atrophy of the ovary follow pituitary removal and have been found in all vertebrate groups in which the operation has been performed.

The literature on the effects of hypophysectomy on the teleost ovary is very sparse and information is available from only six species of fish. The most detailed study is that of Vivien (1941) who described the reproductive cycle of Gobius paganellus and studied the effect of hypophysectomy performed at different periods in the cycle. In this fish the spawning period from April to June is followed by a period of involution which lasts till October. During this time, the ovary volume is reduced and the oocytes are less than 60 μ in diameter. From then on, the volume increases slowly and yolk is deposited in the eggs. The spawning period is preceded by a short "période statique" during which there is little change in the ovary.

Of 45 females, hypophysectomised immediately before spawning, 40 did not lay eggs. Of the five which did spawn, three spawned completely and these three were among the last to be operated. Vivien suggests that as they were very near the spawning season, the level of circulating hormone after pituitary removal, may have been sufficient to maintain the "ovarian function" until after the eggs were shed. He also suggests that spawning may have been due to pituitary fragments left behind after the operation and functioning as intracranial implants. A small number of eggs was shed by the other two fish about 15 days after

operation and Vivien was unable to find any pituitary fragments in serial sections of the pituitary region. Involution of the ovaries was very slow, being first noticeable 281 days after operation.

In operations performed immediately after the spawning season, there was little difference between the gonads of hypophysectomised and control animals autopsied after one month. After two months, however, involution of those ripe eggs not shed at spawning was well under way and atresia was observed in young oocytes which had just started vitellogenesis. After three months, the ovary resembled that of a immature fish although there was little reduction in volume.

Operations performed during the period of vitellogenesis resulted in its being blocked and there was no further increase in ovary weight. Involution did not begin till three or four months after operation and was accompanied by a slow degeneration of the eggs.

The ovaries of fish operated at the beginning of the pre-spawning latent period, when vitellogenesis was complete, were little different from those of controls during the first two months. Involution began about the middle of the third month and resulted in atresia of the ripe eggs. The size of the ovaries was not reduced until eight months after operation.

From a consideration of all the experiments, Vivien (1939, 1941) concludes that effect of ablation of the pituitary varies depending on the state of the ovary at operation. Spawning is completely prevented, ripe eggs degenerate and those in which yolk deposition has begun are transformed into corpora atretica. The normal cycle of ovarian changes

is arrested and the development of young oocytes is stopped at a certain "critical stage". In Gobius paganellus, oocytes at this stage measure 40 to 60 μ. Hypophysectomy of immature fish leaves the ovary unaffected, but development of the eggs is arrested at the same critical stage. The literature of the effect of hypophysectomy in the teleost ovary is summarised in Table 1. (P. 39).

Table 1.

Summary of literature on the effect of hypophysectomy on the teleost ovary.

Author	Species	Time of Operation	No. of animals	Duration of experiment	Results
Buser - Lahaye (1953)	Gobius capito	May - June	Not stated	More than 6 weeks	Involution of ovaries in which practically no ripe oocytes were found.
"	Ameiurus nebulosus	"	"	"	"
Matthews (1939)	Fundulus heteroclitus	Autumn	"	Not stated	Little difference in weight or macroscopic structure of operated and control fish.
		March-April	3	55 days	Ovary lighter than that of control relative to total body weight.
Olivereau (1954)	Anguilla anguilla. Yellow, slightly silvered stage of maturation.	Not stated	45	More than 4 months	Activity of ovaries continued for at least 4 months.
Vivien (1939,1941)	Gobius paganellus	Immediately before natural reproduction	45	Maximum of 467 days	Spawning prevented. Involution of ovaries did not begin until 281 st day.
"	"	Immediately after natural reproduction	18	Maximum of 430 days	Degeneration of ripe oocytes remaining in ovary. Atresia of young oocytes. Little change in volume of ovaries but infantile condition attained after 3 months.

Vivien (1939, 1941)	Gobius paganellus	Period of genital activity	8	Maximum of 229 days	Vitellogenesis blocked. Atresia did not begin till after 3 months.
"	"	Latent pre-spawning period. Ovary full of ripe oocytes	9	Maximum of 217 days	No change in ovary for 2-3 months. Degeneration of ripe oocytes did not begin till after 3 months. Only small oocytes (less than 60 µ) left in ovary. Volume of ovary not reduced till after 8th month.
Vivien (1952)	Xiphophorus helleri	"	"	"	Regression of ovaries to infantile condition in 6 to 9 months.

C. The testis.

The main feature in the testis of seasonally spawning teleosts as the occurrence of a sequence of cyclical changes. Turner's (1919) description of spermtogenesis in Perca flavescens is one of the most detailed and will serve as a basic pattern. Here the testis is depleted and its volume small during the summer months after spawning in April and May. The volume begins to increase in late August and by the end of September the testis weight has increased by a factor of 30. The maximum weight is attained in November at which time it represents 4.5 - 6.0% of the gross body weight. From then until March, there is a gradual decline until the spawning season in late April when the weight decreases rapidly.

The reconstitution of the testis begins in April and is accomplished by a migration of primordial germ cells into the lobules at the periphery of the testes. Their number is increased by mitotic division and by the arrival of new migratory cells. This process continues throughout the summer until in August a solid cord of germ cells fills each lobule of the testis. The transformation of the germ cells into spermatogonia is contemporaneous with the beginning of the increase in the volume of the testis. There is a definite reduction in cell size between germ cells and spermatogonia and the occurrence of an increase in volume of the testis at this time is probably due to mitosis of the spermatogonia. After about 5 or 6 spermatogonial divisions, spermatocytes are produced. The formation

of spermatids and spermatozoa follow rapidly on one another, although the entire period in which the former may be found in the testis lasts from September to mid-December. Spermatozoa are first found in the testis early in September and spermatogenesis is completed in January. These changes are characteristic of species which spawn in spring, but a number of variations from the basic pattern have been noted.

Although the sequence of spermatogonia through spermatocytes and spermatids to spermatozoa is common to all species, the relationship in time which these stages bear to one another is subject to considerable variation. In several species, the stages follow one another rapidly and spermatogenesis is completed in the autumn. The testis consists largely of spermatozoa throughout the winter and spring and a "potential maturity" exists in which ripe sperm capable of fertilising the ova, are present in the testis for several months before the spawning season (Gambusia affinis, (Geiser,1922), (Gasterosteus aculeatus, (Craig-Bennett,1931) and Esox lucius, (Lofts and Marshall,1957). In contrast to this situation, several species have been described in which the sequence of changes occupies most of the year and spermatozoa are not produced until a few weeks before the spawning season (Cottus bairdii (Hann,1927), Phoxinus laevis (Bullough, 1939), Lepomis macrochirus, (James,1946). In these species, the production of one stage is more or less complete before the next one appears. Fundulus heteroclitus is intermediate between the two conditions. (Matthews,1938). In the testis of this species all stages between spermatogonia and spermatids can be

seen from September to March. Mitotic activity of spermatogonia is still present in March and spermatids become increasingly more numerous in proportion to other cell types. Spermatozoa appear in April and May.

Another source of variation in the spermatogenesic cycle of teleosts lies in the origin of each season's germ cells. The reconstitution of the testis by the migration into it of primary germ cells described in P. flavescens by Turner (1919) has been described in other species (Gasterosteus aculeatus, (Craig-Bennett, 1931) and Esox lucius (Lofts and Marshall, 1957)). In Gambusia affinis, on the other hand, spermatogonia develop from inconspicuous germ cells lying in the testicular stroma. These germ cells migrate peripherally and give rise to new spermatogonia by mitotic division. (Geiser,1922). A similar mode of origin of spermatogonia has been described in Cottus bairdii, (Hann,1927), Fundulus heteroclitus, (Matthews, 1938) and Phoxinus laevis, (Bullough,1939).

The presence of cells in the testis of fish, homologous in structure and endocrine function with the mammalian interstitial cells of Leydig, has been the subject of considerable controversy. The earlier literature is reviewed by Oslund (1928) and several writers have stated that such cells are absent from the teleost testis, while others, although recognising their presence, have been uncertain of their secretory nature.

Interstitial tissue is well developed in Gasterosteus aculeatus, (Craig-Bennett,1931). The interstitial cells at their maximum

development just prior to the breeding season and the presence in them of black granules after osmic acid fixation suggests that they contain lipid material. After the spawning season, the cells are reduced in size and when the testis is at its minimum size, they are indistinguishable from connective tissue cells. According to Craig-Bennett, the interstitial tissue at its maximum development is very similar to that of mammals and has the cytological structure which would be expected in a gland of internal secretion.

The amount of interstitial tissue in the testis of Phoxinus laevis was estimated by Bullough (1939) who concluded that it remains fairly constant throughout the year. He could find no evidence to suggest that the development of secondary sexual characters in the breeding season was under the control of hormones secreted by the interstitial cells.

Much of this controversy has been due to the difficulty of distinguishing between interstitial cells and connective tissue cells on morphological grounds and the work of Marshall and Lofts (1956) may help to settle the matter. These authors suggest that fish may be divided into two groups on the basis of their testicular endocrine tissue. In one arrangement, a true interstitium is lacking and secretory cells are found only in the lobule boundaries, while the second arrangement conforms to the typical mammalian pattern. In both types, histochemical methods are essential to distinguish the secretory cells from connective tissue cells. Lofts and Marshall (1957) conclude that the lobule boundary cells influence the production of secondary sex characters and play little or no part in spermatogenesis.

D. The effect of hypophysectomy on the testis.

Hypophysectomy has been found to cause regression of the testis in all vertebrate groups and all workers have reported adverse effects on spermatogenesis. The effect of hypophysectomy on the testis has been studied in only 5 species of teleost fish and the most complete study is that of Vivien (1938,1941) who hypophysectomised adult male Gobius paganellus at different times in their normal spawning cycle. Pituitary removal shortly before the normal spawning season prevented emission of sperm in about 70% of the fish. Spermiation was normal and territory and eggs were guarded in the remainder. Regression of the testis and accessory glands began after about 3 months (at which time the controls were exhibiting their normal involution) and continued for at least 3 months more. When hypophysectomy was carried out immediately after the spawning period, the testis was reduced in volume fairly quickly and only spermatogonia were present after 3-4 months.

When the pituitary was removed during the period of winter genital activity, spermatogenesis was halted. Involution did not begin for 3 or 4 months and was accompanied by resorption of spermatozoa. and spermatocytes and only spermatogonia remained. When the pituitary was removed during the latent pre-spawning period after spermatogenesis was complete, degeneration did not become obvious till after the third month. Only spermatogonia eventually remained and spermatozoa were resorbed. The size of the testis was gradually reduced over a period of 8 months by which time it appeared immature.

Vivien (1941) extended his work on Gobius paganellus to include immature fish and found that the gonads were unaffected by hypophysectomy.

Matthews (1939) hypophysectomised male Fundulus heteroclitus in October/December when the testis was beginning to grow again and in March/April when testis growth is at its maximum. In the first series, although there was little difference in weight between operated and control animals 73 days after the operation, differences in microscopical structure were obvious. Spermatids and spermatozoa were very scarce and cysts of spermatocytes were small 10-13 days after pituitary removal. Differences in testis weight between operated and control fish were obvious after 2 weeks, in March operations, although there was no obvious difference in histological structure. After 26 days, however, the majority of the cells in the testes of operated fish were spermatogonia, with only a few spermatids and spermatozoa. After 206 days, the testes contained only primary spermatogonia and a few secondary spermatogonia.

The results of these and other workers are summarised in Table 2 (pp. 47 - 48) from which it is evident that regression of the testes follows hypophysectomy in the few teleosts so far studied.

Table 2.

The effect of hypophysectomy on the reproduction in male teleosts.

Author	Species	No. of animals	Maximum survival	Results
Matthews 1939	Fundulus heteroclitus	33	206 days	Inhibition of stages beyond secondary spermatogonia noticed after 10 days in autumn operations and after 26 days in spring operations. Mitosis between primary and secondary spermatogonia present after 206 days.
Burger 1941	" "	6+	2 months	Inhibition of spermatogenetic stages beyond those of spermatogonial division. Once spermatogenesis has been initiated, spermiogenesis can continue for some time in the absence of the pituitary.
Vivien 1938	Gobius paganellus	20	168 days	The testes and sex accessories regressed and involution of the testes was complete by about 6 months.
Vivien 1941	" "	11	405 days	Regression of the testes and accessories after 3 months and continuing for at least three more. (Operated just before breeding season).
Vivien 1941	" "	34	416 days	Rapid reduction in testes volume. Only spermatogonia present after three to four months. (Operated just after spawning season).

Vivien 1941	Gobius paganellus	6	230 days	Operations performed during the period of genitial activity. Rapid cessation of growth; spermatogenesis stopped. Involution did not begin till about 3-4 months after which there was a slower degeneration of spermatocytes and spermatozoa.
" "	" "	6	193 days	Little change for 3 months. Spermatozoa eventually resorbed and only spermatogonia present in testes.
Buser-Lahaye 1953	Gobius capito and Ameiurus nebulosus	Not mentioned	More than six weeks	Involution of gonads. Interstitial tissue of testes poorly developed.
Tavolga 1955	Bathygobius soporator	6	30 days	Genital system reduced to winter conditions. Courtship and combat behaviour and territorial behaviour diminished.

E. The teleost pituitary gland.

Despite its basic similarity to that of other classes, the teleost pituitary gland has many structural and histological features peculiar to itself. Many of the staining methods used to study its structure have been developed for use with mammalian material and a brief description of the results obtained in mammalian projects is warranted in view of the attempts which have been made to homologise the various components of the teleost gland with those of the mammalian pituitary. The cells of the mammalian pars anterior can be divided into acidophils, basophils and chromophobes on the basis of their reaction with trichrome staining methods and several workers, by the employment of carefully controlled tinctorial stains, have been able further to subdivide the acidophils and basophils. As many as 6 tinctorially different cell types have been described and it is generally agreed that the basophils are responsible for the secretion of gonadotrophic and thyrotrophic hormones (Dawson and Friedgood 1938, Goldberg and Chaikoff 1952, Purves and Griesbach 1957).

Histochemical methods have also been used in the study of pituitary cytology, especially the periodic acid - Schiff (PAS) reaction which is a test for glycoprotein. The aldehyde fuchsin (AF) reaction has also been used, although its specificity as a glycoprotein stain has not been established. Most authors are agreed that the PAS and AF positive material is located in the basophil cells of the pars

anterior. The results of investigations into the cytology of the mammalian pars anterior are summarised in Table 3, p. 51.

The earlier literature on the cell types in the teleost pituitary has been discussed by Charipper (1937). Three cell types, acidophils, basophils, and chromophobes are present in the meso-adenohypophysis and most recent authors agree that the basophils are similar in function to the glycoprotein-containing cells of the mammalian pars anterior which they often resemble in structure. (Atz (1953), Sokol (1953,1955) and Barrington and Matty (1955)).

Cyclical changes have been described in the teleost pituitary and several authors have correlated them with the changes which occur in the gonads. Matthews (1936) described cyclical changes in the proportions of large basophils in the more posterior portion of the pituitary gland of Fundulus heteroclitus, the number being highest from May to September and lowest in March and April. There is some confusion as to the homology of the regions involved in these changes, as, in a later paper, Matthews (1937) decides that the large anterior region is the pars anterior (pro-adenohypophysis), not the Ubergangsteil (meso-adenohypophysis), although he is doubtful whether it is homologous with the pars anterior of Stendell (1914). The view taken in the later paper is probably the correct one as the pro-adenohypophysis is generally held to be composed largely of acidophils and chromophobes. Scrugg's (1939) description of the pituitary gland of Fundulus heteroclitus supports this view as he maintains that the basophils described by Matthews belong to the meso-adenohypophysis .

Table 3

Summary of the staining reaction of the cell types of the mammalian anterior pituitary.

Author	Species	Stain	Acidophil		Basophil			Chromophobe
Goldberg & Chaikoff 1952	Dog	Trichrome	Alpha Orange	Epsilon Red	Beta Light blue	Delta Light blue	Zeta Violet purple	Gamma unstained
		PAS	-ve	-ve	+ve	+ve	-ve	-ve
		AF	-ve	-ve	+ve	-ve	-ve	-ve
Purves & Griesbach 1957	Dog	Mallory	Orange	Red	Blue	Purple	Pale	unstained
		PAS	-ve	+ve	+ve	+ve	+ve	-ve
		AF	-ve	-ve	+ve	+ve	+ve	-ve
Martins 1933	Rat	Trichrome	Red		Blue			unstained
Purves & Griesbach 1951 & 1954	Rat	PAS	-ve		Central (Beta) +ve	Central +ve	Peripheral (Delta) +ve	-ve
	Rat	AF	-ve		+ve		-ve	-ve
Wilson & Ezrin 1954	Rat	PAS trichrome	-ve		+ve (red)		+ve (purple)	-ve
Halmi 1950, 1952	Rat	AF	-ve		+ve		-ve	-ve

Matthews (1936) suggested that the acidophil changes were associated with the breeding season and that those in the basophils have some relation to the development of the nuptial sex character which in Fundulus heteroclitus consists of a black pigment spot on the dorsal fin.

Kerr (1948) studied the pituitary gland of normal and parasitised roach (Leuciscus rutilus). In normal fish, the basophils of the pro-adenohypophysis showed slight variation in colour, but it was not possible to correlate these differences with seasonal changes. No variation was seen in the acidophils or chromophobes of this region. Some variation was seen in the acidophils of the meso-adenohypophysis but this was irregular and the small size of the cells together with their large numbers made them an unsuitable type for study.

By far the greatest degree of variation was found in the basophil cells of the meso-adenohypophysis. These cells were scattered in irregular groups and varied in size and in the size and staining intensity of their cytoplasmic granules. The basophils were at their maximum in April and May when the gonads were fully ripe. They stained intensely and varied in diameter up to about 13 μ. Their size and density gave the impression that their numbers had increased relative to the acidophils, but Kerr found that accurate cell counts were impossible owing to the small size and large numbers of the acidophils.

After breeding, there was a regression of the basophils and by late June or July they were at their least prominent. Here the proportion of lightly granulated cells was much higher and the maximum

cell diameter was about 8 μ. After July there was a slow increase in granulation and cell size to the maximum in April and May.

Kerr found that the occurrence of the plerocercoid stage of the tapeworm Ligula intestinalis as a parasite in the body cavity of these fish was accompanied by a marked regression in the gonads which resembled these of immature fish. The meso-adenohypophysis basophils differed from the normal ones in the smaller maximum size which they attained (6.5 μ) and in their lower level of granulation. Seasonal changes were either absent or so reduced that they were obscured by individual variation.

Scrugg's (1951) studying the pituitary of the goldfish Carassius auratus and the carp Cyprinus carpio, found striking changes in the basophils of the meso-adenohypophysis and were able to correlate these with the stages in the breeding cycle. In these two species, varying numbers of acidophilic globules are present in the basophils which during the pre-spawning period comprise 68% of the cells. In the early part of this period, the globules stain lightly, but as the spawning season approaches, they increase in size and staining intensity and the basophil cells increase in diameter from 10 to 13 μ. At the same time the cell boundaries of the acidophil cells become more distinct and they also increase in diameter.

During the spawning period, the globules reach their maximum size and coalesce and after pass into the inter cellular spaces. Towards the end of the period, the basophils shrink and their contained globules are reduced in number and size. In July and August, when the

gonads are completely exhausted, the number of basophils decreases to about 5% and there is a corresponding increase in the acidophils. By September, however, the proportions have returned to normal and the globules increase in size and number. During the winter, when there is little activity in the gonads, there is little change in the size of the basophils, but the enlarged globules found in September decrease slowly in size and number to the conditions found in January. The acidophil cells also decrease slowly in size until they reach their most crowded condition in January when the cell outlines are again indistinct.

Scrugge is of the opinion that the globules of the basophils, which have also been described by Kerr (1942), are secretory and that they probably have some functional part in the control of the reproductive cycle.

F. Bioassay of fish pituitary preparations.

1). Amphibia.

It is now well-authenticated that many anurans and urodeles respond to the injection of mammalian and other gonadotrophins by ovulation or spermiation and these responses form the basis of a number of bio-assays for gonadotrophins (Landgrebe 1948, Hobson 1952a,b, Hobson and Landgrebe 1954). Conflicting results have been obtained, however, when such methods have been used to establish the gonadotrophic nature and potency of fish pituitary substances.

The results of these experiments are summarised in Table 4 (pp56-58), but many of the papers quoted are poorly documented and , in some cases, the results are based on the response of a single animal and therefore hardly justify the title of bio-assay. In view of the large number of recipient and donor species involved and the variety of ways in which the material has been administered, it is difficult to draw any definite conclusions. For the most part, the experimental data are inadequate, but the results obtained by Otsuka (1956a) using hypophysectomised newts (Triturus pyrrhogaster) are worthy of more careful consideration. Here, the injection of pituitary material from 3 teleost species was followed by ovulation (Table 4). Several dose levels were used and the minimum dose level which would produce ovulation was 1.0 mg. Doses of 0.25 mg. to 0.5 mg. were sufficient when 40% ethanol extracts were used, but not all the activity lay in the extract as a response was obtained from 7.0 mg. of the residue.

Table 4.

The effect of fish pituitary preparations on the gonads of Amphibia

Author	Donor	Recipient	Method	No. of test animals	Dose	Result
Elasmobranchii Berk & Shapiro 1939	Dogfish	Xenopus laevis (female)	Implantation of fresh anterior lobes into dorsal lymph sac	Unspecified	5 "lobes"	No ovulation
Actinopterygii						
Houssay 1947	Salminus maxillosus	Bufo arenarum (male)	Injection	?	1 and 3 glands	No release of sperm
	Merluccius hubbsi	"	"	?	"	"
	Austromenidia bonariensis	"	"	?	"	"
Atz and Pickford 1954	Urophycis tenius	Rana pipiens (male)	Injection into dorsal lymph sac of glands from post-spawning fish	6	Brei of 6 or 12 frozen glands	No sperm release
	"	"	"	7	Brei of 2 frozen glands	Small to moderate release of sperm within two hours in 3 out of 7 of the test animals
	"	"	"	2	Brei of 0.5. frozen glands	No sperm release

Atz and Pickford (ct) 1954	Urophycis tenius	Rana pipiens (male)	Injection into dorsal lymph sac of glands from post-spawning fish	14	4 mg and 12 mg of acetone dried glands = 2 and 6 glands respectively	No release of sperm
	Gadus morrhua	Rana pipiens (male)	Injection into dorsal lymph sac of glands from pre-spawning fish	4	4 and 12mg of acetone dried glands	No sperm release
	Pollachius virens	"	"	2	12 mg of acetone dried glands	No sperm release
Stroganov and Alpatov 1951	Acipenser guldens-tadti	Rana temporia (male)	Injection of acetone-dried glands into dorsal lymph sac	6	0.3 and 0.8 mg	Sperm emitted within $3\frac{1}{2}$ hours
	"	"	"	3	0.15 mg	Sperm emitted within an hour (2 out of 3 animals)
	"	Rana ridibundi (male)	"	3	0.08 mg	Sperm emitted within one hour (1 out of 3 animals)
	"	Rana esculenta (male)	"	Unspecified	Unspecified	Sperm emitted

Houssay et al 1929	Micropogon opercularis	Bufo arenarum (female)	Implantation of fresh glands	1	1 gland for ten days	No ovulation
Rostrand 1934	Cyprinus carpio	Rana temporia Rana esculenta (female)	Injection of extracts of glands	Unspecified	Unspecified	No ovulation
	Gadus merlangus	"	"	"	"	"
Hansen 1955	Cynoscion nebulosus	Scaphiopus holbrooki (female)	Homogenate of fresh glands in Holtfreter's solution added to in vitro ovary	4	1-5 glands	No ovulation
Wills, Riley and Stubbs 1933	Lepisosteus platostomus	Bufo americanus (female)	Injection	6	2-4 glands	Ovulated on 2nd to 4th day (5 out of 6 animals)
	"	Rana pipiens (female)	Daily injections	1	4 glands	Ovulated after 3 injections
Eliakova 1954	Acipenser guldenstadti and A.stellatus	Mature axolotls (female)	Several intra-muscular injections of triturated acetone-dried or fresh glands from pre-spawning fish	4	Unspecified	Eggs shed

Otsuka 1956a	Inimicus japonicus	Triturus pyrrhogaster (female)	Subcutaneous injection into hypophysectomised animals. Acetone dried glands. Dose given in 2 equal injections and animals autopsied 24 hrs. later	6	3 mg	5 animals ovulated
				"	2 mg	4 animals ovulated
				"	1 mg	3 animals ovulated
				"	0.5 mg	No ovulation
"	"	"	As above. 40% ethanol extract	6	2.0 mg	3 animals ovulated
				"	1.0 mg	2 animals ovulated
				"	0.5 mg	2 animals ovulated
				"	0.25 mg	No ovulation
"	Onchorhyncus keta	"	As above. Acetone dried glands	6	3.0 mg	4 animals ovulated
				"	2.0 mg	3 animals ovulated
				"	1.0 mg	2 animals ovulated
				"	0.5 mg	No ovulation
"	"	"	As above. 40% ethanol extract	6	1.0 mg	3 animals ovulated
				"	0.5 mg	3 animals ovulated
				"	0.25 mg	No ovulation
"	Makaira marlina	"	As above. Acetone dried glands	6	3.0 mg	4 animals ovulated
				"	2.0 mg	3 animals ovulated
				"	1.0 mg	3 animals ovulated
				"	0.5 mg	No ovulation
"	"	"	As above. 40% ethanol extract	6	1.0 mg	3 animals ovulated
				"	0.5 mg	3 animals ovulated
				"	0.25 mg	2 animals ovulated
				"	0.125 mg	No ovulation

At least in the case of the salmon, the pituitary glands were collected in the breeding season of the fish, but as Otsuka does not give the weight of the gland, comparison of his results with those of other authors is difficult. Since the test animals were hypophysectomised 5 days before the start of the injections, it is likely that all endogenous hormone would have been eliminated and that ovulation was indeed induced by the injected material. The breeding season of Triturus pyrrhogaster was not given, but Otsuka stated that many of the newts ovulated spontaneously after hypophysectomy. The animals were collected between April and June, the breeding season of most animals in the northern hemisphere, and the possibility cannot be ruled out that the injections merely acted as a non-specific stimulus to ovulation.

2) Mammals

Increase in uterine weight and ovarian weight in rats and mice have been used extensively as end-points in the bio-assay of gonadotrophic hormones. These methods have also been employed to a small extent in the assay of teleost pituitary material, but as in the case of amphibian test animals, the experimental data are too sparse to allow a satisfactory conclusion to be drawn (see Table 5 pp. 61-62).

Table 5

The effect of fish pituitary preparations on the gonads of rats and mice.

Author	Donor	Recipient	Method	No. of test animals	Dose	Result
Elasmobranchii						
Dodd 1955	Raja batis	Mouse	Sub-cutaneous injection of acetone dried "anterior lobes" into 19-day old animals	6	?	No increase in uterine weight
"	"	"	Sub-cutaneous injection of acetone dried "neuro-intermediate lobes" into 19-day old animals	6	4 mg (2.5 lobes) per animal	Increase in uterine weight from 6.4 to 17.5 mg.
Witschi 1955	Shark	Rat	Injection of acetone-dried powdered gland	Unspecified	More than 50 mg	Vaginal cornification - 1 Rat (FSH) = more than 50 mg
Actinopterygii.						
Callamand 1943	Anguilla anguilla	Rat	Injection of extract of gland	1	30.2 mg over 10 days	Slight enlargement of ovaries
"	"	"	Implantation	1	2 glands	No effect
"	"	Mouse	Injection of acetone-dried glands	1	large amounts	No effect

del Castillo and Novelli 1938.	Menidia platensis	Young Rat	Daily sub-cutaneous	2	10 "anterior lobes"	No increase in ovary weight. Vagina not opened
"	Luciopimelodus pati	"	"	2	"	" "
"	Cynoscion striatus	"	"	2	"	" "
"	Merluccius hubbsi	"	"	1	"	" "
Witschi 1955	Lepisosteus	Rat	Injection of acetone-dried powdered gland	Unspec-ified	Unspec-ified	Vaginal cornification 1 Rat Unit (FSH) = more than 200 mg
"	Salmon	"	" "	Unspec-ified	Unspec-ified	Vaginal cornification 1 Rat Unit = more than 200 mg
Otsuka 1956 b	Makaira marlina	19 day old immature female mice	Subcutaneous injection of acetone dried "anterior pituitary glands"	6	= 30 mg	100% increase in ovary weight: 32% increase in uterus weight
				10	= 15 mg	Increase in ovary weight no significant increase in uterus weight

IV. The Ovary and its associated Structures.

A. Anatomy

The ovaries of the plaice are paired elongated bodies. Each ovary is cone-shaped and the base just projects into the posterior part of the body cavity, while the bulk of the organ tapers backwards towards the tail, lying against the haemal spines and is partially covered by the muscles of the trunk. The ovary, like that of the majority of teleosts, is a sac, the wall of which is continuous with the short oviduct. The oviduct opens into the posterior side of the rectum, just inside the opening of the latter to the exterior. The external oviducal opening leads into a short chamber into which the right and left ovaries open. Unlike most teleosts, the oviduct has no connection with the ureters, ie. there is no urinogenital sinus.

The ova are developed on the ovigerous lamellae which are longitudinal folds in the internal ovary wall and which project into the lumen of the ovary, filling most of the cavity. Externally, the ovary is bounded by a loose connective tissue layer in the thickness of which is a thin sheet of black pigment. Internal to this is a layer of unstriated circular muscle which varies in thickness throughout the year. Within this and filling up the thickness of the lamellae is a network of connective tissue and the internal surface of the ovary is bounded by an epithelial layer.

The length, weight and age at which sexual maturity is attained vary greatly (Bagenal, 1953), so that none of these is a good

criterion for determining the gonadal condition of the fish. For most of the year, however, it is relatively easy to distinguish between immature and mature fish. If a fish be held against a bright light, the ovary can be seen as a dark shadow in the body musculature. The shadow is short and narrow in an immature fish and long and broad in a mature one. In addition, the ovary of a mature fish becomes increasingly swollen as the season progresses.

In the immature fish the ovary measures about one inch in length and projects only slightly into the body musculature. Macroscopically, it appears to be filled with a transparent, jelly-like material. Histologically, the ovary consists of many ovigerous lamellae each containing large numbers of oocytes of different sizes up to a maximum of 140 μ. With the onset of maturity, which may occur in the second or third year (Bagenal,1953), the ovary begins to swell and grow backwards towards the tail. Concomitant with this is the initiation of growth of the oocytes and this process can be divided into a number of stages.

Stage 1. Oogonia.

These cells are present either singly or in small nests in the lamellar epithelium at all times of the year but are present in greatest quantity during the late spring and summer, during which time occasional mitotic divisions are seen. The oogonia are small cells from 3 to 5 μ in diameter and are always found in association with one or more potential follicle cells. The nucleus contains a single, large nucleolus and a distinct nuclear membrane separates it from the narrow

accumulation of colourless cytoplasm. Fine threads of chromatin radiate from the nucleolus to the bulk of the chromatin material at the periphery of the nucleus (Plate 3, Fig. 1).

Stage 2. Primary oocyte.

The early primary oocyte is little bigger than the oogonium, being about 4-5 μ in diameter. The primary oocyte can be easily distinguished from the oogonium by the appearance of distinct chromosomes in the nucleus. At first, the cytoplasm is still colourless and the chromosomes appear as a tangle of thick deeply staining threads in the nucleus (Plate 3, Fig. 2). This is the leptotene stage of meiosis and is followed immediately by the pachytene. Here the chromosomes are visible as deeply staining threads of varying length distributed evenly throughout the nucleus. It is not possible to distinguish between two strand and four strand pachytene.

Shortly after this, the follicular cells organise themselves round the developing oocytes, the cytoplasm of which begins to increase in amount and becomes strongly basophilic. The nucleus becomes enlarged and the chromosomes lose their distinct nature, appearing as loose threads in the granular nucleoplasm. This marks the early stages in the development of the lamp brush chromosomes which are so characteristic of diplotene. With the growth of the oocyte, the basophilic cytoplasm increases considerably in relative volume and at the same time several deeply-staining basophilic nucleoli appear and gradually arrange themselves towards the periphery of the nucleus. At the end of stage 2 maximum diameter of the oocyte is 150-200 μ (Plate 4, Fig. 1).

Stage 3. Yolk precursor stage.

At the end of stage 2 a single layer of thecal cells is present outside the follicular epithelium or granulosa and a "yolk-nucleus" is present in the form of a black dot near the surface of the cytoplasm. Stage 3 is characterised by the presence of a ring of vacuoles in the cytoplasm just inside the cell membrane. Simultaneously a narrow pre-deposit of the zona radiata makes its appearance between the cytoplasm and the follicular layer. Stage 3 oocytes measure between 160 to 200 μ (Plate 4, Fig 1).

Stage 4. Yolk-vesicle stage.

The early cells of this stage are characterised by the presence of yolk globules associated with the ring of vacuoles present in Stage 3. The round germinal vesicle lies in the centre of the oocyte and many nucleoli can be seen around the periphery of the nucleus. Lamp brush chromosomes are present in the granular nucleoplasm. At the end of this stage, yolk globules are present throughout the cytoplasm with the exception of a narrow zone at the periphery and the oocytes measure up to 500 μ (Plate 4, Fig 2).

Stage 5. Yolk stage.

Here the egg is enveloped by two layers, the granulosa and theca, each being one cell thick. There does not appear to be a subdivision of the theca into interna and externa and the granulosa is composed of squamous cells. The zona radiata is well-developed and clearly shows radial striation. The yolk globules, which are much enlarged and stain deeply with haematoxylin, are found throughout

the faintly staining cytoplasm except for a narrow zone at the egg surface. The germinal vesicle assumes a rounded form with an irregular contour and contains faintly staining nucleoli of spherical form. At the end of this stage, when the oocytes measure up to 1000 μ, the lamp brush chromosomes become indistinct and the nucleoli increase in number and become scattered throughout the nucleus (Plate 4, Fig. 2). A layer of homogeneous yolk may surround the nucleus.

Stage 6. Migratory nuclear stage.

The germinal vesicle in the migratory stage, moving towards the pole of the egg, is found surrounded by a zone of apparently viscid substance. After the migration, the yolk globules appear to coalesce, becoming few in number and of very large size. There is little change in size at this stage (Plate 5, Fig. 1).

Stage 7. Maturation.

At this stage there is a rapid increase in the diameter of the oocyte to about 1,400 u. The cytoplasm is distributed in a thin band round the periphery of the egg and is thicker at one pole than the other. The coalescence of the yolk globules continues and the yolk appears as a homogeneous mass filling the interior of the oocyte. The zona radiata becomes thinner due to the increase in size of the egg (Plate 5, Fig. 1). The egg membranes rupture and the ripe oocyte comes to lie free in the lumen of the ovary. The production of the polar bodies has not been observed and it is presumed that this takes place after the ovum has been released from the egg membranes; possibly after fertilisation.

B. Follicular Membranes and their Derivatives.

1. Follicular membranes.

Only 2 membranes, the follicular epithelium and the theca interna, are present in the plaice oocyte, unlike the placental mammals where the developing oocyte is invested by 3 distinct layers. A third tissue - the theca externa is present outside the theca interna, but it does not form a complete membrane. It is continuous with the general ovarian epithelium and appears to be thrown into deep folds where it comes into contact with the oocytes, partly investing them.

Granulosa cells are associated with the smallest oogonia and rapidly become organised into a distinct membrane of squamous epithelium which is never more than one cell thick. The granulosa rests on a basement membrane which separates it from the theca interna. The latter layer, also one cell thick, is first seen in Stage 2 oocytes, when they measure 150 - 200 μ in diameter and is formed by the modification of fibroblasts which organise themselves round the granulosa. Blood capillaries are numerous in the theca interna.

2. Derivatives.

a. Calyx.

The ruptured follicle left after discharge of the ripe ovum does not form a corpus luteum (Plate 5, Fig. 2). It shrinks very considerably and appears as a pocket in the epithelium lining the ovarian cavity. The granulosa cells are thrown out into the lumen of the follicle and begin to disintegrate, the nuclei becoming pale and the cytoplasmic boundaries indistinct. The follicle is lined by thecal cells which have an

irregular arrangement due to the contraction of the follicle (Plate6, Fig. 1). Mitotic divisions, which are absent from the granulosa, are occasionally seen in the theca, but there is no indication that this tissue hypertrophies. The calyx continues to shrink and leucocytes are seen in the lumen. About 2 months after spawning the follicles are present only as small accumulations of thecal cells which eventually disappear completely.

b. Atretic follicles.

Corpora atretica, produced by the degeneration of the oocyte inside the follicle membranes, are present in the plaice ovary. They are not of frequent occurrence and form only a very small proportion of the total oocytes developing in the ovary. Post-ovulation corpora atretica are formed by the degeneration of stage 5 - 7 oocytes which remain in the ovary after natural ovulation has been completed (Plate 6, Fig. 2). They persist for long periods relatively unchanged, but gradually become smaller and disappear (Plate 7, Figs. 1 and 2).

Oölysis was first seen in a few developing oocytes in November (Plate 8, Figs. 1 and 2). It was not present in all the ovaries examined and a similar random occurrence of oölysis was noted throughout the winter and spring until the spawning season. In all such oocytes, the pattern of degeneration was the same as that found in post-ovulatory corpora atretica.

There is a considerable variation in the events associated with atresia, but the general pattern in plaice is as follows. The first visible signs of atresia in a yolked oocyte are seen in the zona

radiata. This membrane begins to disintegrate and its outer surface becomes irregular and pitted. There is little change in the granulosa cells, but these appear to be responsible for the breakdown of the zona radiata (Plate 9, Fig. 1). The zona radiata eventually ruptures and some of the ooplasm and yolk globules escape from the oocyte to form a layer of substance between the granulosa and zona opposite the point of rupture of the zona radiata (Plate 8, Fig. 1). Granulosa cells migrate across this space and the dissolution of the zona is continued (Plate 8, Fig. 2). Invasion of the interior of the oocyte begins and the yolk is phagocytosed by granulosa cells which hypertrophy. There also appears to be some invasion of the oocyte by leucocytes. Small ovoid bodies having a distinct, rather refractive covering and which measure about 1 μ along their long axis make their appearance at this stage. One half of the body is colourless while the other half is filled with dense material which is eosinophilic when eosin is used in conjunction with Mayer's haemalum but stains black when eosin is used with Heidenhain's haematoxylin. These bodies have not been seen in normal oocytes, but in atresia of yolked oocytes, they first appear in association with yolk globules (Plate 9, Fig. 2). As degeneration of the oocyte proceeds, they are often visible in clusters of about 20 surrounded by a distinct membrane. It is possible that they are formed by the breakdown of yolk, as they are generally seen in association with yolk globules.

In the late stages of atresia, the oocyte is completely resorbed and all traces of yolk and the zona radiata have disappeared. The interior of the follicle is filled with a mass of cells with small

round or oval nuclei about 4 μ in diameter surrounded by a weakly eosinophilic cytoplasm, which often contains yolk substance. The ovoid bodies described above are very common at this stage and seem to be inside the cytoplasm of the cells filling the follicle. The whole structure is bounded by a membrane one cell thick which is similar in structure to the theca of a normal oocyte. There is little vascularisation of the atretic follicle which is no larger than the oocyte from which it was derived.

The fate of the theca interna during atresia appears to vary. In the atretic follicles found in early vitellogenesis (October-December) the theca interna remains unchanged as a layer of flattened cells outside the basement membrane of the granulosa. In two atretic follicles found in January, however, there was a considerable increase in the number of thecal cells, particularly opposite a break in the zona radiata, and the membrane was no longer one cell thick (Plate 10, Fig. 1). Mitotic divisions were fairly common in some of the thecal cells which had a denser cytoplasm (Plate 10, Fig. 2). The granulosa in these follicles appeared as a single layer of cells with a distinct basement membrane.

The atretic follicles may persist in the ovary for several months, but gradually become smaller. The "ovoid bodies" degenerate and the remaining cells are absorbed into the lamellar epithelium as strands of interstitial tissue.

C. Cyclical changes in the ovary.

1. Gravimetric variation.

The ovary weight is least just after the spawning season, in March and April. During the summer months there is practically no change, except for small individual variations. Ovary weight begins to increase in August and the greatest rate of increase is between November and February (Fig. 2). At the end of this period, the maximum ovary weight is attained, when it is about fifty times as great as the weight of the spent ovary and may comprise as much as 28 per cent of the gross body weight. The decrease in the percentage of the body weight formed by the ovary from February to May (Fig. 2) does not mean that there is a gradual expulsion of ova by each individual, but rather that some fish discharge their eggs earlier than others. The curve declines as the proportion of spent individuals increases.

The seasonal weight changes indicated in Fig. 2 are based on data from 109 fish collected over a period of 19 months from May 1958 to January 1961 inclusive (Table6, p.73).

While the numbers of animals on which the graph (Fig. 2) is based are insufficient for comprehensive statistical analysis, it is evident that the greatest percentage of the body weight formed by the ovary is found during January and February and the least from May to September.

The absolute ovary weight is not a reliable index of cyclical variation. According to Pickford and Atz (1957) the relationship of the weight of the gonad to the total body weight is an objective, sensitive and reliable indication of gonad-state. It will be seen from table 6 (p.73)

Table 6.

Variation in ovary weight expressed as a percentage of the total body weight.

Date of Collection	N.	M.%	± S.D.
May 1958	3	0.88	0.63
June	3	1.28	0.04
July	2	1.6	0.22
September	3	2.4	0.94
October	4	4.3	1.1
November	4	3.9	0.7
December	4	10.1	1.3
January 1959	4	13.8	1.03
February	4	17.9	2.6
March	4	14.2	11.57
April	4	7.5	7.2
July	6	1.6	0.31
August	8	1.7	0.34
September	7	2.4	1.0
October	10	4.2	2.44
December	27	8.7	2.1
January 1960	10	11.4	3.1

N = Number of animals.

M.% = Mean value for gonad weight expressed as % of total body weight. ("gonosomatic index").

± S.D. = Standard deviation from the mean.

that as the spawning season approaches the variation in "gonosomatic index" encountered in any one month can be as great as that between one month and the preceding one. In view of this, it is felt that the "gonosomatic index", while giving some indication of the gonadal condition of the fish, is not sufficiently sensitive in plaice to provide reliable information on the effect of experimental procedures except where differences are very large.

2. Histological variation.

Oogonia are present in the ovary throughout the year, but appear to be most common in the spring and summer after spawning. The early stages of meiotic prophase are seen from March until September and the resulting primary oocytes (Stage 2) are also present throughout the year. The first oocytes in which the yolk precursor is visible (Stage 3) are found in May, and a few such oocytes are still present in October. They are found in greatest numbers during July and August. The early stages in vitellogenesis (Stage 4) are first found in July and Stage 5 oocytes are present from August until March. Stage 6 oocytes first appear in February and production of ripe secondary oocytes (Stage 7) takes place from February to April although the peak of the spawning period occurs in March. The histological variation in the ovary is represented diagramatically in Fig. 3.

During the year there is a considerable variation in the macroscopic appearance of the ovaries. In the summer months, the ovary is flaccid and its contents appear transparent, but with the onset of vitellogenesis, the ovary increases in size and the developing eggs

are yellow. This condition lasts until February when the swollen ovary contains numerous large transparent eggs.

3. Oocyte proportions.

In addition to the oocytes which will be shed at the current spawning season, both oogonia and reserve primary oocytes are present in the ovary. Estimates of the percentage of the total oocytes formed by each of these three classes were made (see p. 24) and the results are detailed in Figs. 4 and 5.

D. Discussion.

1. Origin of new oocytes.

Most workers are agreed that, in mammals at least, oocytes are derived by the meiotic division of primordial germ cells which can be identified in the embryo. The definitive oocytes which will be shed during the course of the reproductive life of the animal are already present in the ovary at puberty and oocytes do not generally arise *de novo* in the ovary.

In mammals, however, the number of oocytes liberated at each oestrus period is small, in contrast to the situation in teleosts where the number of oocytes spawned may be several million. Female plaice produce about 100,000 oocytes at each spawning season and according to Cunningham (1893) the number of yolkless eggs left in the ovary after spawning is far less than the number of ripe eggs shed in the following season. "Consequently the greater number of the eggs of one season's crop are produced *ab initio* during the year". Cunningham could not account for the origin of these new oocytes but stated (Cunningham, 1897) that mitotic division of oogonia did not occur in plaice.

This discrepancy has been recognised by several authors and a number of hypotheses have been put forward to account for it. The production of new oocytes from cells of the ruptured follicles found after spawning has been described by Wheeler, 1924, Craig Bennett, 1932, Bullough, 1939, and Yamamoto, 1956 (see p. 30). This is certainly not the case in the plaice, where the maximum production of new oocytes is complete before the empty follicle has been completely resorbed. Oogonia and small

oocytes are occasionally associated with the empty follicle, but always with its outermost layer which is continuous with the ovarian epithelium. Oogonia can also be seen in the 'theca externa' where it comes in contact with the developing oocytes and it is possible that the germ cells seen by these authors in association with the empty follicle were already there before the ripe ovum was shed. Most authors agree that the cells of the follicular epithelium degenerate after the egg has been shed and it is highly unlikely for this reason that such cells would be capable of transforming into oocytes.

The direct development of new oocytes from cells of the germinal epithelium is suggested by Wallace (1903) who describes nests of epithelial cells containing one or more oocytes. Such a process is again unlikely, as in a teleost, where the numbers of oocytes shed may be several million, it would lead to a depletion of the epithelium which would be noticeable in older fish.

The present investigation on the plaice has failed to establish unequivocally the origin of new oocytes in the spent ovary. Oogonia are found all the year round, but are most common just under the epithelium lining the ovary during the summer. The occurrence of small nests containing three or four of these germ cells suggests that they may have been derived by mitotic division from a single oogonium. This would account for the nests described by Wallace (1903) in Pleuronectes limanda. Mitotic divisions of oogonia have been observed occasionally in ovaries collected during the summer, but their presence is not a regular feature of the ovary after spawning. It is likely

that the new oocytes are produced by mitosis, as described by Hann (1927) and Matthews (1936) but that this occurs very rapidly in the individual ovary and may easily be missed.

It is probable that the primordial germ cells described by Dodds (1911) do give rise to definitive oocytes in the teleost ovary, but the occurrence of large numbers of first meiotic prophases in the plaice after each spawning period is likely to be a feature common to all teleosts and strongly supports the hypothesis that there is a definite renewal of the oocytes in the ovary each year. This indicates a real difference from the situation in mammals where the early stages of meiosis are complete in the immature animal.

The large size of the yolked eggs in contrast with primary oocytes in ovary sections examined during the winter gives the impression that the number of developing eggs is far in excess of yolkless eggs - which would be in agreement with the statement of Cunningham (see p. 76). The results obtained from oocyte counts in the present investigation indicate that this is not so (Fig. 5). Yolked eggs account for about fifty per cent of the total in pre-spawning ovaries. This means that there are already present in the ovary enough reserve eggs (primary oocytes and oogonia) to provide for the next spawning cycle, but succeeding generations must be produced de novo.

Figs. 4 and 5 show that percentages of oogonia and primary oocytes are almost equal in April, but that in the next two months, primary oocytes account for a much larger percentage of the total. Percentages are again about equal during July and August. These facts can be interpreted as follows:- in April, new oogonia are being

produced without much transformation into primary oocytes. During May and June, the production of new oogonia continues but their metamorphosis to primary oocytes is going on at a rate greater than that of their origin de novo. Between July and September, the percentage difference between oogonia and primary oocytes is reduced partly by a reduction in the rate of metamorphosis between them and partly because an increasing percentage of the total oocytes are later stages produced by the onset of vitellogenesis in primary oocytes (Fig. 4). It is not clear to what extent the new primary oocytes formed during the summer continue to develop and form yolk and whether any of the reserve oocytes present during the previous year remain as primary oocytes.

2. Follicular epithelium and "corpus luteum" formation.

The development of the membranes of the ruptured follicle into a functional endocrine gland in mammals is closely associated with viviparity. Cunningham and Smart (1934) on the basis of their own and other morphological studies, conclude that among the lower vertebrate, only viviparous forms exhibit true corpora lutea, but an examination of the literature (p. 23) indicates that this does not apply strictly to teleosts where hypertrophy of the ruptured follicle after ovulation has been described for some viviparous species but not for others. A similar hypertrophy of the follicular epithelium has also been described for oviparous species. The results of the present study indicate that the follicular membranes remaining in the plaice ovary after ovulation do not hypertrophy, but disintegrate and disappear rapidly. Hoar (1955) concluded that "since these organs are comparable developments of the

follicular cells, it seem logical to call them "corpora lutea", but it is felt that, in accordance with the views of Dodd (1955,1960), until the production of progesterone and its association with gestation has been demonstrated the physiological nature of 'corpora lutea' in fish must remain in doubt.

V. Experimental Studies on the Ovary.

A. The effect of hypophysectomy on the ovary of Pleuronectes.

1. The effect of hypophysectomy on oogenesis.

Hypophysectomy, ('H' in the following description) and mock operations ('HC') in which the pituitary was not removed, were performed on groups of fish at various times of the year. The data in Table 7 (pp. 82-84) and Fig. 6 are derived from fish which were deliberately killed at various intervals after hypophysectomy. Information from post-mortem material confirms that from sacrificed fish, but has not been used in assessing the results.

Results

Group 1H (Operated early October). (Fig. 6a). Mortality was high in this group and the maximum survival obtained was a little more than 5 weeks. The ovary of one fish, sacrificed 9 days after hypophysectomy, contained oocytes in the early stages of vitellogenesis. The general condition of the ovary was very similar to that of a control fish sacrificed at the same time and there was no evidence of atresia in either animal.

In both fish several germ cells were in process of transformation from Stage 1 to Stage 2 as evidenced by the presence of leptotene and pachytene figures in their nuclei.

In two animals sacrificed about 3 weeks after pituitary removal, all oocytes beyond Stage 2 were beginning to degenerate. Stage 2 oocytes were normal with a maximum diameter of 120 μ and the early prophase of meiosis was present in several of the Stage 1 eggs. Stage 4 oocytes

Table 7.

The effect of hypophysectomy on the ovary of mature plaice.

No. (1)	Date killed	Survival in days	Meiotic prophase	Stage 3 (2)	Stage 4	Stage 5-7	Corpora atretica
Operated October 1958							
1H3	13.11.58	30	Present	Absent	Absent	Absent	Present
1H4	23.10.58	9	Present	Present	Present	"	Absent
1H9	21.11.58	38	Absent	Absent	Absent	"	Present
1H10	3.11.58	20	Present	"	"	"	"
1H11	1.11.58	18	Present	"	"	"	"
1H2C	22.11.58	39	Absent	Present	Present	Present	"
1H5C	23.10.58	9	Present	"	"	"	Absent
1H15C	3.11.58	20	"	"	"	Absent	"
1H28C	22.11.58	38	Absent	"	"	Present	"
Operated October/November							
3H1	12.1.59	78	Absent	Absent	Absent	Absent	Present
3H3	17.12.58	52	"	Present	"	"	"
3H12	1.12.58	20	"	Absent	"	"	"
3H15	12.1.59	61	"	Present	"	"	"
3H24	1.12.58	17	"	Absent	"	"	"
3H30	29.11.58	15	"	Present	"	"	"
3H28C	1.12.58	17	"	"	Present	Present	Absent
3H32C	18.12.58	31	"	Absent	Absent	"	Present
3H35C	12.1.59	56	"	Present	"	"	"
Operated January							
6H5	11.4.59	79	Present	Present	Absent	Absent	Present
6H12C	11.4.59	78	"	Absent	"	Present	"

Table 7 cont.

Operated February							
7H6	6.10.59	230	Absent	Absent	Absent	Present	Present
7H7	16.9.59	210	"	"	"	Absent	"
7H8	16.3.59	26	"	"	"	"	"
7H13	20.8.59	167	"	"	"	"	"
7H15	26.8.59	182	"	"	"	"	"
7H21	19.10.59	236	"	"	"	"	"
7H24	24.10.59	241	"	"	"	"	"
7H4C	5.7.59	137	"	Present	"	"	"
7H16C	13.3.59	16	"	Absent	"	"	"
7H26C	24.10.59	240	"	Present	Present	Present	Absent
Operated March							
8H1	10.7.59	127	Absent	Absent	Absent	Absent	Present
8H2	2.11.59	238	Present	"	"	"	"
8H17	18.3.59	7	Absent	"	"	Present	Absent
8H23	19.3.59	9	"	"	"	"	Present
8H27	21.3.60	375	Present	"	"	Absent	Absent
8H32	8.6.59	83	Absent	"	"	"	Present
8H35	13.6.59	85	Present	"	"	"	"
8H6C	12.6.59	93	"	Present	"	Present	"
8H7C	17.3.59	7	Absent	Absent	"	"	"
8H10C	17.2.60	351	"	"	Present	"	Absent
8H12C	18.3.59	8	"	"	Absent	"	Present
8H24C	2.11.59	236	Present	Present	Present	Absent	Absent
Operated April							
9H2	18.8.59	113	Absent	Absent	Absent	Absent	Present
9H17	19.8.59	112	Present	"	"	"	"
9H19C	19.8.59	111	Absent	"	"	"	Absent

Table 7 cont.

Operated May							
10H5	6.6.59	12	Present	Absent	Absent	Absent	Present
10H8	6.12.69	194	Absent	"	"	"	"
10H12	8.6.59	12	Present	"	"	"	Absent
10H13	22.8.59	87	Absent	"	"	"	Present
10H2C	6.12.59	195	"	Present	Present	Present	Absent
10H3C	6.2.60	257	"	"	"	"	"
10H4C	22.8.59	89	"	"	Absent	Absent	"
10H6C	8.6.59	13	Present	"	"	"	"

1) "C" after the fish number denotes control.

2) Stages 1 and 2 were present in all fish.

3) Corpora atretica degenerating and small.

up to 200 μ in diameter were present in the ovary of a control fish sacrificed at the same time and there was no evidence of breakdown of these oocytes.

The ovaries of two hypophysectomised fish sacrificed 4 and 5 weeks after operation contained no normal oocytes beyond Stage 2 and several later stage eggs were showing signs of atresia. A few meiotic prophase figures were present in one fish. Vitellogenesis was well advanced in a control animal killed at the same time. Several Stage 5 oocytes were present, having a maximum diameter of 400 μ. The majority of these secondary oocytes were normal, but a very few (about 1%) were in the early stages of follicular atresia. Transformation from oogonia to primary oocytes was absent.

Group 3H (operated October - November). (Fig.6b). Three fish were sacrificed 15 to 20 days after pituitary removal. Vitellogenesis had been arrested in all 3 fish but there was some variation in the degree of atresia present. In 3H30, 25% of the oocytes contained yolk and the maximum egg diameter (Stage 5) was 490 μ. All of these oocytes were beginning to disintegrate. 3% present of the oocytes were in Stage 3 and these, too, showed signs of breakdown and atresia formation. Both oogonia and primary oocytes were normal and the maximum diameter of the Stage 2 oocytes was 130 μ.

Vitellogenesis had also ceased in the ovary of 3H24, but in this fish only 6% of the oocytes had developed beyond Stage 2 and in these, atresia was well advanced. The atretic follicles measured about 110 μ.

In 3H12 less than 2% of the oocytes had passed Stage2, and in contrast to 3H24, these had just started to degenerate. The maximum diameter of these eggs was 140 μ and no advanced corpora atretica were present.

Less than 2% of the eggs in the ovary of a control sacrificed at the same time as these 3 experimental fish showed any signs of breakdown. Many of the developing oocytes were in Stage 3, but a number of normal Stage 4 and 5 oocytes were present. The maximum diameter of the Stage 5 eggs was 380 μ.

Some variation in the degree of atresia was also found in the ovaries of 3 fish sacrificed between 7 and 11 weeks after hypophysectomy. Two stages in oocyte disintegration were found in the ovary of 3H3. In most of the degenerating eggs, invasion by granulosa cells and resorption were complete, but in a few, invasion of the interior of the egg had not begun (Plate 11, Figs. 1 and 2). Follicle diameter was 90 - 110 μ in the former and 150 μ in the latter. About 1% of the oocytes had entered Stage 3 and these showed no evidence of resorption. The ring of vacuoles which is characteristic of this yolk precursor stage were larger than usual and irregularly distributed round the periphery of the egg, suggesting that development was abnormal in these oocytes.

In 3H15, 30% of the oocytes were in process of resorption. A few of these were fully-formed corpora atretica, measuring about 100 μ, but in the majority resorption of the oocyte was not complete and, in these, the follicles measured about 550 μ (Plate 12, Fig. 1). A few

Stage 3 and 4 oocytes were present in which no abnormalities were evident and, in two Stage 5 eggs resorption had not begun, although there was some hypertrophy of the cells of the follicular epithelium.

Vitellogenesis had been completely arrested in the ovary of 3H1 and normal oocytes beyond Stage 2 were absent. Typical advanced corpora atretica were present measuring about 100 - 120 μ in diameter (Plate 12, Fig. 2; 13, Fig. 1).

Two control fish were killed after 4 and 8 weeks. In one, all the developing oocytes were normal and had reached Stage 5 with a maximum diameter of 700 μ. In the second, however, a few Stage 3 oocytes were still present and more than half of the Stage 5 oocytes were showing the signs of breakdown typical of early atresia. No small atretic follicles were present and the Stage 5 oocytes measured about 700 μ.

Group 6H (operated January). (Fig. 6b). Mortality was very high in this group and information is available from only 2 fish which were killed 11 weeks after operation. Vitellogenesis had ceased in the ovary of 6H5 and atresia was well-advanced in all the developing oocytes (Plate 13, Fig. 2; 14, Fig. 1). A few oocytes, however, had entered Stage 3 and no abnormalities were seen in these eggs. Several cells in meiotic leptotene or pachytene were present and a single oogonium in mitotic metaphase was seen.

Meiotic prophase figures were also seen in the control. Oogenesis was proceeding normally in this fish and the Stage 5 oocytes has a maximum diameter of 870 μ. A few corpora atretica were present,

but these accounted for less than 1% of the eggs and there was no evidence of widespread breakdown. No Stage 3 oocytes were present.

Group 7H (operated February). (Fig. 6c). In the normal cycle of oogenesis, one would expect the secondary oocytes to have reached Stage 5 or 6. This was the case in the ovary of a control animal killed 23 days after operation. The majority of the oocytes were well developed and normal, but a few well-developed corpora atretica were present. The absence of yolk globules in these and their advanced state makes it certain that they were present before the animal was operated. In the ovary of 7HB, a hypophysectomised fish sacrificed 26 days after operation, a few corpora atretica were present similar to those described for the control. All the large developing oocytes were showing signs of breakdown and conversion to corpora atretica.

Six fish were killed between August and October, 24 - 34 weeks after hypophysectomy. In all of these only oogonia and primary oocytes were present, the latter measuring 120 - 140 μ in diameter. Discrete corpora atretica were either entirely absent or present as pieces of tissue about 100 μ in diameter and in many of these, the cell nuclei were pycnotic and beginning to disintegrate (Plate 14, Fig. 2). No meiotic prophase figures were seen.

Meiotic prophase was also absent from the 2 controls sacrificed 19 and 34 weeks after operation. In the former, however, vitellogenesis had begun and more than 4% of the eggs were in Stage 3. Vitellogenesis had progressed much further in the second control and several Stage 5 oocytes with a maximum diameter of 340 μ were present.

Group 8H (operated March). (Fig. 6d). The information from 7 hypophysectomised and 5 control fish is similar to that from group 7H. All secondary oocytes were converted to corpora atretica and no new development of eggs was observed even a year after hypophysectomy (Plate 15, Fig. 1). In the 2 long-term control fish, vitellogenesis, although well-advanced, had not proceeded as far as would be expected from comparison with the normal cycle. No Stage 5 oocytes were present in 8H24C killed in November and the maximum diameter of Stage 5 eggs was only 620 μ in 8H10C killed in February (Plate 15, Fig. 2).

Group 9H (operated April). (Fig. 6e). Mortality was high in this group which was composed of fish in which spawning was in progress, or had been completed, at the time of operation. The ovary of 9H2 was flaccid on palpation when the animal was hypophysectomised, indicating that the ripe eggs had been shed. Oogonia and primary oocytes were present 16 weeks later but there was no new development of eggs. Several ripe oocytes in various stages of corpora atretica formation were present in the lumen of the ovary, but no corpora atretica were seen in the lamellae. Ripe eggs were escaping from the ovary of 9H17 when the pituitary was removed. A few corpora atretica were present in the lamellae at autopay 16 weeks later and large numbers of ripe eggs were found in the lumen of the ovary. These eggs were dull and opaque and were found to be deteriorating. No new vitellogenesis had begun but several meiotic prophase figures were seen.

Corpora atretica and degenerating ripe eggs were absent from a control fish killed at the same time, but vitellogenesis had not

begun, although one would have expected to find some Stage 3 oocytes at this time.

Group 10H (operated May). (Fig. 6e). High mortality rates were again encountered in this group which was composed entirely of spent fish. The ovaries of 2 fish, killed 12 days after hypophysectomy were indistinguishable from those of normal spent fish although no Stage 3 oocytes were present. The ovary of a control killed at the same time contained about 5% of Stage 3 oocytes measuring 120 - 140 μ across.

Both oogonia and primary oocytes were present in the ovary of 10H13, killed 12 weeks after operation. Several of these primary (Stage 2) oocytes had a granular appearance and measured up to 200 μ i.e. larger than those from normal ovaries. Yolk deposition had begun in about 3% of the eggs, but all of these were showing signs of deterioration.

Stage 2 oocytes were normal in 10H8, killed 28 weeks after pituitary removal, but several small corpora atretica measuring about 100 μ were present. These were degenerating and must have been formed before spawning as the fish was spent at the time of operation.

Vitellogenesis was in progress in three controls killed between 12 and 37 weeks after operation, but in all 3 the egg size was less than would have been expected by comparison with the normal cycle.

2) <u>The effect of hypophysectomy on oviposition.</u>

From the results of group 8H, it is clear that pituitary removal shortly before the natural spawning season results in atresia of all the ripening eggs. These degenerate <u>in situ</u> and ovulation and oviposition consequently do not occur. It is not clear, however,

whether oviposition of ripe eggs (i.e. those which have been ovulated into the lumen of the ovary) can continue when the pituitary is removed from ripe fish. The presence of large numbers of degenerating eggs in the lumen of the ovary of 9H17 (see p. 89) suggests that pituitary removal may inhibit ovulation. Accordingly a group of fish was hypophysectomised in March at the height of the spawning season and examined at intervals in order to see whether spawning had taken place. The results of this experiment, detailed in Table 8 (pp. 92-93) indicate that spawning did not occur in the hypophysectomised animals.

3) The formation of corpora atretica after hypophysectomy.

Hypophysectomy results in atresia of all oocytes in which yolk deposition has begun. The pattern of breakdown is the same in all oocytes examined and closely resembles that found in atresia in normal fish. The onset of resorption is marked by the folding and irregular appearance of the nuclear membrane and the outer surface of the zona radiata becomes pitted. At this stage there is little change in either follicular epithelium or theca. The nucleus breaks down and becomes granular and dissolution of the zona radiata by granulosa cells continues. The granulosa changes from a squamous to a cuboidal or columnar epithelium and loses its regular appearance. The cells of the granulosa appear to be responsible for the breakdown of the zona radiata as they are often seen deeply embedded in it. The zona radiata finally ruptures and granulosa cells invade the interior of the egg, breaking down and ingesting the yolk globules. Fragments of zona

Table 8

The effect of hypophysectomy on oviposition.

No.	Date killed	Survival in days	Condition at operation	Condition at death
1HS1	10.7.60	113 days	Ovary swollen no ripe eggs expressed.	Still very swollen. No eggs in lumen. Many atretic oocytes in lamellae.
1HS2	11.4.60	21 days	" "	Still very swollen. No Stage VII eggs. Early atresia in Stage V and VI oocytes.
1HS3	1.4.60	13 days	Ovary swollen. No ripe eggs expressed.	Still swollen. No ripe eggs. Stage V and VI oocytes normal.
1HS4	6.5.60	48 days	Ovary swollen. Ripe eggs expressed with slight pressure.	Still swollen. Degenerating ripe eggs in lumen. Atretic Stage V and VI eggs in lamellae.
1HS5	27.3.60	8 days	Ovary swollen. No ripe eggs expressed.	Still swollen. Stage V and VI oocytes normal. No Stage VII oocytes.
1HS6	11.8.60	145 days	Ovary swollen. No ripe eggs expressed.	Still slightly swollen. Several ripe eggs in advanced stages of deterioration in lumen. Many Stage V and VI oocytes atretic.
1HS7	22.5.60	64 days	Partly spent. Ripe eggs extruded.	Still slightly swollen. Ripe eggs degenerating in lumen. Few atretic follicles in lamellae.
1HS8	8.6.60	81 days	Gonad swollen. No ripe eggs.	Still very swollen. No ripe eggs. Stage V and VI oocytes atretic
1HS9	29.4.60	41 days	" "	" "

Table 8 cont.

1HS10	2.6.60	75 days	Gonad swollen. Ripe eggs expressed	Ovary still swollen. Several ripe eggs degenerating in lumen. Large numbers of corpora atretica in lamellae.
1HS11	25.4.60	37 days	Gonad swollen. No ripe eggs.	Gonad still swollen. Atresia of Stage V and VI eggs in progress.
1HSC1	11.4.60	23 days	Gonad swollen. No ripe eggs expressed.	Still swollen. No ripe eggs. Stage V and VI eggs normal.
1HSC2	25.5.60	67 days	" "	Spent. Ovary flaccid, but still a few ripe eggs in lumen. Degenerating.
1HSC3	20.4.60	32 days	Gonad swollen. No ripe eggs expressed.	Still swollen. Eggs normal, several ripe eggs in lumen.
1HSC4	18.8.60	152 days	Gonad swollen. No ripe eggs expressed.	Ovary volume gradually reduced. Flaccid by end of April. No ripe eggs at death.
1HSC5	2.6.60	75 days	Ovary only slightly swollen, ripe eggs extruded. Partly spent.	Ovary flaccid two weeks later. No ripe eggs found at death.

radiata may remain for several weeks but the resorption of the oocytes is finally completed and the resulting corpus atreticum is composed of a mass of rounded cells derived from the granulosa and is surrounded by the theca interna which remains relatively unchanged. Some degree of vascularisation of the oocytes is found during resorption and phagocytosis may play some part in the breakdown of the egg.

Well developed corpora atretica can still be found in the ovary 6 months or more after hypophysectomy, but they become smaller and eventually are removed either by disintegration or migration of the cells into the surrounding tissue. No trace of corpora atretica was found in the ovary of one fish a year after pituitary removal(Table 7, pp. 82-84).

4) <u>The effect of hypophysectomy on oogonia and primary oocytes</u>.

It is clear that hypophysectomy in plaice results in the breakdown of all secondary oocytes and their conversion into corpora atretica. Both oogonia and primary oocytes are unaffected by pituitary removal and are present in the ovary even a year after hypophysectomy (Plate 15, Fig. 1). The results obtained from group 9H and 10H indicate that, although primary oocytes are themselves unaffected by hormone withdrawal, their development into secondary oocytes is prevented. As already stated, the transformation of oogonia to primary oocytes is marked by leptotene and pachytene figures in the cell nuclei and the lampbrush chromosomes characteristic of diplotene are found in the nuclei of all oocytes between Stages 2 and 6.

In normal fish, meiotic figures marking the production of new Stage 2 oocytes from oogonia are found from March to September (Fig. 3). Such meiotic figures have been recorded in the ovaries of several hypophysectomised fish and are still present more than one year after pituitary ablation (Table 7, pp. 82 -84). In order to determine whether hypophysectomy had any effect on the relative proportions of oocytes in the ovary, oocyte counts were made on all experimental animals. The number of oocytes of different stages expressed as a percentage of the total oocytes is given in Fig.6a-e.

5) The effect of hypophysectomy on immature fish.

Only oogonia and primary oocytes were present in the ovaries of 3 immature fish hypophysectomised in October and examined 3 and 11 weeks later. A few normal Stage 3 oocytes and several Stage 4 eggs were present in the ovary of a fourth fish killed 3 weeks after pituitary removal (Table 9, p.96). Nuclear degeneration and hypertrophy of the follicular epithelium were evident in all of the Stage 4 eggs, but their transformation into corpora atretica had not progressed very far. The ovary of this fish was very small at operation and the animal was thought to be immature.

The ovaries of four controls killed at similar intervals after mock operation contained only oogonia and primary oocytes and like the 3 hypophysectomised fish mentioned above, were indistinguishable from those of normal immature animals (Plate 16, Fig. 1 and 2). The relative proportions of oogonia and primary oocytes were the same in operated and control fish. (Fig. 7).

Table 9.

The effect of hypophysectomy on the oocytes of immature plaice.

No. (1)	Date killed	Survival in days (2)	Meiotic prophase	Stage 3 (3)	Stage 4	Corpora atretica
2H1	15.11.58	19	Absent	Present	Absent	Present
2H8	12.1.59	77	Present	Absent	"	Absent
2H13	20.11.58	21	Absent	"	"	"
2H14	12.1.59	75	"	"	"	"
2H23C	12.1.59	72	"	"	"	"
2H24C	12.1.59	72	"	"	"	"
2H31C	23.11.58	21	"	"	"	"
2H36C	12.1.59	72	"	"	"	"

1. "c" after the fish number indicates control.
2. Operated October/November.
3. Stage 1 and 2 oocytes in normal proportions were present in all fish.

6) The effect of hypophysectomy on ovary and body weights.

The ovary and body weights, body weight gain and ovary body weight ratio are detailed in Table 10, (pp. 98-99). In assessing the effect of pituitary removal on ovary and body weight, one must take into account the normal large variation in ovary weight throughout the year. Several fish autopsied at varying intervals after operation just before the spawning season, show weight losses of more than 100 gm. These losses are largely due to the expulsion or resorption of the eggs. In general, however, it can be said that hypophysectomised fish only maintain their body weight while control fish continue to increase their body weight.

Table 10

Gravimetric data for hypophysectomised and control fish.

No.	Op./Cont.	Weight at op.	Wt. at death	Gain or loss	Gonad wt.	% B. wt.
1H3	Hypo.	385.	390.	+.5	7.2	1.84
1H4	"	285.	260.	-15.	5.3	2.03
1H9	"	995.	960.	-35.	24.7	2.51
1H10	"	585.	580.	-5.	12.1	2.08
1H11	"	495.	450.	-45.	9.4	2.09
1HC2	Control	660.	660.	—	19.0	2.88
1H5C	"	314.	326.	+12.	6.1	1.87
1H15C	"	310.	320.	+10.	7.5	2.33
1H28C	"	535.	570.	+35.	12.9	2.28
3H1	Hypo.	510.	520.	+10.	10.6	2.03
3H3	"	415.	425.	+10.	4.0	0.94
3H12	"	340.	335.	-.5	6.1	1.81
3H15	"	565.	580.	+15.	35.0	6.03
3H24	"	540.	540.	—	6.5	1.12
3H30	"	465.	420.	-45.	16.8	4.00
3H28C	Control	321.	335.	+14.	8.0	2.38
3H32C	"	605.	560.	-45.	28.9	5.16
3H35C	"	465.	460.	-.5	17.7	3.87
6H5	Hypo.	700.	700.	—	25.0	3.57
6H12C	Control	421.	443.	+22.	81.6	18.37
7H6	Hypo.	765.	665.	-100.	8.18	1.23
7H7	"	335.	310.	-25.	1.25	0.43
7H8	"	420.	440.	+20.	64.50	14.65
7H13	"	735.	640.	-95.	9.30	1.45
7H15	"	340.	280.	-60.	4.90	1.75
7H21	"	155.	161.	+6.	1.94	1.21
7H24	"	241.	200.	-41.	0.85	0.42

Table 10 cont.

7H4C	Control	860.	695.	-165.	21.10	2.02
7H16C	"	350.	355.	+5.	55.10	16.45
7H26C	"	455.	413.	-42.	13.90	3.36
8H1	Hypo.	395.	350.	-45.	.500	1.42
8H2	"	425.	460.	+35.	8.02	1.74
8H17	"	420.	400.	-20.	30.74	7.68
8H23	"	555.	537	-18.	140.31	26.10
8H27	"	405.	416.	+11.	4.90	1.17
8H32	"	460.	460.	- -	101.00	21.95
8H35	"	230.	180.	-50.	17.00	9.55
8H6C	Control	725.	600.	-125.	42.50	7.08
8H7C	"	265.	270.	+5.	39.10	14.48
8H10C	"	615.	638.	+23.	29.50	6.19
8H12C	"	470.	474.	+5.	68.43	14.43
8H24C	"	490.	420.	-70.	7.27	1.73
9H2	Hypo.	330.	280.	-50.	7.50	2.67
9H17	"	415.	300.	-115.	15.50	5.16
9H19C	Control	400.	405.	+5.	3.90	0.96
10H5	Hypo.	240.	220.	-20.	7.61	3.45
10H8	"	700.	680.	-20.	.941	1.38
10H12	"	265.	260.	-5.	4.37	1.68
10H13	"	320.	340.	+20.	1.30	0.38
10H2C	Control	355.	428.	+83.	24.6	5.74
10H3C	"	330.	494.	+164.	10.75	2.17
10H4C	"	435.	490.	+55.	6.50	1.32
10H6C	"	360.	360.	- -	3.60	1.00

B. The effect of administration of gonadotrophic preparations on the ovaries of hypophysectomised fish.

1. Introduction.

The effects of injection or implantation of homoplastic or heteroplastic fish pituitary material has been studied in a large number of teleost species. Much of the work has been done by people interested in the commercial aspects of the problem and the design of many of the experiments leaves much to be desired. In many of the experiments the fish have been close to the breeding season or actually in spawning condition (Pickford and Ats, 1957 for references) and the result of administration of pituitary material has been to induce maturation of the eggs and ovulation. Cardoso (1934) and Azevedo and Canale (1938), however, obtained increases in the ovary weight of immature fish and of animals not in the breeding season.

In none of these studies, has the recipient been hypophysectomised before gonadotrophin administration and consequently the part played by secretion of endogenous hormone cannot be estimated. The most detailed study using hypophysectomised animals is that of Vivien (1941), using female Gobius paganellus at different stages in their life cycle.

2. The effect of injection of plaice pituitary material and other gonadotrophic preparations into hypophysectomised plaice.

A group of fish was hypophysectomised in February and kept in an aquarium until the end of August. Four of these fish then received a series of injections of filtered sea water, two were given injections of plaice pituitary material collected in June and two received pituitary material

collected between October and December. The acetone-dried pituitary glands were macerated in filtered sea water in a tissue mortar and injected as a suspension. Three unoperated fish which had been kept under the same conditions since February acted as a second control group (Table 11, p. 102).

The fish were injected every second day. All fish, except 1RH2, received a total of 20 injections and were killed 6 weeks after the first injection. Fish 1RH2 was in poor condition and was killed after 2 weeks having been given a total of 5 injections. The results of histological examination of the ovaries are expressed Table 12, p. 103. Oocyte counts were made and these are given in Fig. 8 in which the number of oocytes of different stages is expressed as a percentage of the total eggs counted. The areas of tissue in which the counts were made are roughly equivalent, but no attempt was made to express them in numbers per unit area.

A second series of experiments was done in which a group of fish was hypophysectomised at the end of January shortly before the normal breeding season. Plaice pituitary material, CG (Pregnyl, Organon) and PMS (Gestyl, Organon) were used in the injections which were begun 3 days after operation. At autopsy the ovary was opened, and examined for ripe oocytes which are normally clearly visible as large transparent eggs among the opaque ripening ones. They were estimated as a percentage of the total large oocytes and the results, together with details of injections, all of which were given in 0.1 ml. filtered sea water, are expressed in Table 13 (pp. 104 - 105).

Table 11.

Treatment of hypophysectomised plaice with plaice pituitary material.

Group	Number of animals	Pre-treatment	Injection	Total dose	No. of injections
1HC	4	Hypophysectomised in February before spawning season	0.1 filtered sea water every second day	- -	20
1C	3	No operation	No injection	- -	—
1HR	2	Hypophysectomised in February before spawning season	2 acetone-dried plaice pituitary glands every second day. Glands collected in June	1HR1 10 glands approximately 5 mg. total.	1HR1 5
				1HR2 40 glands approximately 20 mg. total.	1HR2 20
1HR	2	As above	As above. Glands collected between October and December	40 glands approximately 20 mg. total	20

"HC" = hypophysectomised
"C" = intact
both controls in this experiment

HR = hypophysectomised and injected with plaice glands

Table 12.

The effect of injection of plaice pituitary material into hypophysectomised plaice.

No.	Duration of experiment	Treatment	Meiosis	Stage 3	Stage 4	Maximum egg diameter	% of Body weight
1HC1	6 weeks	Saline	Absent	Absent	Absent	120 µ Stage 2	1.23
1HC2	"	"	"	"	"	"	0.36
1HC3	"	"	"	"	"	"	0.42
1HC4	"	"	"	"	"	"	0.52
1HR1	11 days	Summer pituitary	Present	Present	Absent	140 µ Stage 3	1.16
1HR2	6 weeks	"	Absent	"	Present	180 µ Stage 4	1.20
1HR3	6 weeks	Winter pituitary	Present	"	"	270 µ Stage 4	1.75
1HR4	6 weeks	"	"	"	"	210 µ Stage 4	1.38
1C1	- -	- -	Absent	"	"	180 µ Stage 4	2.04
1C2	- -	- -	"	"	"	220 µ Stage 4	2.18
1C3	- -	- -	Present	"	"	165 µ Stage 4	1.21

Table 13.

Effect of injection of Pregnyl, Gestyl and plaice pituitary material into fish hypophysectomised near the spawning season.

No.	Dose	No. of injections	Total dose	Duration of experiment (1)	Condition of ovary at autopsy
PH6	150u.Pregnyl	10	1500 u.	33 days	No eggs spawned, but about 25% of yolked eggs ripe.
PH17	"	3	450 u.	10 days	Same
PH20	"	8	1200 u.	27 days	Ripe eggs shed. Almost all yolked eggs ripe.
PH22	"	6	900 u.	18 days	No eggs spawned, but about 40% of yolked eggs ripe.
GH7	100u.Gestyl	3	300 u.	11 days	No ripe eggs.
GH23	"	5	500 u.	19 days	Same
GH15	"	10	1000 u.	32 days	Same
GH16	"	2	200 u.	7 days	Same
PGH4	150u.Pregnyl 100u.Gestyl	2	300+200 u.	9 days	No ripe eggs.
PGH5	"	10	1500+1000 u.	33 days	Ripe eggs shed. Fully ripe.
PGH8	"	2	300+200 u.	9 days	No eggs shed, but a few ripe eggs present.
PGH19	"	7	1050+700 u.	24 days	No eggs shed. About 25% ripe eggs.

Table 13 cont.

PIH10	2 pituitary glands from ripe plaice	11	22 glands or about 11.0 mg.	33 days	Eggs shed after fifth injection. Ovary almost empty at end of experiment.
PIH14	"	"	"	"	Eggs shed after fourth injection. Ovary partly spent at end of experiment.
PIH18	"	4	8 glands or about 4 mg.	12 days	No eggs shed. About 25% of yolked eggs ripe.
PIH12	"	2	4 glands or about 2 mg.	6 days	No eggs shed. A few ripe eggs present.
CH1	Filtered sea water	1	--	4 days	No ripe eggs.
CH2	"	2	--	6 days	Same
CH3	"	6	--	19 days	Same
C1	--	--	--	Killed at start of experiment.	No ripe eggs.
C2	--	--	--	"	Same
C3	--	--	--	33 days	Same
C4	--	--	--	"	No eggs shed, but about 25% of yolked eggs ripe.
C5	--	--	--	"	No ripe eggs.

Note (1). Operations were carried out at the end of January, 1959 and the injections commenced four days later. Experiment ended on the 2nd March, 1959.

C. Discussion.

Oogenesis in the normal plaice is a complex process and can be regarded as a dynamic system having 4 major phases. The spent ovary is reconstituted in part by the development of primary oocytes which are already present. The first phase in oogenesis, however, is the production of new oogonia and this is followed by the transformation of oogonia to primary oocytes. Cytoplasmic growth and vitellogenesis of secondary oocytes form the third phase and oogenesis is completed by maturation which involves meiotic division and the production of polar bodies.

The most striking effect of hypophysectomy is its ability to block vitellogenesis. Atresia of developing oocytes followed pituitary removal in the ovaries of all fish operated between October and March and first became noticeable 15 to 20 days after operation. The results also indicate that the degree of atresia reached within a particular time varies considerably in the individual oocytes. In groups 1H and 3H, which were operated during the early stages of vitellogenesis, atresia was well advanced 30 days after hypophysectomy. In groups 7H and 8H, on the other hand, vitellogenesis was well advanced at operation and there was little or no invasion of the oocyte after a similar period of time. By comparison with the normal cycle the oocyte diameter in the former group would be about 200 - 300 μ and 800 - 900 μ in the latter and this size difference may account for the slowness of atresia in the latter group. In the large eggs, too, the zona radiata may be as much as 50 μ in thickness compared with 5 to 10 μ in the small

eggs and this acts as a physical barrier which must be "dissolved" by the granulosa cells before invasion and resorption can begin.

The concept of Vivien (1939,1941) that there is a critical oocyte size, is true also of mammals and amphibia. In the rat (Smith,1930) and the salamander (Burns,1932, Burns and Buyse,1932) development of small oocytes can proceed to this critical size in the absence of the pituitary, but eggs that have reached this size, are transformed into corpora atretica. In Vivien's study, however, although development was blocked at the critical size, atresia did not occur and the apparently normal eggs remained in this state of arrested development for long periods.

The results of the present study, in which development was arrested when the primary oocytes attained a diameter of about 120 µ, are in agreement with those of Vivien. In 3 hypophysectomised fish, however, some development, characterised by the peripheral vacuolation and deposition of yolk normally seen in Stage 3-4 oocytes, was present when the fish were killed in autumn. Two of these 3 (7H13 and 7H24) were operated in February and the other (10H13) in May, after the spawning season. In all 3 fish these developing oocytes were showing signs of breakdown. Serial sections of the pituitary region did not reveal any pituitary tissue and it must therefore be assumed that some degree of development beyond the critical size is possible although in the absence of the pituitary it rapidly results in atresia.

The effect of pituitary removal on the production of new oogonia and their transformation into primary oocytes is more difficult

to assess. Meiotic porphase figures have been found in many operated fish (Table 7, pp. 82-84). No transformations were found in group 3H, but these fish were killed during December and January and meiotic prophase figures are absent in normal ovaries at this time of year. It seems reasonable to conclude that early meiotic prophase activity can continue in the absence of the pituitary gland and that the production of new primary oocytes is not inhibited even a year after hypophysectomy.

The effect of pituitary removal on the pre-vitellogenesis stages of oogenesis depends on the time at which the operation was performed (Fig. 6a-e). In groups 1H and 3H, where vitellogenesis was just beginning, the percentage difference between oogonia and primary oocytes is very great. Here pituitary ablation blocks yolk deposition and the proportion of primary oocytes becomes abnormally large since their numbers are still being added to by the transformation of oogonia. In group 6H, vitellogenesis was complete at operation and the proportions of oogonia and primary oocytes have been unaffected by hypophysectomy, being similar to those of the control fish and normal fish at this time of year.

In those fish in groups 7H and 8H which survived hypophysectomy for more than 6 months, the percentages of the two early stages are again roughly equal, but the absolute number of oocytes in the ovary is much less than that of normal fish at the end of the period of reconstruction of the ovary (compare Plate17, Figs. 1 and 2). Although comparisons are difficult because of the great disparity in the area occupied by the cells counted, it is possible that the number of oocytes present in these

operated fish is no greater than the number of reserve oocytes which would have been expected at the time of operation and that no new production of oocytes has taken place (Fig. 6c and d).

In groups 9H and 10H, operated during the period when the ovary is engaged on the production of new oogonia, the absolute number of oocytes is similar to that of normal fish at the end of the period of reconstitution (compare Plate 17, Fig. 1 and Plate 19, Fig. 1). In these groups the large percentage of the total formed by primary oocytes is again due to the blockage of their further development.

The results discussed above suggest that pituitary removal before the period of reconstitution of the ovary interferes with this process and that it is the production of new oogonia which is inhibited since it has been demonstrated that primary oocytes can be produced from oogonia in the absence of the pituitary.

Oogonia do not disappear from the ovary and it may be that reduced activity of the ovary under the circumstances is a consequence of the reduced metabolic activity of the body.

The results obtained on the effect of hypophysectomy on the ovaries of immature fish confirm that the presence of the pituitary gland is not essential for the maintenance of oogonia and primary oocytes. Since the age at which plaice first mature varies (Bagenal,1958), the question as to whether these immature fish would have entered puberty in the current season cannot be answered definitely. Vitellogenesis was not apparent in any of the control fish and it is probable that they would have remained immature for at least one more

year. In one fish, however, yolk had been laid down in a few eggs and these were in process of resorption. It is probable, therefore, that the findings of Vivien (1941) are true for plaice and that hypophysectomy prevents the subsequent maturation of the gonad.

The present study indicates that pituitary removal before the onset of natural spawning results in the degeneration of the ripening oocytes and their retention in the ovigerous lamellae. The question whether oviposition is under the control of pituitary hormone is more difficult to answer. The results given in Table 8 (p. 92-93) suggest that oviposition does depend on the presence of the pituitary, but such an interpretation must be viewed with caution as a number of other factors may be involved. Firstly, the precise degree of ripeness in both the hypophysectomised and mock operated fish could not be established. The normal spawning season in plaice extends over several weeks and some fish ripen later than others. Also in any one individual, there is a gradual increase in the number of ripe eggs lying free in the ovary and the eggs are not all spawned at once. Consequently, it is possible that those fish in which ripe eggs could not be expressed may have possessed few or no ripe eggs and that hypophysectomy simply resulted in atresia. Secondly, the amount of handling which the fish received during feeding may have adversely affected spawning and the shock and damage consequent on pituitary removal, which is always greater in hypophysectomised than in mock operated fish, may have contributed to the inhibition of oviposition.

The work of Foster et al (1937) on the hypophysectomised rabbit indicates that the capacity of large follicles to develop in response to administered gonadotrophin falls off as the interval between hypophysectomy and injection increases. From the results of the present experiments it is evident that the primary oocytes of hypophysectomised plaice retain their capacity to respond to injected gonadotrophin for at least 6 months after operation. Further, the egg diameter in those fish which received winter pituitary material is greater than that of the fish which received summer glands. (Compare Plate 18, Fig. 2 and Plate 19, Fig. 1). The percentage of the eggs in which vitellogenesis has started is also greater in the former group. Since the ovaries were in the same state of arrested development at the start of the experiment and since both groups received equal amounts of pituitary material, it is evident that the pituitary material collected during the period of winter growth is more potent than that collected during the summer.

The administration to ripening fish of pituitary material obtained from ripe fish resulted in precocious maturation and ovulation of eggs. The atresia and inhibition of ovulation normally consequent on hypophysectomy were prevented, but it is not known whether the tailing off of response as found by Foster et al (loc. cit.) also occurs. The fact that injection of commercial CG but not commercial PMS will also produce precocious maturation suggests that the mechanism of ovulation is a response to luteinizing hormone rather than follicle stimulating hormone. These results given in Table 13 (p. 104-105) are highly suggestive, but it should be borne in mind that

the animals were close to the normal spawning season and that other stimuli, such as release of endogenous hormone at hypophysectomy, may have been responsible for the maturation of the eggs.

VI. The Plaice Testis.

A. Anatomy.

1. Testis.

No detailed description has been made of the testis and its annual cycle in the plaice and the following account is based on an investigation extending over 16 months. The testis of the adult plaice is a paired elongated body situated ventral to the kidney with its long axis lying dorso-ventrally against the posterior wall of the body cavity. It is attached to the body wall by mesenteries and is indistinctly lobed, being often divided dorso-ventrally into 3 regions by deep grooves. The mesenteries carry the genital blood vessels which enter the testis near the testis duct and subdivide as they pass ventrally. The testis is much smaller than the ovary and projects forward only a little way into the body cavity, though even in the male, the alimentary tract is slightly displaced for a few months during the winter when the testis reach its maximum size. Unlike the condition in the female, there is no posterior extension of the gonad into the body musculature.

True seminiferous tubules are not found in teleosts and in common with other fishes, the plaice testis is composed of large numbers of ill-defined lobules, which are separated by a thin connective tissue stroma containing elastic fibres. The lobules radiate out from the main collecting ducts which are situated on the anterior side of the testis. These collecting ducts run longitudinally in the testis and unite to form the testis duct which arises near the ventral end of

the testis. Elongated bodies of adipose tissue are located in the testis duct and the peripheral connective tissue of the testis. The testis duct itself is broad and flattened and is divided by a septum into a number of longitudinal canals. The two ducts unite before their junction with the ureters and open into a urinogenital sinus. Sperm are shed through a urinogenital papilla.

Spermatogonia comprise the bulk of the testis during the summer and early autumn, but they are present singly or in small groups in the connective tissue walls of the lobules at all other stages in the cycle. The spermatogonia are relatively large cells (11 μ) with a nucleus of about 7 μ in diameter. There is a distinct nuclear membrane in the resting stage and the nucleus contains a single large nucleolus from which fine threads of chromatin radiate to the bulk of the chromatin round the periphery (Plate 19, Fig. 2). The spermatogonia, after passing through several mitotic divisions, become reduced in size to form primary spermatocytes. These cells are about 6 μ in diameter and in their earlier stages are morphologically similar to spermatogonia. They soon lose their nucleolus and are most commonly seen in the prophase of the first meiotic division, the various stages of which cannot easily be distinguished. The first meiotic division takes place, giving rise to secondary spermatocytes (Plate 20, Fig. 1). These cells are about 4 μ in diameter and the chromatin of the nucleus is denser than that of the primary spermatocytes. They are of short duration and the immediate result of the second meiotic division is the formation of spermatids which have a diameter of about 2.5 μ. (Plate 20,Fig.1).

The chromatin of the spermatid nucleus gathers at one side to form a cup-shaped mass which contracts to produce a solid sperm head with a diameter of 1 μ. The spermatozoa do not have the uniform arrangement in the lobule which is so typical of the seminiferous tubules of mammals. The cells do not appear to have any orientation and appear as a tangled mass completely filling the lobules (Plate 20, Fig. 2).

2. Interstitial tissue.

The connective tissue of the lobule wall varies in thickness during the year, being only one cell thick during the period when the testis is swollen with sperm. After spermiation the lobules decrease and the consequent thickening of the connective tissue is thought to be due to contraction of the elastic fibres. In addition to the elongated connective tissue cells, small round or oval cells are visible in the walls of the lobules (Plate 21, Fig. 1). These are also common in the interstices between the lobules and may be homologous with the mammalian interstitial tissue (Plate 21, Fig. 2). The number of these cells appears to be fairly constant throughout the year, although it is difficult to compare testes from different times of the year due to the great difference in size. It can certainly be said that there is no hypertrophy of interstitial cells during the period immediately before spawning.

Sections from plaice testes collected between March and October were treated with Sudan Black, but in none of the material so treated was a positive result obtained. Lipid material was found in the sperm and in the adipose tissue in the testis and testis duct.

The material tested was from spawning, spent and maturing testes, but owing to the shortage of material, no tests were done on testes from the period immediately before spawning. Lofts and Marshall (1957) found that lipid-positive material was present in the lobule-boundary cells of Esox lucius between October and April i.e. in the pre-spawning period only. The negative results found in plaice are largely in agreement with this but the demonstration of secretory interstitial tissue in plaice must await a more complete investigation.

B. Cyclical changes in the testis.

1. Gravimetric variation.

The pattern of weight changes found in the testis throughout the year, although less pronounced, is similar to that of the ovary and need not be described in detail. These seasonal changes, indicated in Fig. 9, are based on data from 85 animals collected over a period of 16 months from September 1958 to December 1959 (see Table 14, p. 119).

2. Histological variation.

The small nests of dormant spermatogonia (Plate 20, Fig. 2) found in the testis appear to be solely responsible for the reconstitution of the testis following spermiation. The lobules have been depleted of their sperm and become considerably reduced in size. Mitotic divisions of spermatogonia in the lobule walls are first seen in the testis in June and continue during the summer, forming cysts of spermatogonia which rapidly fill the lumen of the lobules (Plate 19, Fig. 2). The maximum production of spermatogonia occurs in early autumn as evidenced by the increased number of mitotic figures in the lobules and increase in the size of the latter. In view of the considerable increase in the diameter of the lobules, several generations of spermatogonia must be produced, but the precise number has not been estimated.

The transformation of spermatogonia to primary spermatocytes takes place fairly suddenly in October. Meiotic metaphase figures are first seen at this time and secondary spermatocytes and spermatids become increasingly numerous. Spermatozoa are first seen in the testis in December and from then till February the later phases of

Table 14.

Variation in testis weight throughout the year.

Date of collection	Average weight of fish grm. (1)	Average weight of testis grm.	%
12.5.58	151 (2)	0.4	0.25
5.6.58	364 (3)	0.47	0.12
9.7.58	416 (3)	1.0	0.24
5.9.58	563 (2)	1.5	0.23
6.10.58	492 (3)	7.6	1.64
4.11.58	436 (4)	5.0	1.32
1.12.58	461 (4)	25.25	5.37
6.1.59	490 (4)	23.0	4.39
10.2.59	436 (4)	13.0	3.07
2.3.59	369 (4)	10.25	2.79
6.4.59	332 (4)	9.25	2.83
30.6.59	385 (12)	1.21	0.31
10.8.59	404 (8)	1.2	0.29
15.9.59	301 (10)	0.60	0.20
19.10.59	335 (10)	3.00	0.96
14.12.59	306 (8)	8.6	2.78

1. Number in brackets refers to number of animals in group.

spermatogenesis become less common. Fully mature testes can be found by January and by the end of February all the testes examined were in this condition. Spawning takes place between February and the end of April, the peak being in March. During this period, the testis becomes increasingly depleted of sperm, the lobules furthest away from the testis duct emptying first. By the end of May the testis is completely reduced, although residual sperm can still be found in some of the lobules and in the testis duct.

These histological changes can be divided into three phases:-

a) Multiplication of spermatogonia (Mitosis).

b) Spermatogenesis or maturation (Meiosis).

c) Spermiation.

Phases a and b are distinct processes although they follow one another without pause. The presence in January of testes composed entirely of spermatozoa suggests that spermatogenesis is completed fairly rapidly in the individual, but this phase occupies the period between October and February in the population. In contrast to the situation in mitosis, all the cells in a cyst are in the same stage of division at the same time although more than one stage may be present in the testis.

The histological variation in the testis is represented diagramatically in Fig. 10 and it will be seen that there is a period of "potential maturity" from January to March in which ripe motile sperm are present in the testis.

During the year, the testes changes markedly in colour. The ripening testis found from October onwards is milky-white, but this changes to a yellow colour during the period of spermiation, and the exhausted testis from June to August is colourless or pale pink and more or less transparent.

C. Discussion.

1. The testis and spermatogenesis.

It is apparent from the review of the literature that the sequence of changes which comprises spermatogenesis is common to all species of teleosts. There is, however, some variation in the time relations involved. Spermatogenesis is not completed until just before the spawning season in some species, while in others there is a "potential maturity" for several months before the spawning season during which time, mature sperm are present in the testis. According to the evidence presented here, the presence of motile sperm in the plaice testis in January assigns this species to the latter group in which there is a "potential maturity".

The significance of such a variation in the timing of spermatogenesis is not obvious. In Gasterosteus aculeatus, the completion of spermatogenesis in the autumn is dependent on the presence of sufficiently high temperatures. If these are not attained, the later stages will not appear before the next spring. (Craig-Bennett,1931). Both temperature and food supply are known to influence the reproductive cycle (Pickford and Atz,1957) and it is possible that the phenomena discussed above reflect variations in the metabolic processes of the species involved.

2. The origin of the new season's sperm.

According to Dodd (1960) there is no permanent germinal epithelium in the fish testis; "primary germ-cells migrate into the lobule walls after spawning and a new wave of spermatogenesis is

initiated". Dodd does not indicate where these new primary germ-cells have originated. The literature reviewed earlier indicates that their origin is by no means constant in the teleosts. In Perca flavescens, spermatogonia are produced by migration of primary germ cells from "a cord of germ cells outside of the testis". (Turner, 1919). This germ cord was found in a single specimen killed in May, and Turner was unable to find it in specimens caught at other dates. In support of his migration theory, Turner suggests that the elongated and irregular shape of germ cells found along the septum between the lobules suggests amoeboid motion. He also points out that clusters of germ cells found at the periphery of the testis could not give rise to all the cells found there a short time later as there were few mitotic figures. This view is supported by the work of Lofts and Marshall (1957) on Esox lucius and Craig-Bennett (1931) on Gasterosteus aculeatus.

Foley (1927) states that in Umbra limi, new spermatogonia arise from transformation of stroma cells of the testis, which may migrate into previously existing lobules or may transform in situ, forming new lobules.

In several species, however, spermatogonia are present in the testis all the year round and these divide mitotically to form the spermatogonia of the next generation. (for references see p43).

The evidence presented here for Pleuronectes platessa is in accordance with the last of these hypotheses, mitotic divisions of spermatogonia being common during the summer and autumn. Careful

examination of the testis duct and the tissue surrounding the testis failed to demonstrate the presence in them of spermatogonia and migratory germ cells were not evident. This however, cannot be taken as certain evidence of their absence as it is conceivable that such cells would be morphologically indistinguishable from the other cells in the tissue.

It may be that there is a considerable variation in the means by which the teleost testis is reconstituted after spawning, but to postulate a migration of germ cells either from the testis duct (Craig-Bennett,1931) or from some extra-testicular source does seem unnecessary. The more likely explanation is that the small nests of spermatogonia found in the testis throughout the year act as a reserve. These divide mitotically to produce a large number of cells most of which eventually form spermatozoa, but a few remain as spermatogonia and form the next year's reserve, thus maintaining the germinal epithelium. The description of mitotic division of spermatogonia by Turner and Craig-Bennett suggests that at least part of the new season's germ cells are produced in a similar manner in P. flavescens and G. aculeatus.

VII. Experimental Studies on the Testis.

A. The effect of hypophysectomy.

1. The effect of hypophysectomy on spermatogenesis.

Hypophysectomy and control operations were performed on groups of fish at various times of the year. The data shown in Table 15 (pp. 126 - 128) are derived from fish which were deliberately killed while still in good health. Information from post-mortem material confirms that from sacrificed fish, but has not been used in evaluating the results. In the following description, 'H' denotes hypophysectomised animals and 'HC' denotes controls.

Results.

Group IH. (operated early October). The mortality was high in this group and only 2 hypophysectomised fish survived. They were sacrificed 2 weeks after operation. The testes of both fish contained only small cysts of spermatogonia and occasional nests of degenerating sperm were still present from the previous year's spawning. The lobule walls were very thick and the interlobular tissue was well developed. By comparison with the normal sexual cycle, one would have expected to find primary and possibly secondary spermatocytes at this time of year. Multiplicatory divisions of spermatogonia were absent. No controls survived.

Group 3H. (operated October). The mortality was again very high and most of the fish died within 2 weeks of operation. One hypophysectomised animal was sacrificed nearly 4 weeks after operation.

Table 15.

The effect of hypophysectomy on spermatogenesis in P. platessa.

No. (3)	Date killed	Survival in days	Multiplicat. (1) mitoses	Primary S'cytes	Secondary S'cytes	Spermatids	Sperm
16.10.58 1 H 6	29.10.58	13	Absent	Absent	Absent	Absent	Present (2)
16.10.58 1 H 8	31.10.58	15	"	"	"	"	" (2)
27.10.58 3 H 2	20.11.58	24	"	"	"	"	"
24.1.59 6H 24C	2.6.59	129	Present	"	"	"	"
23.1.59 6H 13	2.6.59	130	Absent	"	"	"	"
18.2.59 7H 5C	3.8.59	166	Present	"	"	"	"
25.2.59 7H 22C	6.10.59	223	"	"	"	"	"
26.2.59 7H 28	6.10.59	222	Absent	"	"	"	"
26.2.59 7H 30	6.10.59	222	"	"	"	"	Absent
10.3.59 8H 13C	21.3.60	377	"	Present	Present	Present	Present
11.3.49 8H 15C	21.3.60	376	"	"	"	"	"

Table 15 cont.

11.3.59 8H 20C	21.3.60	376	Present	Present	Absent	Absent	Absent
14.3.59 8H 28C	14.9.59	184	"	Absent	"	"	"
9.3.59 8H 3	31.8.59	175	Absent	"	"	"	Present
9.3.59 8H 4	21.3.60	378	"	"	"	"	Absent
10.3.59 8H 11	1.3.60	357	"	"	"	"	"
11.3.59 8H 22	13.9.59	186	"	"	"	"	"
1.5.59 9H 32C	19.8.59	110	Present	"	"	"	Present (2)
29.4.59 9H 14	19.8.59	112	Absent	"	"	"	Absent
6.11.59 14H 3C	24.11.59	18	"	Present	Present	Present	"
6.11.59 14H 8C	4.12.59	28	"	"	"	"	Present
7.11.59 14H 12	4.12.59	27	"	"	"	Absent	Absent
3.3.60 15H 5C	15.5.60	73	"	Absent	Absent	"	Present
3.3.60 15H 6C	21.3.60	18	"	"	"	"	"

Table 15 cont.

3.3.60 15H 14C	9.4.60	37	Absent	Absent	Absent	Absent	Present
2.3.60 15H 1	25.5.60	84	"	"	"	"	"
2.3.60 15H 2	19.3.60	17	"	"	"	"	"
3.3.60 15H 3	23.5.60	81	"	"	"	"	"
3.3.60 15H 4	9.4.60	37	"	"	"	"	"
3.3.60 15H 7	25.5.60	83	"	"	"	"	"
3.3.60 15H 10	21.3.60	18	"	"	"	"	"
3.3.60 15H 11	10.4.60	38	"	"	"	"	"
3.3.60 15H 13	9.3.60	6	"	"	"	"	"
3.3.60 15H 15	9.4.60	37	"	"	"	"	"
3.3.60 15H 16	9.4.60	37	"	"	"	"	"

(1) Spermatogonia were present in the testes of all animals - both operated and control.

(2) Retained from previous spawning season.

(3) 'H' = hypophysectomised. 'HC' = control.

In this fish, the testis was composed of small cysts of spermatogonia separated by well-developed interlobular tissue. Multiplicatory divisions of spermatogonia were absent. Residual spermatozoa were found in many of the lobules and were present in large numbers in the testis duct. The blood vessels contained large numbers of lymphocytes, and phagocytes were present in the sperm-containing lobules.

No controls survived, but the normal testis at this time would be expected to contain both primary and secondary spermatocytes. The testis duct is normally free of residual sperm in November and only small nests are found in some of the testis lobules.

Group 6H (operated late January). Information from this group is confined to 2 animals, one hypophysectomised and one control, which were killed in June, 130 days after operation. In both animals, motile sperm were present in a smear taken at the time of operation and it is probable that spermatogenesis was complete. The testis volume was considerably reduced at autopsy and histological examination showed that spermiation has taken place although sperm were still present in large numbers in the testis duct and small nests were seen in many of the testis lobules (Plate 22, Figs. 1 and 2).

In both animals, the lobule walls were thick and the interlobular tissue was well developed. The testes of both fish were very similar to those of a normal spent fish, but spermatogonial cysts in the hypophysectomised fish were small and reduced in number compared to the control. Mitotic divisions of spermatogonia, present in the control, were completely absent in the operated animal. Small nests

of degenerating spermatids were present in some of the testis lobules of the hypophysectomised fish.

Group 7H. (operated late February). Motile sperm were present in smears from all but one of the fish in this group and it is fairly certain that in these fish, spermatogenesis was complete at the time of operation. Information is available from 4 animals sacrificed between 5 and 7 months after operation. The testes of both hypophysectomised fish were reduced and the few spermatogonia present occurred singly or in small groups. Residual spermatozoa were still present in the testis (7H28). The lobule boundaries were indistinct and the connective tissue was very well developed. Multiplicatory divisions of spermatogonia were absent.

The testis of a control fish killed in August (7H5C) was very similar to that of a normal fish at this time of year. Large spermatogonial cysts were frequent and mitotic divisions were visible, although not so numerous as would have been expected. The lobule walls were still fairly thick. The animal was in poor condition when killed. The second control fish (7H22C) may have been immature at the time of operation as milt was not expressed after slight pressure on the abdomen. At autopsy, the testis was small, but the lobules were filled with spermatogonial cysts and mitotic figures were frequent. The absence of residual sperm and the compactness of the spermatogonial cysts suggests that the fish was, in fact, immature at the time of operation and was maturing for the first time. Neither primary nor secondary spermatocytes were present, but spermatogenesis is often retarded in fish which are maturing for the first time.

Group 8H. (operated February/March). All the fish in this group were ripe as evidenced by the presence of motile sperm in smears taken before operation. Information is available from 8 fish (4 hypophysectomised and 4 controls) sacrificed between 6 months and one year after operation.

The testes of 2 fish sacrificed in August, 175 and 186 days after hypophysectomy contained very few spermatogonia and these were present either singly or in small cysts of three or four cells. The later stages of spermatogenesis were absent, although a few residual spermatozoa were still present in the testis duct and the testis lobules near it. Multiplicatory mitoses of spermatogonia, present in the normal testis at this time of year, were completely absent. The interlobular tissue was well developed and was composed mainly of connective tissue fibres with large numbers of cells with prominent nuclei and indistinct cytoplasmic boundaries.

Spermatogonia were numerous in the testis of a control fish killed 184 days after the start of the experiment, and mitotic division figures were seen in some of the cells. The lobule boundaries were indistinct and the interlobular tissue was still thick. No further stages of spermatogenesis were present, but although this is consistent with the situation in the normal fish in September, the number of mitotic divisions was fewer and the lobule walls thicker than in a normal fish (taken freshly from the sea).

The testes of the 2 fish sacrificed one year after hypophysectomy were considerably reduced in volume. The lobules were completely

collapsed and the bulk of the testis composed of connective tissue. Occasional spermatogonia were present, either singly or in cysts of three or four cells embedded in the connective tissue (Plate 23, Fig. 1), and a single mitotic metaphase was present in 8H4. "Interstitial" cells with round or oval nuclei and indistinct cytoplasmic boundaries were present in the interlobular tissue. Large numbers of lymphocytes were present in the testicular blood vessels of 8H11. In both animals the testis was small and the ducts were collapsed (Plate 23, Fig. 2).

In the testes of 2 of the 3 control fish sacrificed at the same time, spermatogenesis was well advanced. Primary and secondary spermatocytes, spermatids and sperm were present, the majority of the cells being spermatids (Plate 24, Fig. 1). Spermatogonia were few and multiplicatory mitoses absent. The testes of the third control fish contained spermatogonia and some cysts of primary spermatocytes: a few mitotic division figures were still visible. Spermatogenesis in this fish was considerably retarded since one would expect spermatogenesis to be almost complete at the beginning of March. Spermatogenesis was also slightly retarded in the other two controls.

Group 9H. (operated April/May). Mortality was high in this group and information is available from only 2 fish - 1 control and 1 hypophysectomised - which were killed 3 months after operation. The testis lobules near the testis duct still contained residual sperm in both the operated and the control fish. In both animals, small cysts of spermatogonia were present in the lobules and the lobule walls were thin. Spermatogonial divisions were present only in the control fish,

but the number of spermatogonial cysts in the operated fish was greater than that of hypophysectomised fish from previous groups.

Group 14H. (operated early November). By reference to the normal spermatogenic cycle, it was estimated that spermatogenesis would be underway in this group at the time of operation. Mortality was again high and only 3 fish survived to be sacrificed 3 to 4 weeks after operation.

The testis lobules of the 2 control fish contained cysts of cells in various stages of meiosis. The majority of cells were primary and secondary spermatocytes but a few cysts of spermatids were present in 14H3C and spermiogenesis had just begun in 14H8C. A few nests of spermatogonia were present and spermatids in various stages of transformation to sperm were common. The lobules were broad and the interlobular spaces small; connective tissue fibres and "interstitial" cells were present.

Many of the lobules of the hypophysectomised fish still contained cysts of spermatogonia, but both primary and secondary spermatocytes were present in some of the lobules. Spermatids and spermatozoa were absent although their presence would be expected in the testes of normal fish in December. There was no evidence of deterioration in the spermatocytes.

The results of experiments performed in 1958 and 1959 on the effect of hypophysectomy on the testis suggested that pituitary removal had no effect on the expulsion of ripe spermatozoa from the testis. Accordingly a more detailed study was during the spawning

period of 1960. Fish in which motile sperm were expressed from the testis at the time of hypophysectomy, were sacrificed at various intervals after operation. The results of this experiment are summarised in Table 16 (p. 135).

Three control animals were sacrificed 18, 37 and 73 days after the start of the operation. The testes of all 3 fish were reduced in size and yellowish-pink in colour. Histological examination showed that most of the lobules were reduced and empty although sperm were still present in large numbers in the collecting ducts and in the testis lobules near them. The walls of the empty lobules were thickened and composed of connective tissue and "interstitial" cells. Small cysts of spermatogonia were present in many of the lobule walls, but mitotic divisions were not seen (Plate 24, Fig. 2).

Hypophysectomised fish were sacrificed 1, 3, 5, and 12 weeks after hypophysectomy. In 15H13, killed 6 days after operation, the testes were large and white and the lobules were wide and packed with sperm. Spawning had not yet begun.

Spermiation was well advanced in 15H2 and 15H10 killed 17 and 18 days after pituitary removal. In the former, only a few lobules near the testis duct contained sperm, although the duct itself was still swollen with sperm. The interlobular connective tissue was well developed and there appeared to be an increase in the numbers of "interstitial" cells (Plate 25, Fig. 1). Spermatogonial cysts were small and few in number and mitotic divisions were absent. Some sperm had been expelled from the testis of 15H10, but most of the lobules were

Table 16.

The effect of hypophysectomy on spermiation.

Operated March 1960	Killed	Survival in days	Comments
(1)15H5C	15.5.60	73	Testes lobules were still full of sperm. Motile sperm present in smear. Spawning started.
15H6C	21.3.60	18	Only these lobules near the vas deferens were filled with sperm. Motile sperm present. Partly spent.
15H14C	9.4.60	37	Most lobules reduced, but a few near the vas deferens full of sperm. Motile sperm present. Partly spent.
15H1	25.5.60	84	Testes reduced in volume. Sperm still present in vas deferens. Motile sperm present in smear. Spent.
15H2	19.3.60	17	Testes considerably reduced in volume. Masses of sperm in vas deferens. Motile sperm in smear. Partly spent.
15H3	23.5.60	81	Testes in vas deferens reduced in volume with few sperm present. Motile sperm in smear. Spent.
15H4	9.4.60	37	Many lobules swollen with sperm, but some reduced in volume. Motile sperm present. Spawning started.
15H7	25.5.60	83	Only residual sperm in testes and vas deferens. No sperm in smear. Spent.
15H10	21.3.60	18	Some lobules reduced but most still swollen with sperm. Motile sperm present. Spawning started.
15H11	10.4.60	38	Very similar to 15H10. Spawning started.
15H13	9.3.60	6	Testes and vas deferens full of sperm. Motile sperm present in smear. Spawning not started.
15H15	9.4.60	37	Testes and vas deferens reduced volume. No sperm in smear. Spent.
15H16	9.4.60	37	Testes reduced in volume. Sperm plentiful in vas deferens. Motile sperm in smear. Spent.

(1) 'H' denotes hypophysectomised and 'HC' denotes control.

still wide and full of sperm: some thickening of the lobule wall was apparent, especially in the lobules furthest from the testis duct.

Four fish were killed 3 weeks after hypophysectomy and in these the testis was reduced in size and yellowish-pink in colour. In 15H15 and 16, spermiation was more or less complete. The testis lobules were collapsed and only occasional nests of sperm were present, although sperm were plentiful in the testis duct. The interlobular connective tissue was plentiful and "interstitial" cells common. A few spermatogonia were present either singly or in small cysts but multiplicatory mitoses of spermatogonia were absent. Spermiation had begun in the other 2 animals sacrificed at this time. Most of the lobules were wide and full of sperm, but the lobules distal to the testis duct were reduced to some extent.

Spermiation was complete in the 3 fish killed 12 weeks after pituitary removal. The testis and its duct were very small and yellowish-pink in colour. Only residual sperm were present in the testis lobules and either absent or few in number in the duct. The testis were composed mainly of connective tissue fibres and "interstitial" cells were common. Spermatogonia were few and mitotic divisions were absent.

It is evident from the above description that there was a considerable variation in the degree to which sperm had been expelled from the testis in both control and hypophysectomised fish. Spermiation had proceeded further in some fish examined in April than in another examined in May. This variation, however, is consistent

with the variation found in normal animals taken from the sea between March and May.

2. The effect of hypophysectomy on the testis of immature plaice.

The experiments on adult fish were extended to include the effect of pituitary removal on the testes of immature fish. Mortality was high among the fish in this group and information is available from only 6 fish which were sacrificed 3 to 4 weeks after operation (Table 17, p. 138).

In both operated and control fish, the testes were small and undeveloped. Histological examination showed that the testis lobules were filled with "resting" spermatogonia. These cells in the hypophysectomised fish appeared healthy and normal and there was no evidence of deterioration of the cell contents. The testes were indistinguishable from those of control fish and from normal immature fish (Plate 25, Fig. 2; Pl. 26, Fig. 1).

3. The effect of hypophysectomy on testis and body weights.

Information on body and testis weights from all groups is detailed in Table 18,(pp.139-140). It is evident that the hypophysectomised animals have either lost weight or at best maintained their body weight. Weight increases are recorded for 6 animals which had their pituitary gland removed, but the increase in weight are small and are probably not significant.

Conversely, it is apparent that most of the control animals have increased their body weight during the course of the experiment. This is particularly true of the controls in group 8H where weight

Table 17.

The effect of hypophysectomy on immature fish.

No. (1)	Survival in days (2)	Testes weight at death	Testes as % total body wt.	Comment
2H5C	16	0.14	0.09	The testes lobules were filled with spermatogonia. No evidence of cell division. Testes typical of an immature fish.
2H21C	32	0.18	0.12	
2H32C	21	0.15	0.11	
			Average 0.10	
2H2	20	0.09	0.08	The spermatogonia filling the testes lobules appeared normal and healthy. Indistinguishable from the controls and from normal immature fish.
2H9	19	0.20	0.19	
2H10	20	0.12	0.11	
			Average 0.12	

(1) 'H' denotes hypophysectomised and 'HC' denotes control.

(2) The operations were performed between 27th October and 2nd November, 1958.

Table 18.

Total Body and Testis Weight of Experimental Males.

No. (1)	Weight gm. at operation	Weight gm. at death	Gain or loss	Testis gm. weight	% Testes wt.
1H6	325	340	+ 15	0.75g	0.22
1H8	410	410	-	0.6g	0.146
6H24C	230	290	+ 60	0.43	0.148
6H13	270	280	+ 10	0.41	0.146
7H5C	220	170	- 50	0.52	0.306
7H22C	200	240	+ 40	0.11	0.045
7H28	340	360	+ 20	0.68	0.18
7H30	175	150	- 25	0.19	0.12
8H13C	275	342	+ 67	1.18	0.34
8H15C	360	412	+ 52	1.15	0.27
8H20C	300	378	+ 78	0.67	0.17
8H28C	245	295	+ 50	0.30	0.10
8H3	245	260	+ 15	0.2	0.07
8H4	220	182	- 38	0.27	0.14
8H11	340	348	+ 8	0.36	0.10
8H22	375	380	+ 5	0.54	0.14
9H22C	265	285	+ 20	0.9	0.31
9H14	180	140	- 40	0.1	0.07
14H3C	210	246	+ 36	3.0	1.42
14H8C	190	220	+ 30	2.9	1.52
14H12	280	270	- 10	2.4	0.95

Table 18 cont.

15H5C	310	332	+ 22	4.24	1.27
15H6C	316	308	- 8	2.12	0.68
15H14C	340	328	- 14	4.95	1.50
15H1	266	244	- 22	0.51	0.20
15H3	314	315	+ 1	0.725	0.23
15H4	310	310	-	4.65	1.5
15H7	368	334	- 34	0.67	0.2
15H10	408	408	-	3.35	0.82
15H11	264	244	- 20	4.6	1.47
15H13	232	204	- 28	1.82	0.89
15H15	164	146	- 18	0.40	0.27
15H16	280	252	- 28	0.95	0.37

(1) 'H' = hypophysectomised

'HC'= control

increases of 50-78 g. were recorded over a period of one year. The body weight of 3 of the animals was reduced, though probably not significantly. The decrease in weight of 50 g. recorded for 7H50 may be due to the fact that the animal was in poor condition and suffering from fin-rot when sacrificed.

The testis weights of hypophysectomised fish were also less than those of controls especially in long-term operated fish.

B. Discussion.

All workers on pituitary-gonad relationships are agreed that the presence of the pituitary gland is essential for normal development and functioning of the gonads and that pituitary hormones exert a controlling influence on spermatogenesis. There is, however, little information on the precise stages in spermatogenesis which are affected by hypophysectomy.

Matthews (1939) and Pickford (1953), both working with Fundulus heteroclitus, found that spermatogonia were unaffected by pituitary removal, but that few spermatocytes or spermatids and no spermatozoa were present in the testis five months or more after operation. Matthews found that mitotic divisions of spermatogonia were reduced after hypophysectomy, but dividing spermatogonia were still present 206 days after operation. He concludes that the influence of the pituitary is of greater importance in maturation than in proliferation of the germ cells.

The results obtained by Burger (1941) agree with those of Matthews and Pickford in so far as the later stages of spermatogenesis are concerned, but there appears to be some contradiction in the conclusions drawn from Burger concerning the earlier stages. In summarising his results Burger states that "Fundulus, hypophysectomised shortly after maximal testicular development, show an inhibition of spermatogenesis for stages beyond those of spermatogonial multiplication. Spermatogonial divisions do not become numerous". However, in decribing the effect of hypophysectomy at this period, he states that

"at no time were spermatogonial multiplications suppressed. These divisions formed a well defined cortical zone of spermatogonia". From a critical examination of Burger's results, it would appear that although spermatogonial divisions are considerably reduced so that the autumnal build-up of spermatogonia in the testes does not occur, they are not completely suppressed, as evidenced by the well defined zone of spermatogonia in the cortex of the testis.

Vivien (1941) found that the testis of adult Gobius paganellus appeared immature 5½ months after hypophysectomy and essentially similar results are reported by Tavolga (1955), using Bathygobius soporator. The former author also reported that spermiation and territorial behaviour were prevented in 70% of a group of Gobius paganellus hypophysectomised just prior to the natural breeding season. Vivien was unable to account for successful spermiation in the other 30%. The most obvious suggestion is that the pituitary gland had not been completely removed in these fish and the amount of hormone produced by the remnants was sufficient to allow the animals to spermiate. The occurrence of a time-lag between the removal of the pituitary and the initiation of the effect on the target organ has been noted in all groups of vertebrates. It is possible that some of Vivien's fish were so near to their natural spawning that spermiation had taken place before pituitary ablation had become effective. This, however, would not account for the ability of the fish to continue their territorial behaviour, assuming that this is basically under pituitary control.

The evidence presented here constitutes a strong case for the hypothesis that spermatogenesis in Pleuronectes is controlled by the secretion of gonadotrophic hormone(s) by the pituitary gland. There is, however, a considerable variation in the effect of hypophysectomy at different times of the year.

The results obtained in Groups 1H and 3H indicate that the transformation of spermatogonia to spermatocytes is under pituitary control, since stages beyond spermatogonia were absent from the testis in hypophysectomised animals of these groups. It is impossible to be certain that maturation had not started at the beginning of the experiment, but the testes of many of the fish in their natural environment would be composed entirely of spermatogonia.

The results obtained from Group 14H suggest that once spermatogenesis has started, meiosis can continue for some time in the absence of the pituitary gland. Most of the testes lobules of 14H12 contained spermatogonia and their development had again been arrested. The absence of meiotic divisions between spermatocytes and spermatids suggests that this transformation is inhibited in the absence of the pituitary. The duration of the experiment was too short to answer this question satisfactorily. Whether spermatogenesis once started can go on to completion after hypophysectomy can only be determined by longer term experiments. When numbers of experimental animals are small and mortality rates high, one is always faced with the problem of whether to be certain of short-term information from sacrificed fish or risk losing the animals in the hope of obtaining long term data.

When the pituitary gland is removed after spermatogenesis is complete, (Groups 6, 7 and 8) sperm are shed normally, but subsequent spermatogonial multiplication is almost completely arrested. The testis of such hypophysectomised animals examined at periods up to one year after hypophysectomy, are composed of scattered cysts of spermatogonia embedded in a very thick connective tissue stroma and mitotic divisions are rare. The post-spawning recrudescence of the testis is suppressed entirely: the few spermatogonia present are those which did not develop in the preceeding maturation period; which, as reserve cells would have been the source of the succeeding generation of spermatogonia. The presence of a few cysts of spermatids in the testes of 6H13, more than 4 months after pituitary removal, suggests that spermiogenesis was not quite complete at the time of operation and that the transformation to sperm of the spermatids present in the testis was inhibited by hypophysectomy.

It is evident from the results that spermatogenesis was inhibited to some extent in some of the control fish. By comparison with the normal spawning cycle the testes of Group 8 controls should have contained nothing but sperm. In one such fish, however, spermatogenesis had just started and in another, the transformation of spermatids to sperm had not progressed very far. It is difficult to provide a satisfactory explanation for this as a great many factors could be involved. The most likely suggestions are that the fish were not receiving a balanced diet (see methods p. 11). In view of this it is felt that all the results discussed above must be interpreted

in the light of possible inanition and captivity effects.

In contrast with the situation in Gobius paganellus where Vivien (1941) found that spermiation was inhibited in 70% of a group of hypophysectomised fish, the results obtained in Groups 6H, 7H and 8H indicate that spermiation in plaice can proceed normally in the absence of the pituitary. That this is the case, is confirmed by the results of Group 15H where fish hypophysectomised during the breeding season were autopsied at varying intervals after operation. Here the absence of any indication of resorption of sperm as found by Vivien and the occurrence of various degrees of expulsion of sperm suggest strongly that spermiation is not under pituitary control in Pleuronectes.

Pituitary gonad relationships in Scyliorhinus caniculus have also been studied at the Gatty Marine Laboratory and the results discussed above may be compared with those of Goddard and Dodd, (1960) and Dodd, Evernett and Goddard,(1960). The testis of S. caniculus has a zonate structure in which ampullae containing germ cells at a particular stage occupy more or less parallel zones concentric with the spermatogonial ampullae. The effect of ventral lobectomy is to cause breakdown of the transitional zone of ampullae which lies between spermatogonia and primary spermatocytes. The tubulogenic zone appears normal as do the cells of the spermatogonial ampullae nearest it. More distal ampullae show a reduction in the number of nuclei. Primary spermatocytes, already established at the time of operation, and all succeeding stages develop in their normal fashion.

These results are in full agreement with those obtained in

P. platessa in so far as the latter can be interpreted safely in view of the difficulties associated with Pleuronectes.

It may be concluded, then, that pituitary gonadotrophin is essential for the normal transformation which occurs when a spermatogonium, which has been dividing by mitosis, becomes a primary spermatocyte which will divide by meiosis. Primary spermatocytes, if already established at the time of operation, can complete their development and pituitary removal does not interfere with spermiation. Several workers have suggested that pituitary removal stops mitotic division of spermatogonia but this does not appear to be the case in plaice, and the reduced number of spermatogonia found may be a more general metabolic effect.

VIII. The Plaice Pituitary Gland.

A. Anatomy.

The plaice pituitary gland is a pear shaped organ, closely attached to the base of the mid-brain by fibres passing into the neural lobe, and the cavity of the third ventricle extends downwards as a shallow hypophyseal process, lined by ependyma cells, in the centre of this oval ring of fibres. The gland lies in a slight hollow in the cranial floor enclosed in the endocranial membranes: but no structure comparable to the mammalian sella turcica is present. A distinct transverse groove, similar to that described in Anguilla anguilla (Waring, 1940) separates the gland into anterior and posterior portions (Plate 26, Fig. 2) but the 3 regions of the adenohypophysis cannot be distinguished externally. In the following description, the nomenclature proposed by Pickford and Atz (1957) is used.

1. Pro-adenohypophysis.

This is the most anterior and the smallest of the 3 glandular regions. It is composed of about equal numbers of acidophils and chromophobes which form a closely-packed mass of small cells. Basophils cells are entirely absent. Antero-dorsal to the main bulk of cells is a band of large chromophobes. The round or oval nuclei of these cells are about 4.5 u in diameter and the cell boundaries are indistinct. The acidophils and chromophobes of the main bulk of tissue are about the same size and have a nucleus about 3.5 u across. The acidophils occupy the periphery of the zone and although often rounded, tend to show an elongation towards connective tissue septa or blood vessels

(Plate 27, Fig. 1). Sections to either side of the median plane show this zone disappearing and its cells becoming contiguous and intermingling with those of the meso-adenohypophysis.

The connective tissue boundary of the pro-adenohypophysis with the neurohypophysis is unbroken and several connective tissue sheets form an irregular framework within the body of the region. The processes from the neural lobe into the pro-adenohypophysis are mainly few and short, but may sometimes penetrate to the outer surface of the gland. The blood supply of the region is not rich, but capillaries are present in the processes of the neurohypophysis and in the substance of the tissue.

2. Meso-adenohypophysis.

Anteriorly, this region extends a short distance below and on either side of the previous region, but posteriorly it is clearly delimited from the meta-adenohypophysis. This posterior boundary is marked on the outside by the transverse groove mentioned earlier. Deep, irregularly shaped processes from the neurohypophysis cause characteristic, though irregular, indentations into the region. The three cell types characteristic of the mammalian pars anterior are present and their distribution is constant. The acidophils are arranged more or less side by side along the boundaries with the neurohypophysis and blood vessels forming a sheet. A few cells may occur separately or in small groups below this sheet, but the bulk of the acidophils are confined to the more dorsal part of this region where they form 60 to 70% of the cells (Plate 27, Fig. 1). Where the acidophils

are associated with neural tissue or blood vessels, they are generally columnar in shape and measure about 7 μ by 11 μ. They may also be round or oval in shape and in this case measure 7 to 8 μ. The chromophil substance in the cytoplasm consists of very fine granules showing a strong affinity for acid fuchsin. The sheets of acidophils are separated mainly by round chromophobe cells which measure about 7 μ. The boundaries of these cells are indistinct making them difficult to measure accurately and their cytoplasm has little demonstrable structure.

The distribution of basophils in the meso-adenohypophysis will be described in some detail as it appears to differ from that in previously described teleosts (Fig. 11a). A narrow zone (zone 1) of basophils is found in the antero-dorsal part of the region immediately posterior to the pro-adenohypophysis and not cut off from the neurohypophyseal processes by connective tissue (Plate 28, Fig. 1). It is most obvious in sagittal sections and becomes intermingled ventrally and laterally with the bulk of the meso-adenohypophyseal cells. The nuclei of these cells are about 3 μ in diameter and the cytoplasm is attenuated. Well-marked cell boundaries are not visible even at very high magnifications (x 2400). The basophilic material in the cytoplasm is extremely fine and cannot be resolved into discrete granules.

The second zone (zone 2) of basophils occupies the ventral and lateral regions of meso-adenohypophysis where they are found intermingled with chromophobes. The concentration of basophils is greatest close to the meta-adenohypophysis and the outer surface of the

gland and decreases gradually towards the acidophil zone (Fig. 11a). Some basophils are found intermingled with the chromophobes between the sheets of acidophils. The cytoplasm of these basophils surrounds a central nucleus and is filled with coarse, irregular granules. The cells are usually large, but a considerable variation in size from about 6 u to 12 μ is found. Small and large cells are randomly distributed throughout the basophil region.

The blood supply of the meso-adenohypophysis is variable. Capillaries are common in association with groups of acidophils and larger vessels are often present in the processes of neural tissue.

3. Meta-adenohypophysis.

This is probably the largest region of the gland and is irregular in shape being in complex association with the branches of the neurohypophysis which may extend to the outer surface of the gland. It is clearly marked off from the previous region mainly by virtue of the difference in staining reaction of the two regions, but an irregular connective tissue boundary is also present. Strong staining reactions are not found in this region and the cells are mainly chromophobes and dull cells which react with both the aniline blue and orange G of the Azan technique. The latter cells are generally orientated towards the neural lobe processes and their cytoplasm contains very fine granules. In this situation, the cells tend to become elongated, measuring 6 μ by 12 μ and the pockets between them are filled by chromophobes (Plate 28, Fig. 2). The blood supply is poor, consisting mainly of capillaries from the neurohypophysis. Occasional

cells with very large (8 μ) round or oval nuclei are seen close to the processes or neural tissue. No cytoplasm is visible in these cells.

4. Neurohypophysis.

This region consists mainly of a network of fibres extending downwards from the brain and spreading horizontally near the dorsal surface of the pituitary and then passing down into the branches of the lobe (Plate 29, Fig. 1). Nuclei surrounded by scanty cytoplasm are scattered throughout the tissue in considerable numbers and the floor of the infundibulum contains similar nuclei of the ependymal layer. A fine connective tissue forms a boundary with the 3 regions of the adenohypophysis.

Herring material is always present and is generally confined to those branches which are associated with the meta-adenohypophysis. It varies in amount and is generally present as a fine granulation although larger masses may be found in the more dorsal branches close to the infundibulum.

In addition to the above histological study, a histochemical investigation was carried out using the periodic acid-Schiff (PAS) reaction. PAS positive cells are found to be confined to the meso- and meta-adenohypophysis. In the former region the distribution of such cells is identical with that of the basophils of both zones (Plate 29, Fig 2 and Plate 30, Fig. 1). In zone 1, the red coloration is diffuse and it is impossible to discern the boundaries of the individual cells (Plate 30, Fig. 2). The cells of the postero-ventral region (zone 2) are intensely PAS positive for most of the year and stand out

clearly against the unstained chromophobes. (Plate31, Fig. 1). In the meta-adenohypophysis, the PAS reaction is confined to those cells which are found on the borders of the neurohypophyseal tissue (Plate 29, Fig. 2).

PAS in conjunction with methyl blue has been used in mammalian studies to differentiate between purple gonadotroph cells and red thyrotrophs. (Wilson and Barin,1954). No such differentiation was found when this technique was used in the present study. The basophil cells of both zones in the meso-adenohypophysis remain red after counterstaining with methyl blue as did the PAS positive cells of the meta-adenohypophysis.

The aldehyde fuchsin (AF) reaction, although not strictly a histochemical test, has been used in teleost studies to differentiate between gonadotrophs and thyrotrophs (Barrington and Matty, 1956). According to these authors both the basophil cell types in the meso-adenohypophysis of Phoxinus laevis are PAS positive but only one of these will react with AF. In the present investigation it was found that all the basophils of the meso-adenohypophysis reacted positively to AF and that these cells also reacted to PAS. The herring material of the neurohypophysis is also AF positive but the PAS positive cells of the meta-adenohypophysis are AF negative (Plate 31, Fig. 2 and Plate 32, Fig. 1).

B. Cyclical changes in the meso-adenohypophysis.

An examination of pituitary glands collected from adult plaice at regular monthly intervals throughout the year suggested that there was a considerable variation in the number, size and staining intensity of the basophils of the meso-adenohypophysis. Zone 1 is small and compact (Fig 11a) and its basophils are randomly intermingled with chromophobes. Most of its cells could be counted in a single field, but the indistinct cell boundaries made accurate cell counts extremely difficult and in some instances impossible. Between July and March, the number of basophils is relatively constant and there is little change in the size or staining intensity of the cells (Table 19, p. 155). Pituitary glands examined in May and June, 1958 were completely devoid of zone 1 basophils and PAS and AF tests were negative, although positive cells were found in sections of glands from other months stained at the same time. Zone 1 basophils were present however, in glands collected in May and June, 1959, and were PAS and AF positive. The cells were too diffuse to count, but appeared to be present in numbers similar to those found in other months.

The basophils of zone 2 are well defined and easy to count at most times of the year, but their distribution within the zone is not random. In order to determine their precise distribution, a series of counts was made in several sections on either side of the median plane in one gland. Approximately 10 fields were counted to include most of zone 2 and the dorsal acidophil zone. The results given in Table 20 (p. 156) indicate that the greatest proportion of basophils is

Table 19.

Numbers of zone 1 basophils present in the meso-adenohypophysis.

Date of collection.	Number of glands counted (1)	Mean % basophils
May 1958	3	-
June	6	-
July	6	50
August	1	45
September	6	52
October	8	54
November	8	49
January	1	51
March	3	42
April	1 (Ripe)	44
	1 (Spent)	63
May	3	50
June	3	45

(1) In many instances, other glands were examined, but the cells were too diffuse to count.

Table 20.

Distribution of zone 2 basophils in the meso-adenohypophysis.

Field (1) Number	Location of section					Mean % basophils ± Stand. Dev.
	150 u	75 u	Median	75 u	150 u	
1.	7.1%	5.7%	7.0%	1.2%	9.6%	6.1 ± 3.09
2.	22.3%	9.1%	5.4%	11.2%	1.3%	9.8 ± 7.74
3.	19.9%	13.1%	24.8%	17.4%	18.9%	18.8 ± 4.23
4.	34.5%	29.1%	38.6%	36.8%	30.3%	33.8 ± 4.08
5.	7.3%	1.4%	0.0%	6.1%	2.1%	3.4 ± 2.93
6.	15.8%	11.7%	5.1%	13.6%	9.9%	11.2 ± 4.06
7.	21.2%	31.3%	29.6%	24.8%	19.4%	25.2 ± 5.15
8.	8.6%	5.4%	0.0%	1.9%	6.4%	4.4 ± 3.45
9.	14.9%	11.1%	0.0%	6.7%	3.4%	7.2 ± 5.93
10.	18.8%	20.6%	27.7%	11.3%	24.5%	20.6 ± 6.22
11.	-	9.4%	21.6%	7.1%	-	12.7 ± 7.23

1. Approximately 250 cells were counted in each field and the percentage of basophils present calculated (see Fig. 11b for exact location of fields).

found in the postero-ventral region of zone 2 (Fig. 11a). It is also evident that the standard deviation from the mean is smaller there than in the more dorsal regions. Since the labour involved precludes the counting of all the cells of the meso-adenohypophysis, it was thought that a single count in the postero-ventral region, where the number of basophils is less variable, would give a reliable estimate of any cyclical change. The figures given in Table 20 (p. 156) also show that there is little variation in the percentage of zone 2 basophils on either side of the median plane. Accordingly, a single field was counted in the sagittal section and in one section 150 μ on either side of it. The results obtained from atotal of 85 pituitaries collected at monthly intervals, from May, 1958 to June, 1959 inclusive, are given in Table 21 (p.158). It is clear that a considerable variation obtains in the number of zone 2 basophils present in the plaice pituitary gland and that this variation is cyclical in nature. During the summer months the stainability of the basophils is completely absent making them impossible to distinguish from the chromophobes which are always intermingled with them. From July until March the basophils appear as large, intensely staining cells and the figures given in Table 21 (p. 158) suggest that the maximum proportion of such cells is reached in January, after which there is a gradual decrease. The standard deviations obtained, however, are very high and there may be no significant difference between one month and the next. During March and April, the staining intensity of the basophils is reduced and their numbers are fewer than in earlier months.

Table 21.

Variation in the percentage of zone 2 basophils in the meso-adenohypophysis of adult plaice.

Date of collection	Condition of gonads	Number (1) of glands counted	Mean % basophils ± Stand. Dev.
May 1958	New gonadal development just beginning	3	-
June	"	6	-
July	Vitellogenesis in females	6	22 ± 10.58
August	Spermatogenesis Vitellogenesis	8	20 ± 7.48
September	"	4	47 ± 22.09
October	"	8	33 ± 4.78
November	"	8	29 ± 5.02
January 1959	"	6	46 ± 6.79
February	"	8	30 ± 6.30
March	"	6	26 ± 4.30
April	Ripe	6	18 ± 11.09
	Spent	4	9.2± 6.65
May	Spent, new gonadal development in some animals	1 (2)	22
June	"	8	-

1. In most cases, equal numbers of glands from males and females were used.

2. Six glands were examined, but basophils were present only in one which was not completely spent.

A similar pattern of events was obtained when the PAS and AF reactions were employed:- PAS and AF positive cells were absent during May and June, intensive from then until March and April, at which time the staining reaction of the cells was reduced.

To ensure that there was no differential variability within the region, cell counts were made from time to time in other parts of the region. The pattern obtained agrees with that described above.

C. Discussion.

The presence of 3 cell types in the vertebrate meso-adenohypophysis is well-established and the present study indicates that the plaice is no exception to the rule. The chromophobes, with a non-granular cytoplasm are generally held to be reserve cells without secretory function.

The basophils have a granular cytoplasm and the glycoprotein nature of their inclusions has been conclusively demonstrated, at least in mammals, by the application of the PAS method. (Halmi (1950,1952), Purves and Griesbach, (1951). These authors have also shown that the 3 glycoprotein-bound hormones (FSH, LH and TSH) of the mammalian pituitary must be associated with the basophilic cells. It does not follow, however, that PAS-positive material is confined to these cells. Purves and Griesbach (1957) obtained a positive PAS reaction in the "epsilon" cells of the dog which are normally regarded as acidophils (Table 3). Similar findings have been obtained from amphibian material. Ortmann (1956) studying the anterior lobe of Rana pipiens, found PAS-positive material dispersed through the cytoplasm of the azocarmine acidophils as well as in the basophils, and certain orange G acidophils contained PAS-positive material in vacuoles.

Two distinct basophilic cell types have been differentiated by suitable staining procedures in the rat (Halmi, and Purves and Griesbach loc. cit.). Those basophils which are selectively stained by the AF method are held by these authors to be thyrotrophs, while the gonadotrophs are AF negative. The gonadotrophs have been further

divided into two groups, one associated with the production of FSH and the other with LH, by a combination of experimental procedures and differential staining (Purves and Griesbach,1954). The use of the AF reaction as a selective stain for thyrotrophs does not appear to be justified in view of the conflicting results obtained by Goldberg and Chaikoff (1952) and Purves and Griesbach (1957), both working on the dog. The former authors found that only the "beta" basophils were AF positive, while the latter obtained AF positive results in all 3 basophil cell types (Table 3).

The basophils of the plaice meso-adenohypophysis can be divided into 2 morphologically distinct zones. The antero-dorsal zone 1 cells were described as "a central mass of apparently degenerating basophils" in a brief description of the plaice pituitary given by Kerr (1942). No evidence is given in support of this hypothesis and the fish examined by Kerr were collected in April. The findings of the present study indicate that this zone has a constant location in the pituitary and that its cells can be identified during the whole year, although there is some variation in the amount of basophilia present. The nuclei of these basophils appear normal at all times of the year and similar in structure to those of adjacent regions but the region as a whole does appear somewhat necrotic. It is unusual to find such a distinct group of cells at the same time so constant in position and yet undergoing degeneration, but such a possibility cannot be excluded on the evidence presented above.

Several authors, using the PAS technique, have found that

glycoprotein containing cells are present in the meso-adenohypophysis of teleosts and that they are identical with the basophils. Experimental procedures or differential staining or a combination of both have enabled a number of workers to identify two basophilic cell types, one gonadotrophic and the other thyrotrophic in function, in the teleost pituitary. (Atz (1953), Sokol (1953,1955) and Barrington and Matty (1955)). Both basophil zones of the plaice pituitary are PAS positive, but attempts to differentiate between them using the AF reaction have been unsuccessful: both zones are AF positive, in agreement with the results obtained by Atz and Sokol (loc. cit.).

The function of the zone 1 basophils and the significance of the changes found in them is not clear. The proportion of these cells appears fairly constant for most of the year, although the accuracy of the cell counts must remain suspect owing to the difficulties associated with establishing the boundaries of the cells. The degranulation and loss of glycoprotein found in them in May and June of 1958 was not found in the same months of the following year and it is difficult to find any explanation for it. If the zone 1 cells should prove to be degenerating, then the presence of PAS positive material in them cannot be taken as evidence of hormone function since PAS reactions are known to occur in necrotic tissue (Dodd and Kerr, personal communication).

The cyclical variation found in the zone 2 basophils of the plaice pituitary suggests that these cells may be associated with the production of gonadotrophin. In support of this hypothesis it can be

seen that the degranulation of these cells and their loss of glycoprotein material is found in glands from post-spawning fish when there is little or no activity in the gonads. The period at which glycoprotein material again appears in the basophils coincides with the onset of vitellogenesis and spermatogonial multiplication and the maximum numbers of basophils is reached at the time when the activity of the gonads is at its highest. The onset of spawning in March and April is associated with the reduction in numbers of zone 2 basophils and a loss in their staining intensity, although it should be noted that their degranulation is not complete for some time after spawning.

IX. Bioassay of Plaice Pituitary Material.

A. The spermiation response of Xenopus laevis.

The assay method and the pre-treatment of the pituitary glands is described in the section on methods (pp.25-27). The degree of purity of the test substance is extremely important in any bioassay and this is particularly true of the assay of pituitary gonadotrophin, where the presence of several other hormones as 'contaminants' may interfere with its efficiency. The method of extraction used in the present study is relatively simple and can be used to deal with small amounts of material.

1. Effectiveness of the extraction technique.

In order to test the efficiency of the technique, approximately equal numbers of male and female plaice glands collected in October were powdered in an agate mortar. Some of the material was extracted in acetic acid and acetone and some was macerated in saline in a tissue grinder. Both preparations were assayed on male toads. The results of the experiment are detailed in Table 22 (p. 165). Since the amount of extracted material required to produce a particular response is less than that of saline suspension, it can be concluded that the extraction technique is more efficient than saline in extracting gonadotrophic material. In addition, it has the advantage over the suspension that the material is injected as a solution and has also less contamination with other pituitary hormones (The extracted material has a reduced oxytocic activity as measured by the isolated rat uterus method).

To ensure that the bulk of the gonadotrophin was extracted, the

Table 22.

Effectiveness of the extraction technique compared with saline suspension.

Material	No. of glands	Weight (dry).	Extraction method	Dose	Response	%
Plaice October 1959	76 Male 78 Female	81.0 mg. mean = 0.52 mg.	Saline suspension	= 10gl/toad (5.2mg.)	2/5	40
			Acetic acid/acetone extract. Fractions 1 and 2 combined	= 6gl/toad (3.12mg.)	2/5	40
				= 12gl/toad (6.24mg.)	3/5	60
Plaice: collection from several winter months	241 glands from male fish	97.7 mg. mean = 0.405 mg.	Acetic acid/acetone extract. Fractions 1 and 2 combined	= 15gl/toad (6.07 mg.)	4/7	57
			Residue from stage 2.	= 30gl/toad	1/7	14

(1). The presence of sperm constitutes a positive response and the fraction indicates the number of positive individuals in each group.

insoluble residue left after stage 2 (see page27) was tested on a number of occasions. In such tests, the insoluble material was collected and dried. A saline suspension was then made with a concentration equivalent to 30 glands. Most of the tests were negative although in 1 test, one toad out of a group of 7 spermiated. Since the dose level was twice that used in the assay of the soluble fractions, it was concluded that the activity of the insoluble residue was negligible. (Table 22).

2. Variation in gonadotrophic potency throughout the year.

Collections of plaice pituitary glands were made at approximately monthly intervals and the reproductive condition of the animals noted. Each monthly collection was assayed separately and in each assay, the numbers of glands from each sex were kept approximately equal. Since the numbers of glands were limited it was not always possible to use the same dose levels and, in a few assays, the lack of material precluded the use of the extraction technique. The results of these assays are given in Table 23 (pp. 167-168). Since material from fish in different stages in their reproductive cycle was assayed separately, it is possible to compare the response to a standard dose of each monthly collection. The result is illustrated in Fig. 12 which gives the response to a standard dose (10 glands) of acetic acid-acetone extracted material. All the above assays were done at the Pregnancy Diagnosis Laboratory, Edinburgh and the test animals used are employed in the routine assay of pituitary and chorionic gonadotrophins. During the course of a long series of experiments, no animal was observed to spermiate

Table 23.

Results of assays of pituitary material from mature plaice.

Date of collection	Condition of fish	Details of extraction	Dose (1)	Response (2)	%
Jan. 1959	Ripening	Acetic/acetone	= 5 glands	0/5	-
			= 10 glands x	3/5	60
Feb./Mar. 1959	Ripening	Acetic/acetone	= 5 glands	0/5	-
			= 10 glands x	2/5	40
Aug./Sept. 1959	Ripening	Acetic/acetone	= 10 glands	1/5	20
Oct. 1959	Ripening	Acetic/acetone	= 10 glands x	2/5	40
Oct. 1959	Ripening	Acetic/acetone	= 6 glands	2/5	40
			= 12 glands	3/5	60
Dec. 1959	Ripening	Acetic/acetone	= 5 glands	1/5	20
			= 10 glands x	3/5	60
Jan. 1960	Ripening	Saline suspension	= 10 glands	5/5	100
"	"	" "	= 2.5 glands	0/5	-
	"	" "	= 5 glands	2/5	40
Jan. 1960	Ripening	Acetic/acetone	= 5 glands	2/5	40
			= 10 glands x	4/5	80

Table 23 cont.

Mar. 1960	Ripening	Acetic/acetone	= 5 glands	$^{1}/_{6}$	17
			= 10 glands	$^{2}/_{6}$	33
"	Ripe	Acetic/acetone	= 5 glands	$^{2}/_{7}$	28
			= 10 glands x	$^{2}/_{5}$	40
April 1960	Spent	Acetic/acetone	= 10 glands x	$^{0}/_{9}$	-
			= 20 glands	$^{3}/_{5}$	60
June 1960	Spent	Acetic/acetone	= 10 glands x	$^{1}/_{5}$	20
"	"	" "	= 10 glands	$^{1}/_{5}$	20
May 1958	Spent	Acetic/acetone	= 10 glands x	$^{0}/_{5}$	-
June 1958	Spent	Acetic/acetone	= 10 glands	$^{1}/_{5}$	20
July 1958	Spent	Acetic/acetone	= 12 glands	$^{5}/_{5}$	100
Mixed (Winter)	Mixed	Acetic/acetone	= 15 glands	F_1 $^{0}/_{7}$	-
				F_2 $^{0}/_{7}$	-
				F_{1+2} $^{4}/_{7}$	57

1. Data marked 'x' used in Figure 12.

2. The presence of sperm in the urine constitutes a positive response and the fraction indicates the proportion of positive individuals in the group.

spontaneously (Hobson,unpublished). It can therefore be safely assumed that the responses obtained in the present study were not due to spontaneous spermiation. Since these assays were performed over a period of several months, the possibility of a seasonal variation in the response of the toads must be considered. That no such variation did occur is shown in the response of the toads to standard doses of C.G. over the same period (Fig. 13 and Table 24, p.170). In those plaice assays in which the 2 dose levels were used, a dose-response curve was constructed and these are given in Fig. 14 together with the dose-response curve for international preparation ALP (ox) for comparison.

3. Assay of glands from immature fish.

In addition to the assays on mature plaice, several assays were done using glands from immature fish. From Fig. 14, the minimum effective dose which will induce ovulation in 50% of a group of toads (MED 50) is about 7.5 mature plaice glands. No response was obtained when similar or higher dose levels of 'immature glands' were used, except in one assay where a dose level of 25 glands per toad was used (Table 25, p. 171). Most of the glands used in this assay were collected during the autumn and it is possible that some of the fish, although macroscopically immature were maturing for the first time and consequently had begun to secrete hormone.

4. Assay of divided glands.

The technique of separating the glands is described in Methods (p.25). Glands collected in October and December, 1959 were divided into anterior and posterior portions. Saline suspensions were

TABLE 24.

Response of male Xenopus to Laboratory Standard C.C.

Date 1960	Dose	Response	% Response	Probit Analysis
January	0.5 mg	11/20	55	5.12.
	1.0 mg	24/30	80	5.84.
February	0.5 mg	11/20	55	5.12.
	1.0 mg	18/20	90	6.28.
May	0.5 mg	6/10	60	5.25.
	1.0 mg	8/10	80	5.84.
June	0.5 mg	24/40	60	5.25.
	1.0 mg	18/20	90	6.28.
July	0.5 mg	6/10	60	5.25.
	1.0 mg	9/10	90	6.28.
August	0.5 mg	11/20	55	5.12.
	1.0 mg	19/20	95	6.64.
Mean of all observations	0.5 mg	69/120	57.5	5.18.
	1.0 mg	115/130	88.4	6.19.

Table 25.

Bioassay of immature plaice pituitary glands using Xenopus laevis.

Material	No. of glands	Total wt.	Extraction	Dose	Response (1)
Jan/Feb. 1959	146	4.6 mg	Acetic/acetone	= 5 gl (1.5 mg)	0/5
	23 male	ave = 0.31 mg		= 10 gl (3.1 mg)	0/5
	121 female				
April 1959	133	4.3 mg	"	= 10 gl (3.8 mg)	0/5
	31 male	ave = 0.38 mg		= 14 gl (5.3 mg)	0/5
	102 female				
Pooled 1959 (autumn and winter(	77 glands mostly male	26.6 mg	"	= 25 gl (8.5 mg)	1/3
		ave = 0.34 mg			

1. The presence of sperm in the urine constitutes a positive response and the fraction indicate the proportion of positive individuals in the group.

made and the portions assayed separately. The results are expressed in Table 26 (p. 173). A positive response was obtained from both regions of the glands suggesting that a separation of the glands into morphologically and physiologically distinct portions is not possible.

5. Assay of plaice pituitary extracts on hypophysectomised toads.

The use of intact test animals is always open to criticism on the ground that the part played by secretion of hormone from the animal's own pituitary cannot be estimated. Plaice pituitary extracts were therefore assayed on hypophysectomised animals. Since the toads employed in this experiment had not been used for some time, it was thought advisable to test their response to gonadotrophin. Accordingly, 30 toads were injected with 30 units of C.G. and examined for sperm 24 hours later. Twenty-five toads were found to be positive and these were again negative 10 days later. Fifteen of this group of 25 toads were selected at random, hypophysectomised, (see p.19) and placed in a separate tank. The other 10 toads were left intact as controls. Plaice pituitary material and international preparation ALP (ox) (for comparison) were assayed on intact and hypophysectomised animals. Both substances were made up as saline suspensions and left for 24 hours at 5°C. The injections were made on the day following hypophysectomy and the animals were examined for sperm 24 hours later. The results, expressed in Table 27, (p. 174) indicate that plaice pituitary material will induce spermiation in hypophysectomised toads although there is some reduction in the response.

6. The response of female Xenopus laevis to plaice pituitary material.

In view of the results obtained by Otsuka (1956a) (Table 4)

Table 26.

Assay of separated glands on Xenopus laevis.

Material(1)	No. of glands	Total Wt.	Extraction	Dose	Response (2)
Oct - Dec. 1959					
Ant. portion	58	19 mg	Saline suspension	= 2.5 gl (0.76 mg)	0/5
		ave = 0.32 mg		= 7 gl (2.2 mg)	1/5
Post. portion	59	29 mg	Saline suspension	= 10 gl (4.9 mg)	3/5

1. The glands were divided along a transverse groove before drying (see p. 28.)

2. The presence of sperm in the urine constitutes a positive response and the fraction indicates the proportion of positive individuals in each group.

Table 27.

The response of hypophysectomised Xenopus laevis to plaice pituitary material and mammalian gonadotrophin.

Material	No. of glands	Weight	Extraction	Dose	Condition of animal (1)	Response (2)	%
Plaice glands (pooled from several months)	63	34 mg	Saline suspension	= 12 glands (6.48 mg)	Hypophysectomised	3/5	60
	132	64 mg		= 24 glands (11.76 mg)	"	5/5	100
	63	34 mg		= 12 glands (6.48 mg)	Intact	4/5	80
International preparation (ALP (ox))	-	-	Saline suspension	4 mg/toad	Hypophysectomised	5/5	100
"			"	4 mg/toad	Intact	5/5	100

1. The material was given in a single injection 24 hours after hypophysectomy and the animals examined for sperm 24 hours later.

2. The presence of sperm constitutes a positive response and the fraction indicates the proportion of positive individuals in the group.

the response of female toads to plaice pituitary glands was investigated. Dose levels which resulted in spermiation in the male toad failed to produce oviposition in the female. No response was obtained even when the dose level was increased to 30 glands from mature plaice collected in January.

7. The response of male toads to pituitary glands from other teleosts.

Most preparations of plaice pituitary glands induced spermiation in male Xenopus laevis in contrast to the negative results obtained by the majority of other workers and it was decided to investigate the response of the male toad to glands from other species of fish. The results, expressed in Tables 28 and 29 (pp. 176-177) indicate that all the species investigated are capable of inducing spermiation in male Xenopus laevis. More detailed information was not obtained owing to lack of material, but the results show that the dose levels required - 1 to 5 mg - are of the same order in all the species. This weight represents several glands in all species except the cod where a single gland weighs as much as 25 mg. It is interesting to note that a large amount (150 mg) of cod pituitary material failed to elicit oviposition in a single female toad. This toad had ovulated to an injection of C.G. 10 days previously and was therefore considered to be capable of responding to gonadotrophin.

The generous gift of cod pituitary material from Mrs. Peter Woodhead, Fishery Laboratory, Lowestoft is gratefully acknowledged.

Table 28.

Response of male Xenopus laevis to pituitary glands of several species of teleost fish.

Material	Condition of fish	Extraction	Dose glands	Dose in mg.	Response
Hippoglossoides platessoides March 1958	Almost ripe male	Saline suspension	= 5 glands per toad acetone dried	2.1 mg	1/5
			= 10 glands per toad	4.2 mg	2/2
Scomber scomber September 1960	Early vitellogenesis female	"	= 8 glands per toad acetone dried	10.4 mg	1/1
Gadus aeglefinus September 1960	"	"	= 8 glands per toad acetone dried	5.36 mg	3/3
Salmo trutta June 1961	Pre-spawning female	"	= 1 gland per toad fresh	-	2/2

1. The presence of sperm constitutes a positive response and the fraction indicates the number of positive individuals in the group.

Table 29.

The response of male and female Xenopus laevis to pituitary glands from pre-spawning arctic cod.

Assay No.	Material	Weight of glands	Extraction	Dose mg.	Dose glands	Response	%
1	Acetone-dried whole glands from pre-spawning arctic cod. (Gadus morrhua) (2)	4 glands = 65.1 mg. ave. = 16.3 mg.	Saline suspension	= 8mg/toad	= 0.48gl/toad	4/7	57
2	"	30 glands= 190.5mg. ave. = 6.35mg.	"	= 6mg/toad = 12mg/toad	= 0.9gl/toad = 1.88gl/toad	6/10 8/10	60 80
3	"	7 glands= 33.3 mg. ave. = 4.75mg.	"	= 3mg/toad	= 0.63gl/toad	6/10	60
4	"	4 glands= 35.8 mg. ave. = 8.95mg.	"	=1.12mg/toad = 2.24mg/toad	= 0.125gl/toad = 0.25gl/toad	1/10 5/10	10 50
5	"	39 gl = 164.7mg.	"	= 150 mg. injected into 1 female toad	35 glands	0/1	—

(1) The presence of sperm(or oviposition) constitutes a positive response. Fractions indicate the number of positive animals in each group.

(2) Grateful acknowledgement is made of the generous gift of cod pituitary material from Mrs. P. Woodhead, Fishery Laboratory, Lowestoft.

B. Immature mouse uterus assay.

Increase of uterine weight in immature mice has been used for the bioassay of pituitary gonadotrophins by a number of workers. (Levin and Tyndale,1937, Claringbold and Lamond,1957, Lamond and Emmens, 1959). Details of the assay method and the pre-treatment of the pituitary material in the present study are given in Methods (pp.25-27).

It is clear from the summary of the literature given in Table 5 that most mammalian test animals are insensitive to the administration of teleost pituitary material. In view of the success obtained in the present study, using the male toad assay, it was decided to investigate the response of a mammalian test species to plaice pituitary glands.

Most workers using the mouse uterus assay have divided the total dose into 3 equal quantities injected 24 hours apart, but some workers have administered the total dose in a single injection. Claringbold and Lamond (1957), using the latter technique, found that the sensitivity of the assay was reduced, but the slope of the dose-response curve was steeper when the animals were killed 24 hours after 1 injection, than when the same total dose was given in 3 equal injections 24 hours apart and the animals killed 24 hours later. Sensitivity and steepness of the slope are important when the materials under test are scarce since a steep dose-response slope means that smaller quantities of material can be employed. Accordingly the response of the Schofield mice used in the present study was measured when standard amounts of international preparation AIP (ox) were given (a) in a single injection and (b) in 3 injections each containing one third of the total dose. The results,

given in Table 30 (p. 180), indicate that the slope of the dose-response curve is steeper when the material is given in 3 equal injections and this technique was used in all subsequent assays.

Owing to scarcity of material and in view of the large numbers of glands required, only a few assays were performed using plaice pituitary material. In the first assay, the response to a saline suspension of glands was compared to that of an acetic acid-acetone extract (Table 31, p. 181). The responses of the two techniques were not significantly different from one another and only the response to the saline suspension was significantly above that of the saline-injected control group. Accordingly only saline suspensions were used in subsequent assays although the uteri of both the treated groups in the above assay were hyperaemic, suggesting that some stimulation had occurred. In another assay the response to pre-spawning plaice glands was compared with that of international preparation AIP (ox). (Table 32, p.182). The response to both substances was significantly above that of a control group which was injected with saline solution.

Although the intact immature mouse is thought to have little or no circulating gonadotrophin, it has been suggested that the administration of gonadotrophin may stimulate endogenous gonadotrophin production (see Lamond and Emmens,1959). If such is the case, the use of intact mice is suspect in the assay of any gonadotrophic preparation and it was decided to investigate the response of hypophysectomised mice to plaice pituitary material. Lamond and Emmens(1959) found that sheep anterior pituitary preparations showed

Table 30

Comparison of the increase in uterus weight of immature mice when standard doses of international preparation ALP (ox) are given in a single injection or divided into 3 equal injections.

Group No.	No. of animals	Dose	Uterine hyperaemia	Mean uterus weight	t.	
1	5	Saline suspension Single injection = 2.5 mg ALP (ox)	Present	8.0 mg ± 0.1		
2	5	As above = 5.0 mg	"	8.2 mg ± 0.8		
3	5	As above = 10.0 mg	"	8.8 mg ± 0.75		
4	4	3 injections total = 2.5 mg (ALP ox)	"	8.75mg ± 1.9	4 and 5 = 2.1	x
5	4	As above total = 5.0 mg	"	12.00mg ± 2.45	5 and 6	
6	4	As above total = 10.0.mg	"	14.75mg ± 1.29	= 1.99	x
7	6	Saline (control)	Absent	4.0mg ± 0.1		

t. (6 degrees of freedom) = 1.94 - 2.44 (P = 0.10 - 0.05).

x. denotes calculated values of t. which exceed the value corresponding to P = 0.01 i.e. significant difference.

Table 31.

Comparison of immature mouse uterus to a standard dose of plaice pituitary glands given as a suspension and as an acetic acid-acetone extract.

Group No.	No. of animals	Treatment of glands	Dose	Mean uterus weight	t.	Uterine hyperaemia
1	6	Saline suspension of pre-spawning plaice glands	= 12.5 glands = 5.75 mf per mouse	21.5 mg ± 8.6	1 and 3 = 2.89 x	Present
2	6	Acetic acid/acetone extract. Pre-spawning glands	= 12.5 glands per mouse	14.2 mg ± 5.5	2 and 3	Present
3	6	-	Saline (control)	10.2 mg ± 2.6	1 and 2 = 1.75	Absent

t. (10 degrees of freedom) = 2.76 (P = 0.02).

x. denotes calculated values of t. which exceed the value corresponding to P = 0.02. i.e. significant difference.

Table 32.

Response of immature mouse uterus to immature and mature plaice glands compared with international preparation AIP (ox).

Group No.	Treatment of glands	Dose	Mean uterus weight	t.	Uterine hyperaemia	Ovary weight	t.
1 (4)	Saline suspension of pre-spawning glands	= 24 glands = 7.68 mg/mouse	6.75 ± 0.25	1 and 5x =6.81(8)	Present	5.75 ± 0.43	1 and 5 = 4.0(8) x
2 (5)	Saline suspension of glands from immature fish	= 18 glands = 5.58 mg/mouse	4.70 ± 0.5		Absent	4.3 ± 0.4	
3 (6)	Saline suspension	5.0 mg AIP (ox)	10.9 ± 1.4	3 and 5x = 17.3 (10)	Present	5.8 ± 0.4	3 and 5 = 4.41(10)x
4 (6)	"	10.0 mg AIP (ox)	13.8 ± 1.75	4 and 5x = 12.0 (10)	"	6.0 ± 1.09	4 and 5 = 3.20(10)x
5 (6)	-	Saline (control)	4.5 ± 0.76		Absent	4.3 ± 0.74	

Figure in brackets in column 1 is the number of animals in each group.

Figure in brackets in column 5 and 8 is the number of degrees of freedom.

x denotes significant difference (P = 0.05).

little or no increase in E.D. (the effective dose required to produce 100% increase in uterus weight) at hypophysectomy but the E.D. was increased by a factor of from 10 to 20 when the injection was delayed until 24 hours after pituitary removal. The significance of such an increase is obvious when one is dealing with limited quantities of material as in the present study and a pilot assay was done using international preparation ALP (ox). The results of this assay, expressed in Table 33 (p. 184), indicate that both hypophysectomised and intact mice will respond to this substance when injected immediately after hypophysectomy, but that the response is reduced in those mice in which the pituitary has been removed. The hypophysectomised mice were examined for pituitary remnants at the end of the assay and only those mice in which the pituitary gland had been completely removed were included in the results.

Hypophysectomised and intact mice were used to assay pre-spawning plaice glands and the results of this assay, in which international preparation ALP (ox) was used for comparison, are given in Table 34 (p.185). The response of the hypophysectomised mice to two dose levels of plaice pituitary is not significantly above that of the control group, while a significant increase was obtained in intact mice.

Table 33.

Response of the uterus of hypophysectomised and intact mice to international preparation AIP (ox).

Group No.	No. of animals	Consitions	Dose	Mean uterus weight	t	Uterine hyperaemia
1	5	Hypophysect-omised	Saline suspension of AIP (ox) ≡ 5.0 mg/mouse	9.5 mg ± 3.14	1 and 2 = 0.52 (8)	Present
2	5	"	As above ≡ 10.0 mg/mouse	10.4 mg ± 2.2	3 and 4 = 2.81 (8) x	"
3	5	Intact	As above ≡ 5.0 mg/mouse	11.1 mg ± 1.65	1 and 5 = 3.36 (7) x	"
4	5	"	As above ≡ 10.0 mg/mouse	14.2 mg ± 1.86	2 and 5 = 5.65 (7) x	"
5	4	"	Saline (control)	4.75 mg± 0.43	3 and 5 = 8.35 (7) x 4 and 5 = 11.9 (7) x	Absent

x denotes significant difference (P = 0.05).

Figure in brackets refers to degrees of freedom.

Table 34.

Response of the uterus of hypophysectomised and intact mice to pre-spawning plaice glands and international preparation ALP (ox).

Group No.	No. of animals	Condition	Dose	Uterine hyperaemia	Mean uterus weight	t.
1	6	Hypophysectomised	Saline suspension = 24 glands plaice 12 mg/mouse	Absent	4.6 ± 0.63	
2	5	"	As above = 12 glands 6 mg/mouse	"	4.7 ± 0.75	
3	4	"	= 12 mg ALP (ox)	Present	11.9 ± 1.05	3 and 5 = 13.5 (7)x
4	5	Intact	= 12 glands 6 mg/mouse	Present	6.3 ± 0.59	
5	5	"	Saline (control)	Absent	4.6 ± 0.37	4 and 5 = 5.66(8) x

x denotes significant difference (P = 0.02).

Figure in brackets refers to degrees of freedom.

C. Discussion.

1. Bioassay of fish pituitary material.

The literature on gonadotrophin(s) in fish pituitaries shows how sparse the information is. The available data are often poorly documented and details of dose levels and numbers of test animals used are often lacking. From a consideration of the data summarised in Table 4, it will be seen that few positive responses have been produced in male Anura to which fish pituitary material has been administered. Only 15 of the 53 individuals of Rana and Bufo species injected spermiated and in 12 of these, the response was induced by sturgeon pituitaries. The response of the 3 other could not be repeated at higher dose levels and therefore must remain suspect.

With regard to the use of female Anura in the bioassay of fish pituitary glands, the situation is similar. Positive responses have been obtained in only 11 out of at least 18 individuals and all of these were induced by sturgeon pituitaries (Table 4).

2. The factor responsible for the spermiation response in male Anura.

The observation by Galli-Mainini (1947), that C.G. will elicit the release of sperm when injected into male anurans has been used in human pregnancy diagnosis. Male anurans have also been reported to react positively to mammalian pituitary gonadotrophins, but there is considerable discussion as to whether the response is due to F.S.H. or L.H. (Houssay,1949). Creze (1949) reported that L.H. caused the discharge of sperm in Rana esculenta and both F.S.H. and L.H. gave positive reactions in the Indian toad Bufo melanostictus (Bhaduri,1951).

Robbins (1951) and Robbins and Parker (1952) found that F.S.H. caused the discharge of sperm in Rana pipiens, but Thorburg and Hansen (1951) found that Bufo bufo did not respond to F.S.H. at a concentration equivalent to 8 Mouse Units.

Atz and Pickford (1954) studied the response of male Rana pipiens to mammalian chorionic and pituitary gonadotrophins. They found that 0.04 mg (10 int. units) of Antuitrin S (Parke Davis) was the minimum dose of C.G. which would elicit sperm release. Luteinising hormone(L.H. Armour) was active at the same dose level. A "weak response" was obtained from F.S.H. (Armour) at a much higher concentration. They suggested that the response induced by the latter preparation was due to contamination of the material with L.H. since its activity was roughly proportional (100:1) to the amount of L.H. present. On the basis of these and other results, Atz and Pickford suggested that the spermiation response of male anurans and Rana pipiens in particular was elicited specifically by L.H. and that the responses obtained from preparations of F.S.H. and prolaction by other workers was due to contamination of their material with L.H.

Hobson (1952a and personal communication) has found that the dose levels of purified F.S.H. and L.H. required to produce the same response in Xenopus laevis are the same. These animals are extremely sensitive to purified pituitary gonadotrophins.

It would appear that the situation with regard to F.S.H. and L.H. requires further clarification and the significance of the work described above is difficult to assess as the part played by endogenous hormone was

not considered. It is possible that the factor responsible for the release of sperm varies with the anuran species used and that some species will respond either to one or the other, while others will respond to both. A great deal of comparative work is necessary using purified preparations on hypophysectomised test animals before any definite conclusions can be drawn.

3. The bioassay of plaice pituitary material.

It will be seen from Table 23 that plaice pituitary glands contain a hormone (or hormones) which are capable of releasing sperm when injected into male Xenopus laevis. The percentage response to a standard dose (10 glands) of plaice material collected at different times of the year and extracted with acetic acid and acetone is graphed in Figure 4 and it would appear that the gonadotrophic content of the pituitary is highest between late autumn and early spring, while the amount present during the late spring and summer is low. The only exception is the figure for July, 1958 where the response to 12 glands was 100%. A 100% response was obtained in one test in which material from January, 1960 was injected as a suspension. The general shape of the graph strongly resembles that of the variations in gonad weight throughout the year. The higher responses obtained for winter material correspond to the period of maximum gonad growth while the low spring and summer responses correspond to the post-spawning period when the gonad weight is least. On this basis it might be suggested that the pituitary activity is greatest during spermatogenesis and ovum development and decreases when the reproduction products are formed and shed. The high response to

material collected in July is difficult to explain, but it is significant that this corresponds to the period when growth is initiated in the gonads and there may be a sudden build-up in the hormone in the pituitary at this time.

Variations in pituitary hormone levels can be interpreted in two radically different ways. One is that the pituitary content is low during periods of gonadal activity, the hormone being liberated into the circulation as quickly as it is secreted, and high during quiescent periods when it is stored in the gland. The second alternative is that a high hormone titre is indicative of increased activity and vice versa. The results obtained using plaice material could indicate that the latter is more correct but the true interpretation will only be obtained when it is possible to assay the gonadotrophic levels in blood samples taken throughout the year and when these results are compared with assays from pituitary material collected at the same time.

The results discussed above are based on data from the assay of nearly 2,000 pituitary glands. Much more information is necessary, but the large numbers of glands required and the difficulties associated with their collection make the work extremely laborious.

The dose-response curves in Figure 14 are based on assays in which positive responses were obtained at two dose levels. By extrapolation it is possible to obtain an estimate of the M.E.D. 50 (Minimum Effective Dose required to obtain a response in 50% of the animals) for plaice material at different times of the year. It will be seen that the M.E.D. 50 varies between about 3 mg (6 glands) and 7.5 mg (15 glands). The M.E.D. 50 for international preparation ALP (ox),

assayed for comparison is 1.1 mg and the slope of the curve is similar to those of plaice pituitary material. It is not justifiable at the moment to assume that the gonadotrophin(s) of the plaice pituitary gland are chemically similar to those of the AIP (ox) but the similarity of the dose-response curves is suggestive. The gonadotrophic potency of the plaice pituitary material cannot therefore be stated in terms of AIP (ox) units and the most that can be said is than an equivalent response is obtained from 1.1 mg AIP (ox) and 3 mg pre-spawning plaice glands when both preparations are assayed on male Xenopus laevis.

4. The nature of plaice gonadotrophins.

As stated earlier, it is not known whether the spermiation of male Anura is due to F.S.H. or L.H. Rana temporia and Rana esculenta both have been held to be specific for L.H. If this is so then it would appear that sturgeon pituitary material contains L.H. (see Table 4). Spermiation in male Xenopus laevis appears to be elicited equally by either L.H. or F.S.H. but ovulation in female Xenopus laevis seems to be induced only by L.H. (Hobson, personal communication). No response has been obtained when plaice pituitary material was injected into female Xenopus laevis. Only 2 tests have been done, each on a single animal, and the doses given were approximately equivalent to 30 plaice glands per animal. Recent work (Barr, unpublished) indicates that male Xenopus laevis is at least 5 times as sensitive to Xenopus pituitary glands as is the female and it is possible that the amount of plaice material injected was not sufficient to induce ovulation.

The results obtained using cod glands, where a ten to twenty-fold increase in dose failed to produce ovulation, suggest that this is not the case. The alternative is that the plaice gland contains mainly an F.S.H.-like hormone and consequently could not be expected to give a positive response in the female toad. The difficulties concerned with continuing this problem become obvious when one considers that about 500 glands would be needed to do a single assay using 2 dose levels.

5. The site of production of plaice gonadotrophin.

There is no physiological evidence for the site of production of gonadotrophin in the meso-adenohypophysis. Such evidence as exists is cytological, based on the histochemical demonstration of mucopolysaccharide in the meso-adenohypophysis of teleost. The plaice pituitary gland is divided into anterior and posterior portions by a narrow groove round the gland at the level of the infundibular stalk. A number of pituitary glands were divided along the groove and the 2 portions assayed separately (see Table 26). Since neural tissue was absent, or at the most present in very small quantities in the anterior portion, the source of gonadotrophin cannot lie in the neurohypophysis or the meta-adenohypophysis. This suggests that the site of gonadotrophin lies largely in the meso-adenohypophysis as would be expected from the histochemical studies discussed earlier (p.162), but it does not preclude the possibility of gonadotrophin secretion by the pro-adenohypophysis.

6. Gonadotrophin content of the immature pituitary.

The results of a number of assays of pituitary glands from immature fish are presented in Table 25. Pituitary glands collected

in January, 1959 from mature plaice gave a response at a dose level of 10 glands per toad, but similar dose levels of glands collected in the same period from immature fish failed to evoke a response. A positive response was obtained in one of 3 animals injected with a dose equivalent to 25 glands. The material was collected at various times of the year and may have been contaminated with glands from animals which were spent or maturing for the first time: these animals are very difficult to distinguish from mature fish during the summer and early autumn before the gonad growth begins. The results indicate the gonadotrophins are either absent in the immature plaice pituitary or are present in quantities insufficient to induce spermiation in male Xenopus laevis.

7. Assay of plaice pituitary material using immature female mice.

Several assays of plaice pituitary material were carried out using the increase in uterine weight of 19 day old immature mice as an end point. A total of 1,000 glands was used and almost 250 of these were used in the assays detailed in Tables 31, 32 and 23. Dose levels of 5 and 10 glands per animal were used in several other assays (not detailed), in none of which was the uterine weight significantly above that of the controls. From the results expressed in Table 32, it is clear that plaice pituitary glands are capable of stimulating the immature mouse uterus. A dose level of 24 glands resulted in a 50% increase in the weight of the uteri which were distinctly hyperaemic. In order to determine whether the response was a direct one or whether the co-operation of endogenous F.S.H. was necessary for steroid production (hence uterine

growth), plaice glands were assayed on hypophysectomised mice. Lamond and Emmens (1959) found that the response of hypophysectomised mice to placental gonadotrophins was considerably reduced and that to pituitary gonadotrophin reduced to some extent when the injections were delayed until 24 hours after pituitary removal. They suggest that the limiting factor in the response of intact mice to human C.G. is endogenous F.S.H. and that to P.M.S. is endogenous L.H. The reduced activity of pituitary gonadotrophin is not due to absence of endogenous gonadotrophin but to decreased sensivity of the test animal or to "a factor or factors not yet recognised".

It will be seen from Table 33, that the response of hypophysectomised mice to mammalian pituitary gonadotrophin is reduced but by no means abolished, in accordance with the results of Lamond and Emmens. Plaice pituitary material elicited no response in hypophysectomised mice at dose levels equivalent to 12 and 24 glands per mouse. The response of intact mice to 12 glands was significantly above that of the controls and the uteri were hyperaemic. (Table 34).

The response of intact mice to plaice gonadotrophin, although significant, is slight when compared with that of mammalian pituitary material. This suggests that the dose levels have not been sufficiently high and are of a threshold nature. Similar results have been obtained from salmon and tunny pituitary by Otsuka (1956b and c). The dose levels used by Otsuka produced a considerable increase in the ovary weight of immature mice although only slight increases were noted in uterine weight (Table 5). This is contrary to the results obtained

in the present study and is in direct contrast to the results of most other workers where the maximum uterine response is reached before there is any increase in ovary weight. It is clear from the above discussion that a great deal of comparative work is required before any definite conclusions can be reached.

8. Species specificity of gonadotrophins.

The early evidence for species specificity of piscine gonadotrophins came from the observation by a number of workers that various species of female anurans did not respond to fish pituitary material (review by Creaser and Gorbman,1939). Experiments of a reciprocal nature, in which pituitary preparations from other vertebrate classes were administered to various species of fish, were also negative (Tuchman,1936, von Ihering and de Azevedo,1937, de Azevedo and Canale,1938, Matthews,1939). Although there were reports of positive results from the treatment of fishes with amphibian and mammalian gonadotrophin (Craig-Bennett,1931, Wills et al,1933, Noble and Kumpf,1936), it was generally held that considerable differences existed between the gonadotrophins of fishes and those of Amphibia and mammals and that these differences affected the biological activity of the hormones when there was a considerable taxonomic difference between recipient and donor species.

The large volume of subsequent work, much of it contradictory and poorly documented, in which the effect of injection of pituitary material from fish and amphibia into fish has been studied, has been reviewed by Pickford and Atz (1957). These authors, considering all

the available data state that "experiments involving administration of amphibian pituitaries into teleost fishes were more successful (in producing gonad stimulation) than those involving pituitary glands from different orders and families of fishes and apparently as successful as when donor and recipient belonged to the same species of fish". They conclude that no absolute biological specificity of vertebrate gonadotrophin has been demonstrated.

Wills, Riley and Stubbs (1933) and Stroganov and Alpatov (1951), using anuran recipients, and Witschi (1955), using rats, obtained positive responses from piscine gonadotrophin (see Table 4 and 5). It is interesting to note that the donor species belong to two groups of primitive fishes (Holostei and Chondrostei) which are believed to be phylogenetically closer to the Amphibia than any of the "higher" teleosts tested. These facts have been used as evidence in support of the hypothesis of species specificity. The evidence presented in the present study, in which the donor species belong to the Teleostei, a group which bears only a remote evolutionary relationship to the Amphibia, considerably reduce the importance of the above findings as evidence for such a specificity.

It is clear that a great deal of further information is needed before it is possible to draw any definite conclusions as to the existence of taxonomic specificity of gonadotrophins. There is evidence that the amount of hormone present in the teleost pituitary gland varies throughout the year (Barr,1960 in press, Gerbilskii,1940) and this may account for many of the negative results. More attention

should be paid to the reproductive state of both donor and recipient species, to the way in which the material is administered and to the proper husbandry and care of test animals.

In spite of the above criticisms, it is felt that some degree of specificity does exist since most critical studies have shown that any test species is more sensitive to gonadotrophin obtained from other individuals of the same species than to that from species belonging to other classes. That differences do exist in the chemical composition of gonadotrophins of various vertebrate groups is highly probable. This is to be expected on the basis of the existing knowledge of the immune properties and biochemistry of proteins in general and of the anterior pituitary hormones of mammals in particular (Evans and Simpson (1950), Hays and Steelman (1955)). It seems reasonable, therefore, that such differences should be of some importance in comparative bioassays.

X. General Discussion and Conclusions.

The events associated with oogenesis and spermatogenesis in the plaice are fundamentally the same as in other vertebrate groups. In common with many other teleosts, this species has a single sexual cycle in which ovulation and spermiation occur only once per year. Unlike the anoestrous period of mammals, the long interval between successive spawnings is occupied by a phase of active growth in which the sex products gradually mature. By comparison with mammals, the numbers of gametes liberated at each spawning period are extremely large, particularly in the female where several tens of thousands of eggs are shed. As a consequence of this, and in direct contrast to the mammalian position, the number of primordial germ cells present in the plaice gonad at puberty is not sufficient to supply all the gametes produced during the reproductive life of the individual. This deficiency has been met by an annual mitotic increase in the number of primary germ cells and their subsequent meiotic division during oogenesis and spermatogenesis. Although the sequence of events which comprise the plaice sexual cycle is the same in both cases, there is some difference in the length of time which each phase occupies. The spent gonad is reconstituted by the mitotic division of reserve cells and this phase is followed by the meiotic division of the newly produced generations of spermatogonia and primary oocytes. This phase, which begins shortly after spawning in the female, is not initiated in the male till the autumn. Meiosis in the male is completed in the population in January and ripe spermatozoa are present for several

weeks before spawning begins. In the female, the primary oocytes enter the first meiotic prophase, but remain in the diplotene condition until the following spring. Meiotic division, accompanied by the production of polar bodies has not been observed in the plaice and it probably occurs after the oocytes have been expelled from the ovary. During the long diplotene period, vitellogenesis takes place in the oocytes which at this stage are homologous with the primary spermatocytes. The climax of the sexual cycle occurs in both sexes in the spring with the liberation of the sex products.

The functional significance of the corpus atreticum in the teleost ovary has been the subject of a considerable controversy. The corpus atreticum, or so-called "pre-ovulation corpus luteum", is of widespread occurrence in fishes and most of our knowledge of its formation and possible functions comes from the work of Bretschneider and de Wit (1947) on Rhodeus amarus. These authors found that changes in the pituitary basophils and the formation of corpora atretica following the immersion of female Rhodeus amarus in solutions of steroid hormones were indicative of the influence of gonadotrophin on the formation of these structures since they were not produced in a single hypophysectomised animal which received the same treatment. Further evidence for the secretory nature of the corpus atreticum was indicated by the correlations which were found between the number of such structures present at various stages of development, the number of basophils in the pituitary and the length of the female bitterling's ovipositor. Bretschneider and de Wit concluded that the corpus atreticum was the site of production of oestrogen in the ovary

and this view is also held by Hoar (1955). The work of the Dutch authors has been severely criticised (see Pickford and Atz,1957), but it has received some support in a recent paper by Ball (1960). This author suggests that the changes consequent on the immersion of Rhodeus amarus in steroid solutions were reactions to stress and agrees that the special pituitary factor causing follicular atresia postulated by Bretschneider and de Wit is improbable since the most effective way of inducing atresia is to remove the pituitary.

Ball also subscribes to the view that the corpus atreticum is the source of oestrogen, but he gives no new evidence in support of this hypothesis, basing his conclusions on the re-interpretation of the existing literature on the subject. During the earlier stages of the formation of corpora atretica, the component cells are cholesterol negative in the Schultz test, but they are strongly positive in the later phases of atresia. From this, Ball infers that the cells of the earlier phases are actively utilising cholesterol and after the degeneration of the oocyte, the cholesterol accumulates in amounts sufficient to be detected histochemically. He then suggests that the activity of the cells leads to the elaboration of ovarian steroid hormones. The maximum number of corpora atretica occurs when, or just before, the ovary is fully developed and this is used as confirmation of Ball's hypothesis since the production of oestrogen would be expected to be high at this time by analogy with the situation in mammals.

The results obtained in the present study have a direct bearing on this question. The events associated with follicular

atresia in the normal plaice ovary are similar to these described in other teleosts. Degeneration of the oocyte is accompanied by phagocytosis of the zona radiata by cells of the granulosa. Yolk material is resorbed and the interior of the egg is completely filled by invading granulosa cells. Atresia occurs at two different stages in the sexual cycle of the mature female. The mature oocytes which are retained in the ovary after the natural ovulation has been completed are transformed into corpora atretica and persist for several months as masses of tissue much larger than the surrounding oocytes of the post-spawning ovary. These atretic follicles are not of frequent occurrence and are not universally present. Atresia of small developing oocytes has not been observed during the summer and is next seen in a few developing oocytes in November. From then until the spawning season occasional atretic follicles in various stages of development are present. They have not been noticed in all the ovaries examined and it should be emphasised that there is no suggestion of an increase in the frequency of their occurrence as the breeding season approaches.

Atretic follicles are not present in the ovaries of immature plaice. "Aborted eggs" have been described in the immature plaice ovary by Cunningham (1897) but these degenerate without being converted into corpora atretica.

The atresia found in the oocytes of hypophysectomised plaice closely resembles those of normal ovaries in structure and mode of formation and is confined to those oocytes in which vitellogenesis has begun.

The evidence from the present study suggests that the corpus atreticum is formed as a consequence of gonadotrophin withdrawal since it is found in the post-spawning ovary when pituitary gonadotrophin secretion is low. The small number found in the maturing ovary may be accounted for by mechanial failure of the follicle to provide sufficient nourishment for the developing oocyte. The secretory nature of the corpus atreticum must therefore remain in doubt until physiological data demonstrating their role in the production of oestrogen are forthcoming.

The general arrangement of the regions of the plaice pituitary gland is basically the same as that found in other teleost species. Most authors are agreed that the source of gonadotrophin is in one of the basophil cell types of the meso-adenohypophysis. This view is based on the observation that this cell type increases in number and activity during the maturation phase of the gonads (Pickford and Ata,1957).

The basophils of the plaice pituitary are divided into 2 morphologically distinct zones which are situated in different regions of the meso-adenohypophysis. Cyclical changes have been found in the zone 2 cells, which increase in number and activity during the autumn and winter . During the breeding season, these cells become shrunken and their numbers are reduced although they are by no means absent in the pituitary immediately after spawning. The cyclical activity of the basophils is slightly out of phase with that of the gonads during the early summer, since maturation of the gonads, characterised by the onset of vitellogenesis in the female and spermatogonial multiplication in the

male, is initiated before the basophilia of the zone 2 cells again becomes noticeable.

Gonadotrophins and thyrotrophins are known to be glycoproteins and the PAS reaction has been successfully used to locate such substances in the basophils of the plaice pituitary. Several authors, using mammalian and teleost pituitary material have considered the AF reaction to differentiate between gonadotrophs and thyrotrophs since both are PAS positive but only the latter are AF positive. Both zones of basophils in the plaice have been found to be AF positive and as several other workers have been unable to differentiate between the two types in other teleost species, the selectiveness of this technique must be suspect.

That the gonadotrophs of the plaice pituitary are PAS positive basophil cells located in the meso-adenohypophysis appears to be demonstrated by the tinctorial and histochemical reactions discussed above. The plaice pituitary gland can be separated into anterior and posterior portions along a transverse groove. The results given in Table 26 (p. 173) suggest that the anterior portion contains a larger amount of gonadotrophin when the two portions were assayed separately on male Xenopus laevis. In agreement with the findings of Kasanskii and Persov (1948), this can be taken as physiological evidence in support of the above view since the anterior portion consists largely of the pro- and meso-adenohypophysis. This does not rule out the possibility that gonadotrophin is secreted by both of these regions. This interpretation must be borne in mind when considering the great secretory activity in the follicles of the pro-adenohypophysis of the herring at

spawning time (Buchmann,1940).

The results of the bioassay of plaice pituitary glands in male Xenopus laevis indicate that gonadotrophin is present in the plaice pituitary and that it is subject to fluctuation in amount throughout the year. This variation corresponds closely with the cyclical variation of the some 2 basophils of the pituitary and there is little doubt that both are closely related to the cycle of activity found in the gonads.

In agreement with the findings of other workers, the present investigation on the effect of hypophysectomy on the gonads has shown that the plaice sexual cycle, at least at some periods, is under the influence of gonadotrophin secreted by the pituitary. It has been conclusively demonstrated that the initiation of vitellogenesis in the developing primary oocyte is induced by gonadotrophin since it is completely inhibited by hypophysectomy. The maintenance and further development of those oocytes in which vitellogenesis has started is also under pituitary control since such development is arrested after hypophysectomy and the oocytes are converted into corpora atretica. The results of the present study indicate that the pituitary gland is not involved in the maintenance of the primary oocytes and their development up to the stage of yolk deposition.

In view of the difficulty of determining the exact degree of maturation of the oocytes of the pre-spawning ovary, it has not been conclusively demonstrated that the ovulation and oviposition of ripe oocytes is under pituitary control in the plaice. Hypophysectomy is followed by atresia when performed during the breeding season, but it is

known that the eggs do not all ripen at once and a partial spawning may have escaped unnoticed. Other factors, such as the adverse effects of handling, may also have been involved in the atresia which resulted.

The intergrity of the pituitary gland is an essential requirement in the initiation of spermatogenesis in the male, since pituitary ablation before the onset of this process inhibits the production of any stages beyond spermatogonia. It is not clear from the present study whether those cells which have entered spermatogenesis can continue the process in the absence of gonadotrophin secretion, but spermiation takes place normally if hypophysectomy is delayed until spermatogenesis is complete.

As with the primary oocytes of the female, spermatogonia are unaffected by hypophysectomy and can persist indefinitely in the immature fish and in the post-spawning adult male. There is, however, some indication that the mitotic division of spermatogonia is also inhibited but this may reflect a decreased metabolism consequent on the withdrawal of other pituitary hormones.

The effect of administration of fish pituitary glands into fish has received considerable attention, perhaps because of its important commercial aspects. The stimulating effect on gonads of both sexes by implantation and injection of fish pituitary material is well established. (see review by Dodd,1960). The work of Robertson and Rinfret (1957) is particularly important since they have used purified fractions of pituitary glands. These authors have produced full maturation of the testis, including shedding of motile spermatozoa,

of immature male Salmo gairdneri after 2 months of treatment with such extracts administered in cholesterol pellets. There are several records showing that the gonads are relatively insensitive at certain periods of the breeding cycle (Pickford and Atz,1957), but there is little doubt that both the development of the gametes and their emission can be induced.

These findings are confirmed by the results of the present study which differ from these discussed above in that the recipient individuals were hypophysectomised. Vitellogenesis has been initiated in the primary oocytes of plaice which were hypophysectomised more than 6 months before the start of the experiment, confirming that primary oocytes are unaffected by pituitary ablation. The oviposition of healthy ripe oocytes has also been produced by the injection of plaice pituitary material into hypophysectomised plaice. This suggests that natural oviposition is under pituitary control, but such an interpretation must be viewed with caution in view of the nearness of the recipients to the spawning period.

The literature on the effect of administering mammalian pituitary and placental gonadotrophins is very extensive and has been reviewed recently by Pickford and Atz (1957) and Dodd (1960). Many authors have shown that mammalian anterior lobe pituitary (ALP) preparations are capable of accelerating the maturation of the teleost gonad and inducing the emission of mature gametes, but completely negative results have also been obtained. It is possible that these discordant results can be explained by hormone specificity and

seasonal unresponsiveness of the gonads, though there are probably real differences in the ability of different species to respond to one and the same gonadotrophin. Several recent workers, however, have obtained considerable success and the relative effectiveness of mammalian FSH and LH has been studied. Pickford (1953 and in Pickford and Atz,1957) injected FSH and LH fractions of swine pituitaries into hypophysectomised male Fundulus heteroclitus and found that their testes increased in weight. The FSH preparation was only one fifth to one tenth as active as the LH preparation in increasing the "gonosomatic index" and the former preparation contains 3-4% LH, which is probably sufficient to account for the degree of stimulation produced by the FSH preparation.

Several species of fish have been treated with chorionic gonadotrophin (CG) and pregnant mare's serum (PMS). Although the results are somewhat discordant, there is no doubt that under good experimental conditions, CG is capable of activating the germinal tissue and inducing spawning in a wide range of teleosts. (Pickford and Atz,1957). Fewer data are available on the effects of PMS on fish gonads and the results are again contradictory. Stimulation of the gonads has been obtained, but several species have been found to be refactory, even in the breeding season.

The results obtained in the present study suggests that CG (Pregnyl, Organon) is capable of inducing premature maturation and oviposition in female plaice but that these responses are not induced by PMS (Oestyl, Organon).

In much of the work on mammalian gonadotrophins and their effects in fish, an attempt has been made to interpret experimental results in the light of the endocrine control of reproduction in mammals. This attempt has only been partly successful, but there is little doubt that fish gonadotrophin(s) perform similar functions to their mammalian counterparts. Mammalian LH has been found to induce spermiation and ovulation in fish, but it also appears to have an FSH-like effect on the germinal tissue. This view is corroborated by the results of experiments with CG which has an LH-like action in mammals. There is little evidence in the literature for the existence of an FSH-like gonadotrophin in fish, though the germinal epithelium is clearly under the influence of the pituitary.

Witschi (1955), on the basis of the vaginal cornification test in rats and the feather reaction in weaver-finches, attempted to compare the relative amounts of FSH and LH in representatives of different vertebrate classes. He concluded that the fish pituitary is low in FSH, but contains an amount of LH similar to that found in mammals and suggests that this indicates that the fish pituitary holds a store of LH whereas it produces FSH only for immediate use. Witschi also suggests that LH may be of greater importance than FSH in the lower vertebrates and that in the female, induction of ovulation is a more ancient function than its role in corpus luteum formation. The evidence for this hypothesis is based on the measurement of chemically diverse hormones by assays on mammals and birds and it is difficult to assess its significance. The importance of the pituitary during

follicular growth, however, has been conclusively demonstrated by hypophysectomy and vitellogenesis can be induced by the administration of fish pituitary extracts suggesting that an FSH-like hormone is stored, at least to some extent, in the pituitary.

In conclusion, it can be said that the sexual cycle in plaice is under the control of pituitary gonadotrophin. The suppression of vitellogenesis and spermatogenesis by hypophysectomy, and the capacity of plaice pituitary extracts to induce follicular growth can be taken as evidence of the presence of an FSH-like hormone in the plaice pituitary gland. Supporting evidence of a circumstantial nature is provided by the differential nature of the response of male and female Xenopus laevis to injected plaice pituitary extracts. The ability of plaice pituitary material and C.G. to produce ovulation in hypophysectomised subjects indicates that an LH-like hormone is concerned in the control of the sexual cycle, although the ability of hypophysectomised males to spermiate does not fit in with this hypothesis since this function is thought to be under the control of LH in mammals. It is not possible, therefore, to say whether more than one gonadotrophin exists in fish and the final answer must await the chemical characterisation of the hormones.

XI. Summary.

A detailed study has been made of the sexual cycle in the plaice, Pleuronectes platessa. The spawning season is in spring and the liberation of the reproductive products is followed by a period of mitotic activity during which the numbers of primary germ cells is increased. The meiotic division of spermatogonia in the testis begins in autumn and is paralleled by the initiation of vitellogenesis in the ovary. Motile sperm are present in the testis for several weeks before the spawning period, but ripe oocytes are not produced until the spawning season is about to begin.

The cytology of the corpus atreticum and the ruptured follicle or "corpus luteum" is described and their functional significance is discussed. It is concluded that the endocrine nature of these structures has not been demonstrated.

A technique for the removal of the pituitary gland has been developed and post-operative survivals of more than one year have been obtained. Much post-operative care is required and all experimental fish had to be force-fed.

The effect of hypophysectomy has been studied on the gonads of plaice at different phases of their annual sexual cycle. In the female, it is clear that the presence of pituitary gonadotrophin is essential for the initiation of vitellogenesis and for the maintenance and continued development of yolked eggs. Gonadotrophin withdrawal results in the conversion of all yolked eggs into corpora atretica. Although the oviposition of ripe oocytes is inhibited after hypophysectomy,

it has been conclusively demonstrated that this phase of the cycle is under the control of gonadotrophin. Pituitary ablation has no effect on the primary oocytes of the immature fish and these cells in the post-spawning adult female can continue their development up to the stage of yolk deposition at which point, further development is arrested. The capacity of primary oocytes to enter vitellogenesis is retained in the absence of the pituitary since yolk deposition can be stimulated by the injection of extract of plaice pituitary glands into hypophysectomised animals.

In the male, hypophysectomy results in the inhibition of spermatogenesis, although there is some evidence that this process, once started, can continue in the absence of pituitary gonadotrophin. In contrast to the situation in the female, spermiation is not prevented by pituitary removal. In both mature and immature fish, the spermatogonia are not influenced by pituitary ablation, but there is some evidence that mitotic division of these cells is prevented.

The cytology of the plaice pituitary gland is described and 2 zones of basophils have been identified in the meso-adenohypophysis. The presence of glycoprotein material in these cells has been established by the use of the periodic acid Schiff reaction and the granules of both cell types are aldehyde fuchsin positive. Considerable variation was found in the numbers of zone 2 basophils throughout the year and evidence is presented that these basophils are responsible for the secretion of gonadotrophin.

A technique for the bioassay of plaice gonadotrophin is described using male Xenopus laevis as a test animal. The results indicate that the amount of gonadotrophin present in the pituitary fluctuates during the year, being least in the post-spawning period of the fish and increasing in the autumn and winter during spermatogenesis and oogenesis.

Pituitary glands from several other teleost species have also been assayed on the same test animal and the MED 50 (minimum effective dose producing a 50% response) is of the same order (5 - 10 mg.) for all species except the cod where it is about 2.25 mg.

Plaice gonadotrophin is capable of stimulating the uterus of the immature female mouse, but the amount of material required are large and the response is considered to be of a threshold nature. In view of this, mammalian test species are thought to be unsuitable for the assay of teleost gonadotrophin.

The nature of the gonadotrophins of the plaice pituitary is discussed and evidence is presented which indicates that the pituitary contains a hormone, the follicle stimulating properties of which resembles those of mammalian follicle stimulating hormone. The evidence for the presence of a hormone comparable to mammalian luteinising hormone is not conclusive.

The concept of the species specificity of vertebrate gonadotrophin is discussed. A comparison of the MED 50 obtained when plaice and Xenopus laevis pituitary material is assayed on male Xenopus laevis suggests that such a specificity does exist.

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Explanation of Plates.

Plate 1	Fig 1.	Apparatus used in force-feeding.
	Fig 2.	Force-feeding technique.
Plate 2	Fig 1.	View of buccal cavity showing position of incision for hypophysectomy.
	Fig 2.	View of buccal cavity showing trephine hole. Operculum removed.
Plate 3	Fig 1.	T.S. ovary showing stages in oogenesis. (x850).
	Fig 2.	T.S. ovary showing leptotene stage of meiosis. (x1500).
Plate 4	Fig 1.	T.S. ovary of ripening fish showing stages in oogenesis. (x150).
	Fig 2.	T.S. ovary of ripening fish showing vitellogenesis. (x85).
Plate 5	Fig 1	T.S. ovary of fish approaching spawning condition. (x250).
	Fig 2.	T.S. ovary of spent fish showing empty follicles. (x250).
Plate 6	Fig 1.	T.S. ovary of spent fish showing structure of empty follicle. (x650).
	Fig 2.	T.S. ovary of spent fish showing an early stage in the conversion of an unshed oocyte to a corpus atreticum. (x70).
Plate 7	Fig 1.	T.S. ovary of spent fish from June, showing later stage in the development of a post ovulation corpus atreticum. (x130).
	Fig 2.	Detail of Fig 1 showing relationship of theca interna and granulosa. (x875).

Plate 8 Fig 1. T.S. ovary from November showing an early stage of atresia in a developing oocyte. (x120).

Fig 2. T.S. ovary from January showing a later stage in atresia. (x100).

Plate 9 Fig 1. Detail of corpus atreticum shown in Plate 8, Fig. 2, showing phagocytosis of zona radiata by granulosa cells. (x1500).

Fig 2. As Fig 1 showing 'ovoid bodies' in association with yolk globules. (x1500).

Plate 10 Fig 1. Detail of same corpus atreticum showing multiplication and hypertrophy of theca and granulosa. (x850).

Fig 2. As Fig 1 showing mitotic division of theca. (x1500).

Plate 11 Fig 1. T.S. ovary 52 days after hypophysectomy in October(3H3) showing two stages in the formation of corpora atretica. (x140).

Fig 2. Detail of early corpus atreticum shown in Fig 1 showing invasion of anterior by granulosa cells. (x1500).

Plate 12 Fig 1. T.S. ovary 61 days after hypophysectomy in November(3H15). Note that all developing oocytes are being converted into corpora atretica. (x70).

Fig 2. T.S. ovary 78 days after hypophysectomy in October (3H1) showing fully formed corpora atretica. (x90).

Plate 13 Fig 1. Detail of atretic follicle in 3H1 showing relationship of theca and granulosa (x1500).

Fig 2. T.S. ovary 79 days after hypophysectomy in January (6H5) showing fully formed corpora atretica and absence of ripening oocytes. (x70).

Plate 14 Fig 1. Detail of atretic follicle in 6H5 showing interior of corpus atreticum. (x1500).

Fig 2. T.S. ovary 241 days after hypophysectomy in February (7H24) showing degeneration of corpus atreticum. (x140).

Plate 15 Fig 1. T.S. ovary 375 days after hypophysectomy in March (8H27) showing complete absence of vitellogenesis. Note presence of normal primary oocytes (x110).

Fig 2. T.S. ovary 351 days after mock operation in March (8H10C) showing normal vitellogenesis. (x100).

Plate 16 Fig 1. T.S. ovary of immature fish 77 days after hypophysectomy in October (2H8) showing absence of breakdown. (x130).

Fig 2. T.S. ovary of immature fish 72 days after mock operation in October (2H23C) showing normal primary oocytes. (x130).

Plate 17 Fig 1. T.S. normal ovary at beginning of vitellogenesis (x75). Compare with Plate 17, Fig. 2 and Plate 18, Fig. 1.

Fig 2. T.S. ovary 182 days after hypophysectomy in February, before beginning of period of reconstitution. Note reduction in number of oocytes. Compare with Plate 17, Fig. 1 and Plate 18, Fig. 1. (x75).

Plate 18 Fig 1. T.S. ovary 112 days after hypophysectomy during period of reconstitution in April (9H17). Note some reduction in the number of oocytes. Compare with Plate 17, Figs. 1 and 2. (x75).

Fig 2. T.S. ovary 6 months after hypophysectomy (1HR2) showing induction of vitellogenesis by injection of plaice pituitary material collected in summer. Compare with Plate 19, Fig. 1. (x90).

Plate 19 Fig 1. T.S. ovary 6 months after hypophysectomy (1HR3). showing induction of vitellogenesis by injection of plaice pituitary material collected in winter . Compare with Plate 18, Fig. 2. (x90).

Fig 2. T.S. testis of post spawning fish showing spermatogonia and mitotic division. (x1500).

Plate 20 Fig 1. T.S. testis from December showing stages in spermatogenesis. (x650).

Fig 2. T.S. testis of ripe fish showing sperm. Note cyst of reserve spermatogonia. (x500).

Plate 21 Fig 1. T.S. testis of ripe fish showing lobule boundary cells. (x1500).

Fig 2. T.S. testis of spent fish showing interstitial cells and reconstitution of testis. (x1500).

Plate 22 Fig 1. T.S. testis 130 days after hypophysectomy in January (6H13), showing residual sperm and reserve spermatogonia. (x110).

Fig 2. T.S. testis 129 days after mock operation in January (6H24C). Note similarity to Fig 1. (x110).

Plate 23 Fig 1. T.S. testis 378 days after hypophysectomy in March (8H4). Note absence of spermatogenesis and development of connective tissue. Compare with Plate 24, Fig. 1. (x700).

Fig 2. Testis of 8H4 and testis from a normal fish taken from the sea at the same time for comparison. (x2).

Plate 24 Fig 1. T.S. testis 376 days after mock operation in March (8H15C). Note development of sperm. Compare with Plate 23, Fig. 1. (x650).

Fig 2. T.S. testis 73 days after mock operation in March (15H5C) showing reduction in lobule size after spermiation. The connective tissue stains strongly with Heidenhain. Compare with Plate 25, Fig. 1. (x70).

Plate 25 Fig 1. T.S. testis 81 days after hypophysectomy in March (15H3) showing reduction in lobule size after spermiation. Connective fibres do not stain with Mayer's haemalum. Compare with previous figure. (x70).

Fig 2. T.S. testis of immature fish 20 days after hypophysectomy (2H2). Note similarity to Plate 26, Fig. 1. (x550).

Plate 26 Fig 1. T.S. testis of immature fish 21 days after mock operation (2H320). Note typical cysts of spermatogonia. Compare with Plate 25, Fig. 2. (x550).

Fig 2. Dorsal view of pituitary gland showing transverse groove.

Plate 27 Fig 1. L.S. pituitary showing arrangement of cells in pro-adenohypophysis. Azan (x600).

Fig 2. L.S. pituitary showing arrangement of cells in meso-adenohypophysis. Note regularity of acidophils. Azan (x600).

Plate 28 Fig 1. L.S. pituitary showing basophilic material in zone 1 of meso-adenohypophysis. Azan (x600).

Fig 2. L.S. pituitary showing arrangement of cells in meta-adenohypophysis. Azan (x600).

Plate 29 Fig 1. L.S. pituitary showing arrangement of regions. Azan (x50).

Fig 2. L.S. pituitary showing distribution of PAS positive cells. Note PAS cells in meta-adenohypophysis. Pituitary detached and turned over. PAS. (x55).

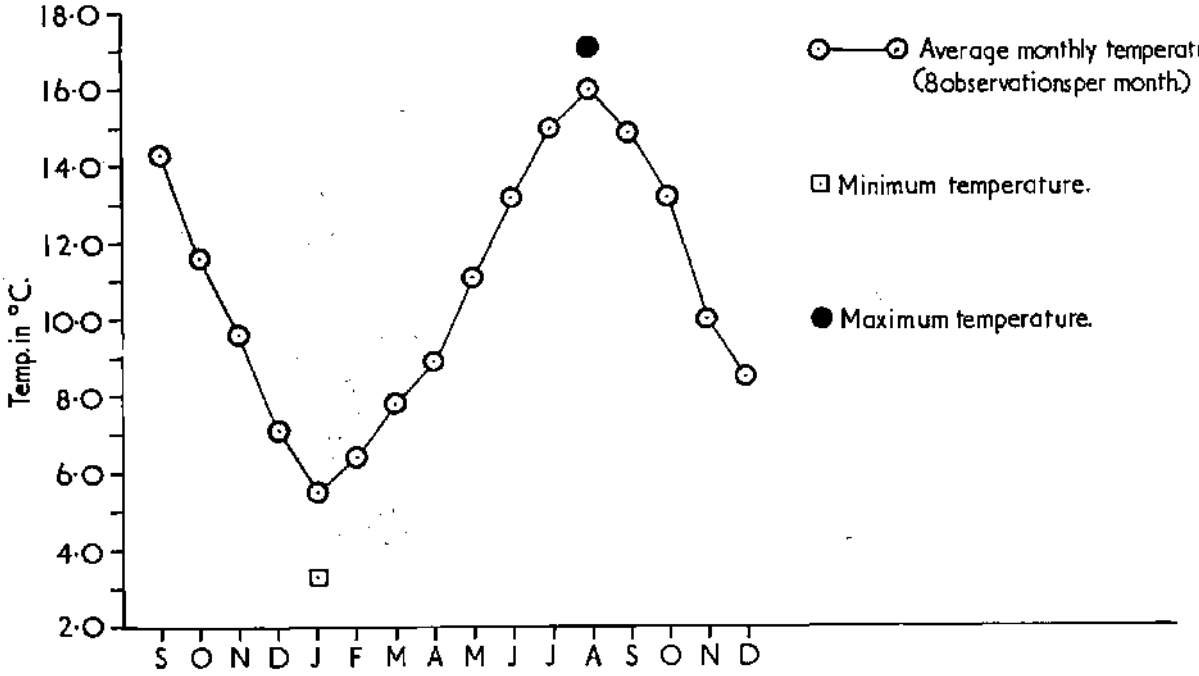
Plate 30 Fig 1. Detail of Plate 29, Fig 2 showing distribution of PAS cells in meso-adenohypophysis. PAS. (x225).

Fig 2. Detail of zone 1 showing distribution of PAS material. PAS. (x900).

Plate 31 Fig 1. Detail of zone 2 showing distribution of PAS material. PAS. (x900).

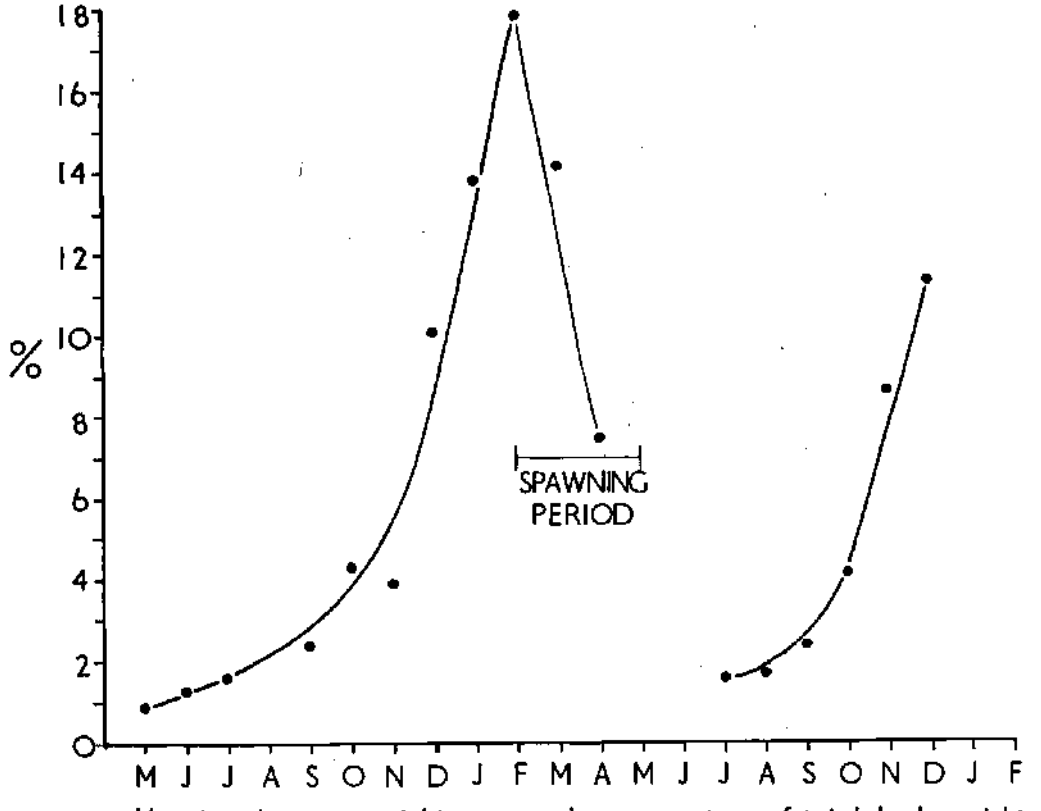
Fig 2. L.S. pituitary showing distribution of AF cells. Note their absence from meta-adenohypophysis. AF. (x55).

Plate 32 Fig 1. Detail of meso-adenohypophysis showing distribution of AF cells in zone 1 and 2. AF (x225).



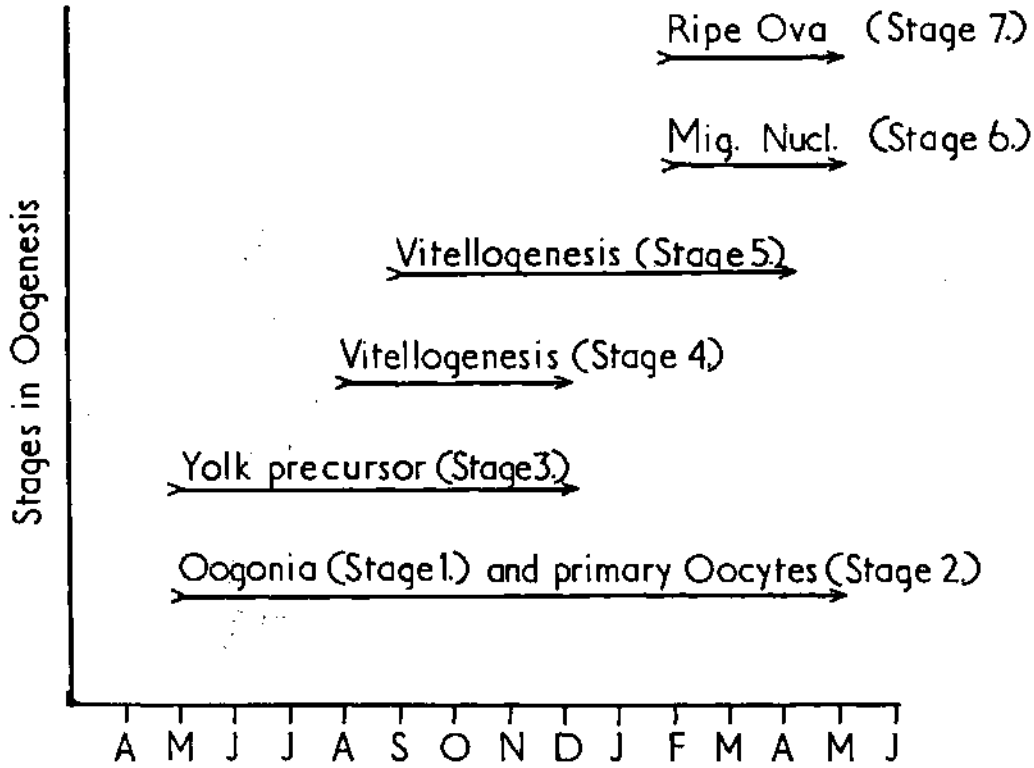
Temperature variation in the aquaria at St. Andrews. September, 1958 — December, 1959

Fig. 1



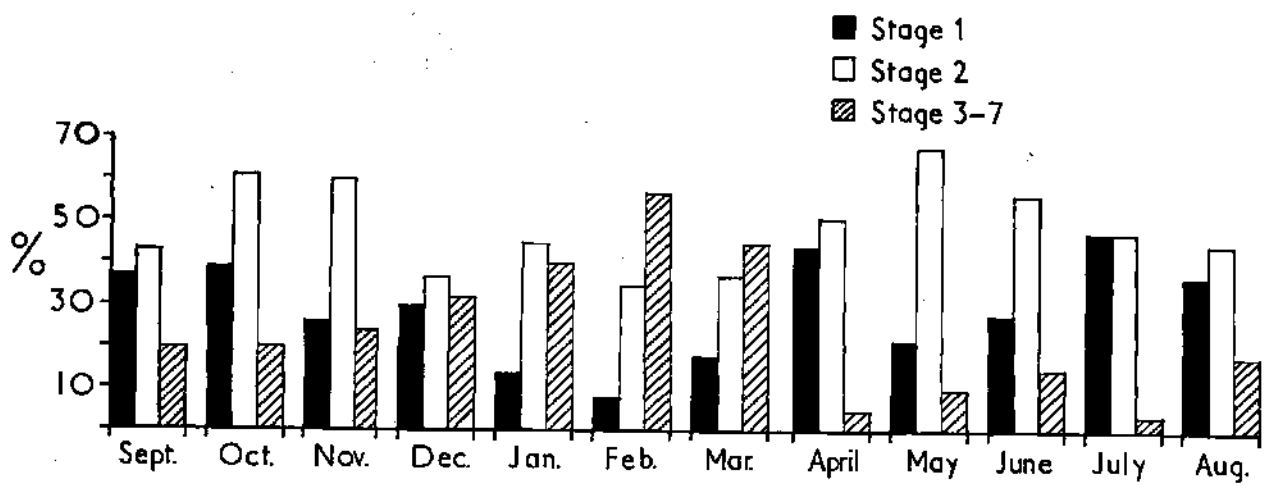
Variation in ovary weight expressed as percentage of total body weight.

Fig. 2



Diagrammatic representation of stages in Oogenesis

Fig. 3



Variation in the proportion of oocytes of different stages present in the ovary.

Fig. 4

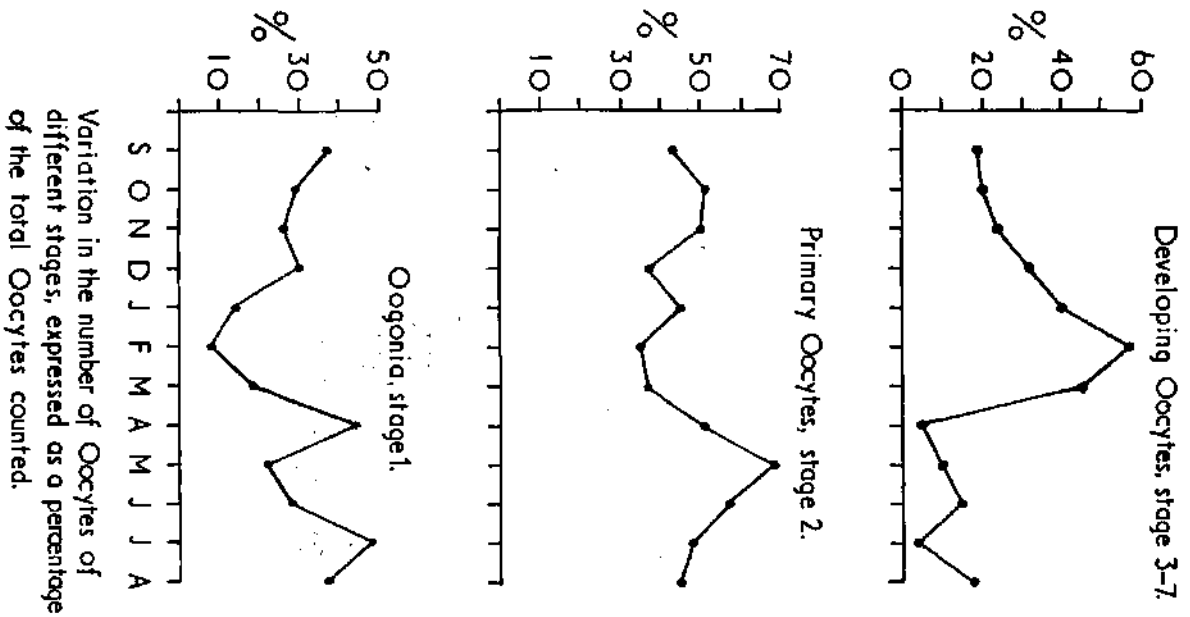

Developing Oocytes, stage 3-7.
Primary Oocytes, stage 2.
Oogonia, stage1.
%
S O N D J F M A M J J A
Variation in the number of Oocytes of different stages, expressed as a percentage of the total Oocytes counted.

Fig. 5

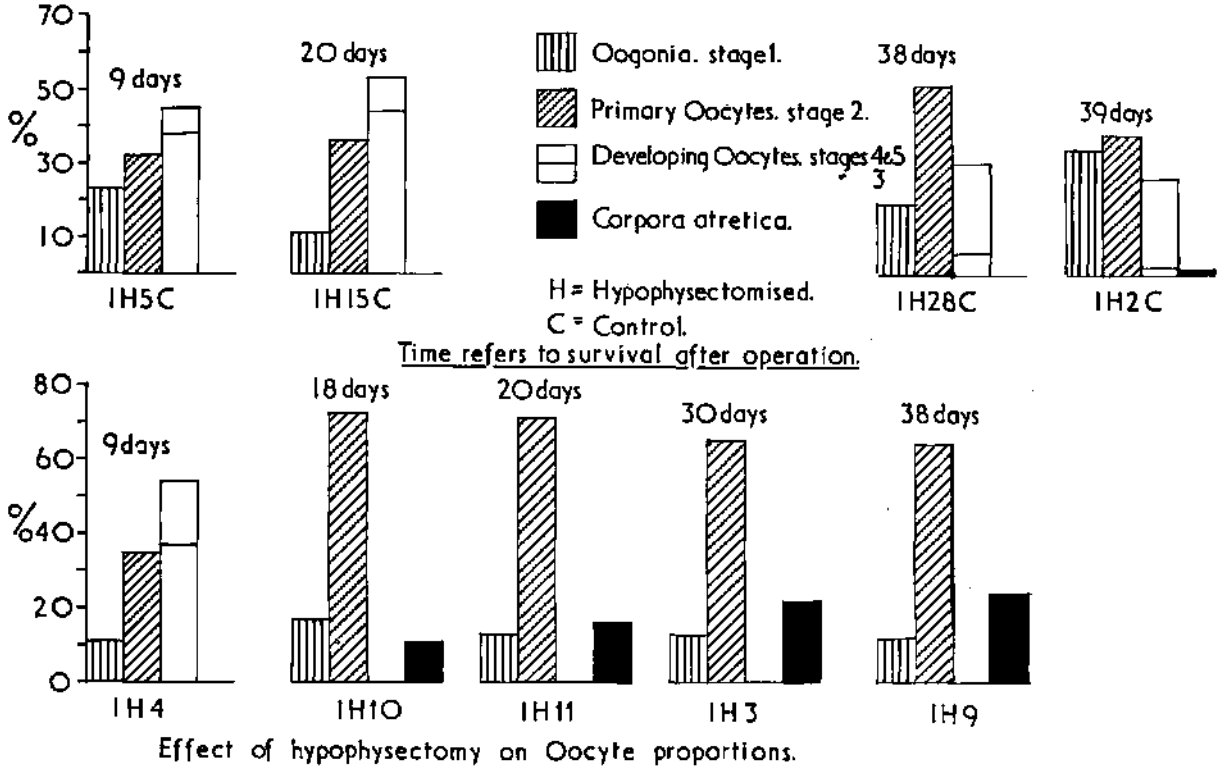

Oogonia, stage1.
Primary Oocytes, stage 2.
Developing Oocytes, stages 4&5
3
Corpora atretica.
H = Hypophysectomised.
C = Control.
Time refers to survival after operation.
9 days
20 days
38 days
39 days
1H5C
1H15C
1H28C
1H2C
9days
18 days
20 days
30 days
38 days
1H4
1H10
1H11
1H3
1H9
%
Effect of hypophysectomy on Oocyte proportions.

Fig. 6a

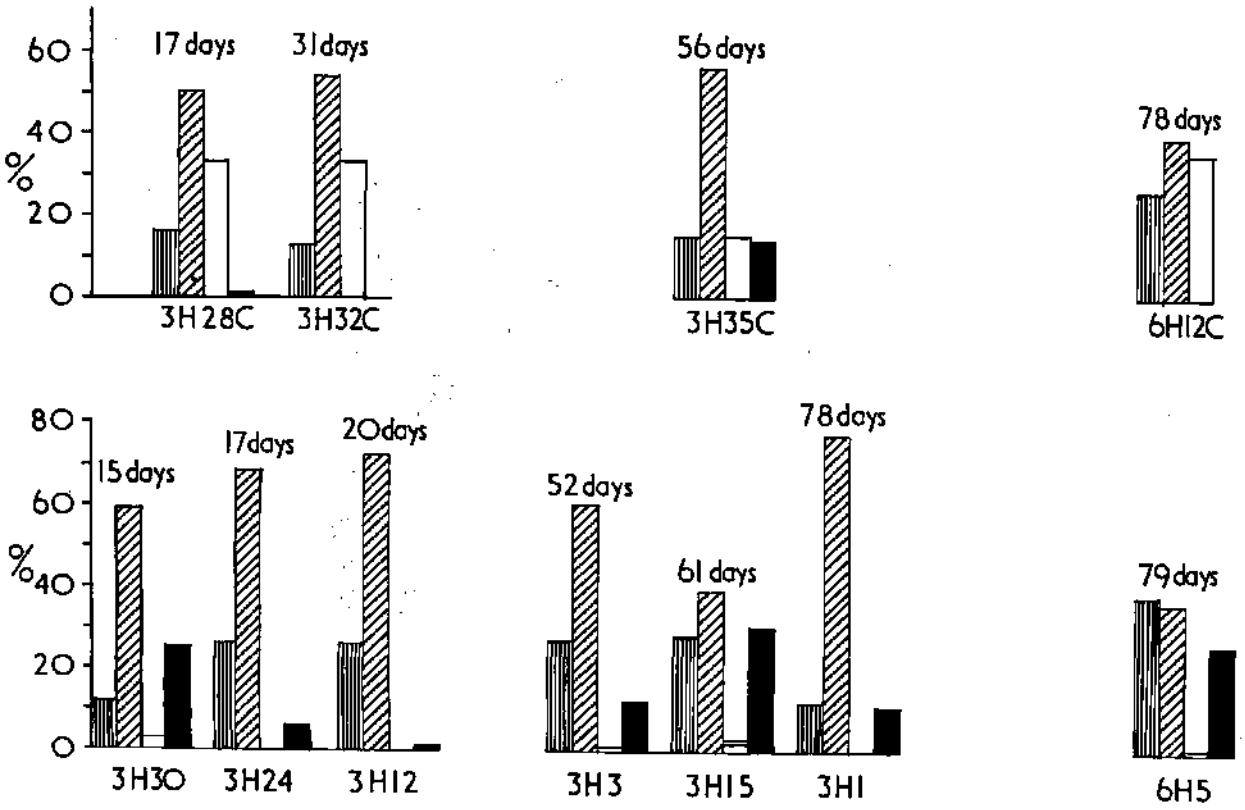

60
40
%
20
0
17 days
31days
56 days
78 days
3H 28C
3H32C
3H35C
6H12C
80
60
%40
20
0
15 days
17days
20days
52 days
78days
61 days
79days
3H30
3H24
3H12
3H3
3H15
3H1
6H5

Fig. 6b

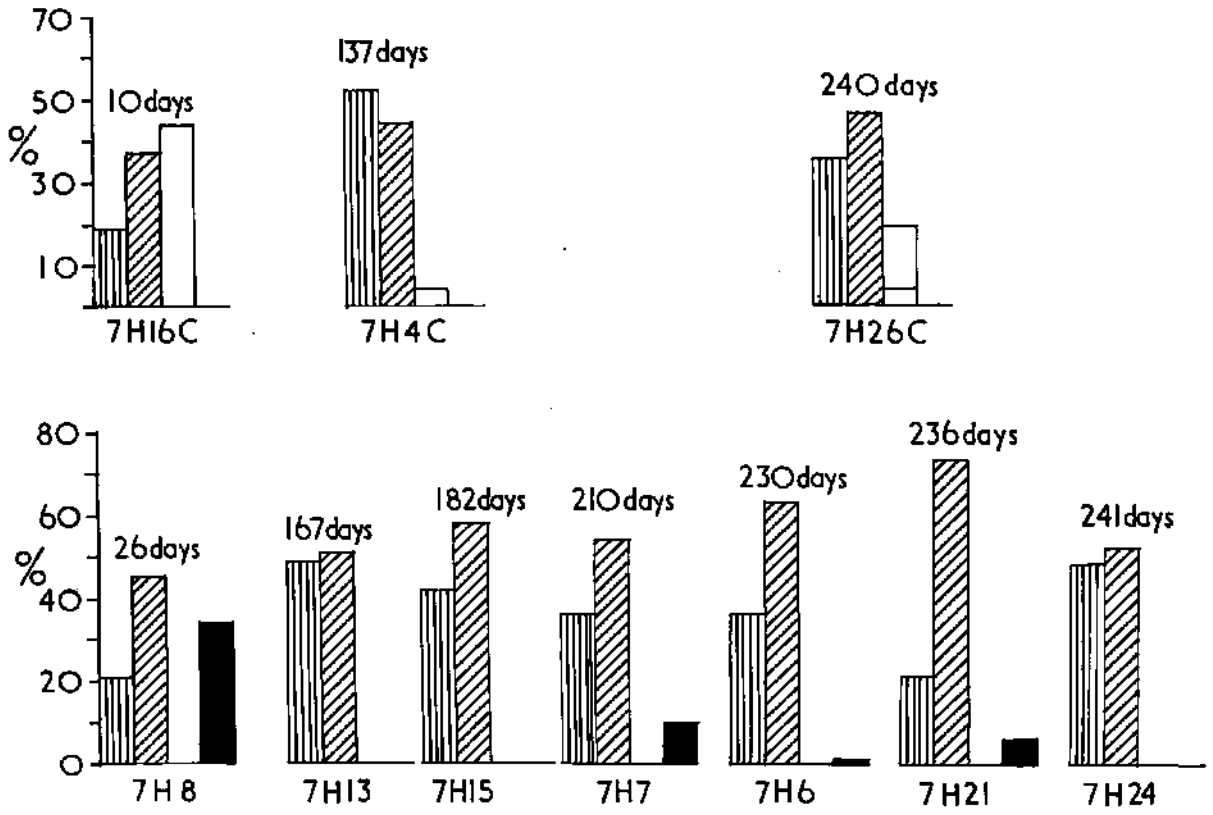

70
50
%
30
10
10days
137days
240 days
7H16C
7H4 C
7H26C
80
60
%40
20
0
26days
167days
182days
210days
230days
236days
241days
7H 8
7H13
7H15
7H7
7H6
7H21
7H24

Fig. 6c

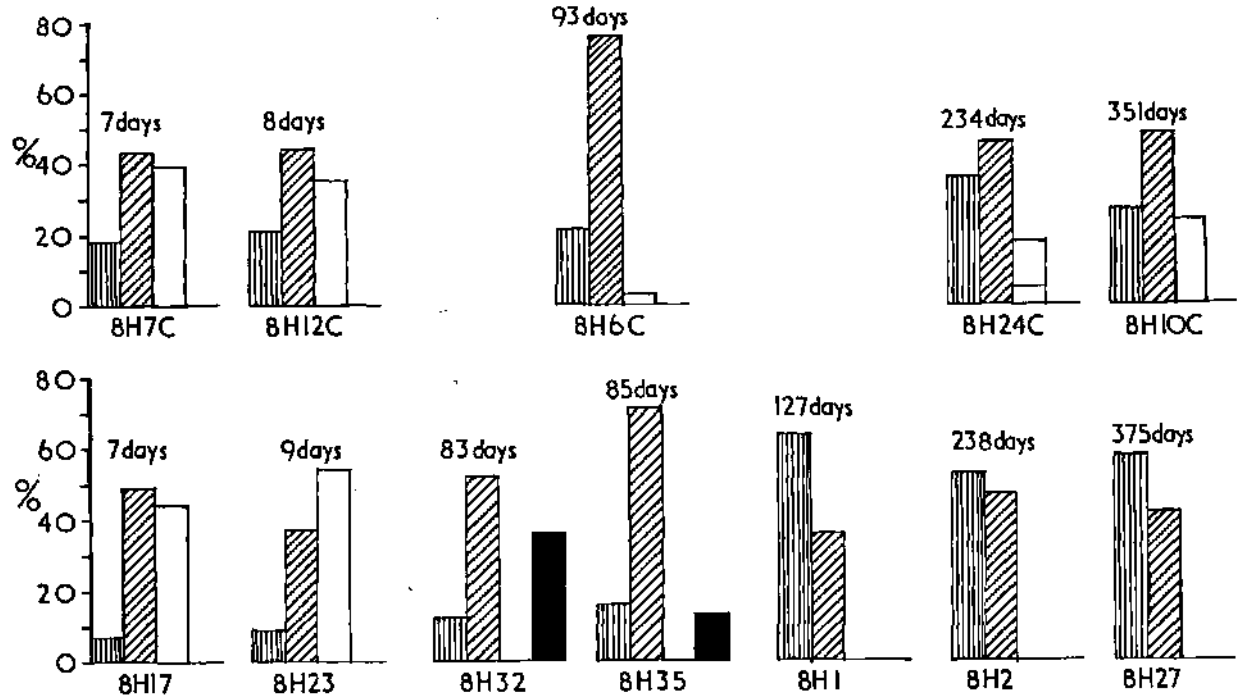

80
60
%40
20
0
7days
8days
93days
234days
351days
8H7C
8H12C
8H6C
8H24C
8H10C
80
60
%
40
20
0
7days
9days
83days
85days
127days
238days
375days
8H17
8H23
8H32
8H35
8H1
8H2
8H27

Fig. 6d

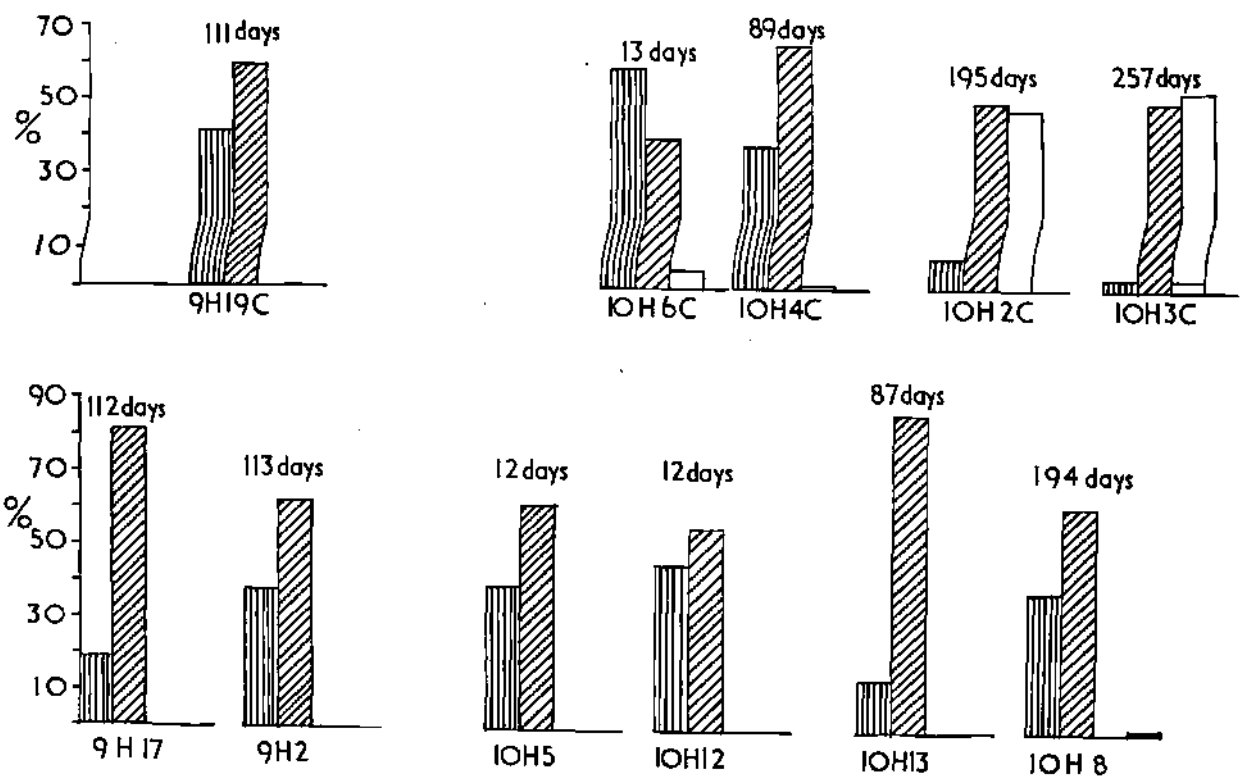
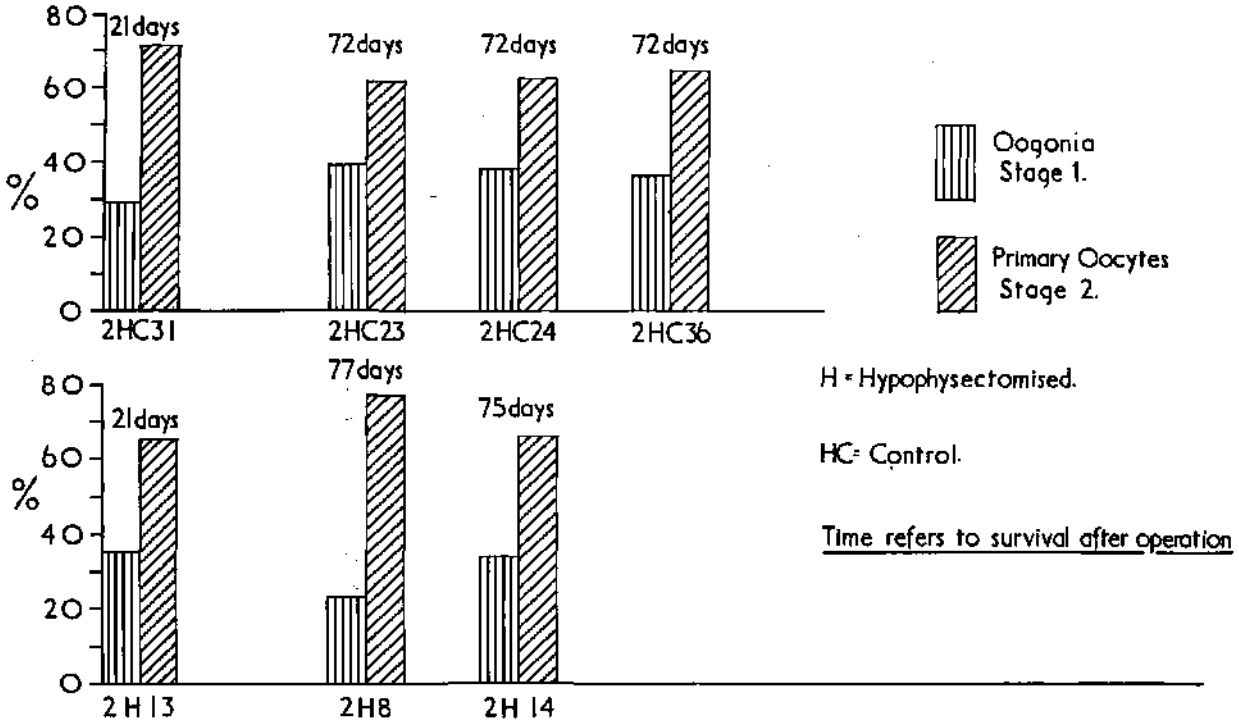
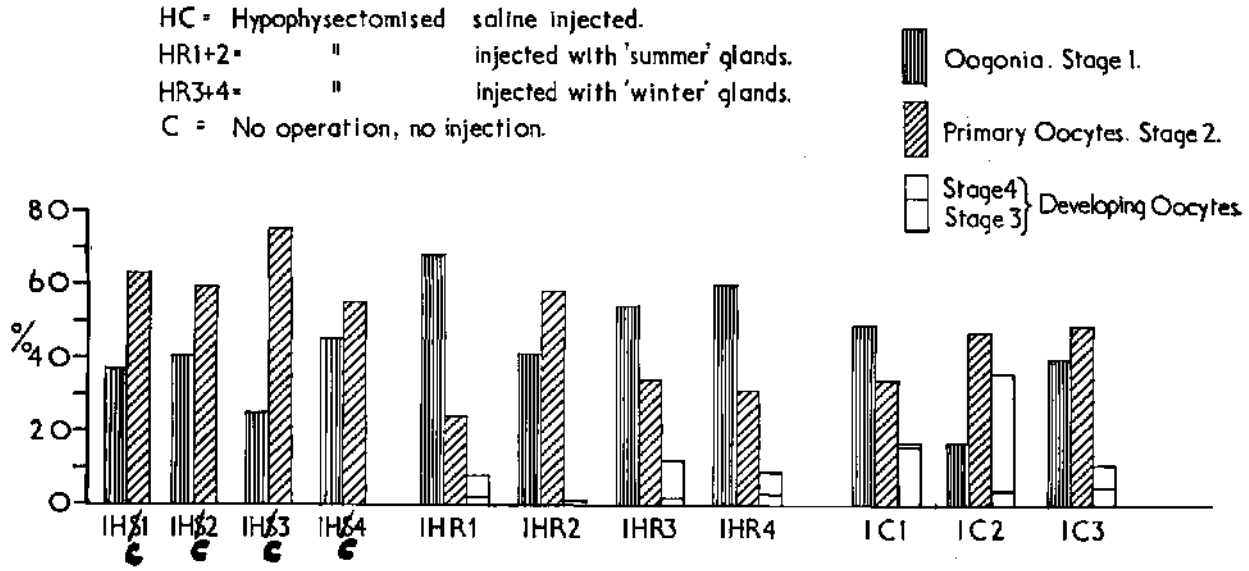

70
50
%
30
10
111days
9H19C
13 days
89days
195days
257days
10H6C
10H4C
10H2C
10H3C
90
70
%50
30
10
112days
113days
12days
12days
87days
194 days
9 H 17
9H2
10H5
10H12
10H13
10H 8

Fig. 6e



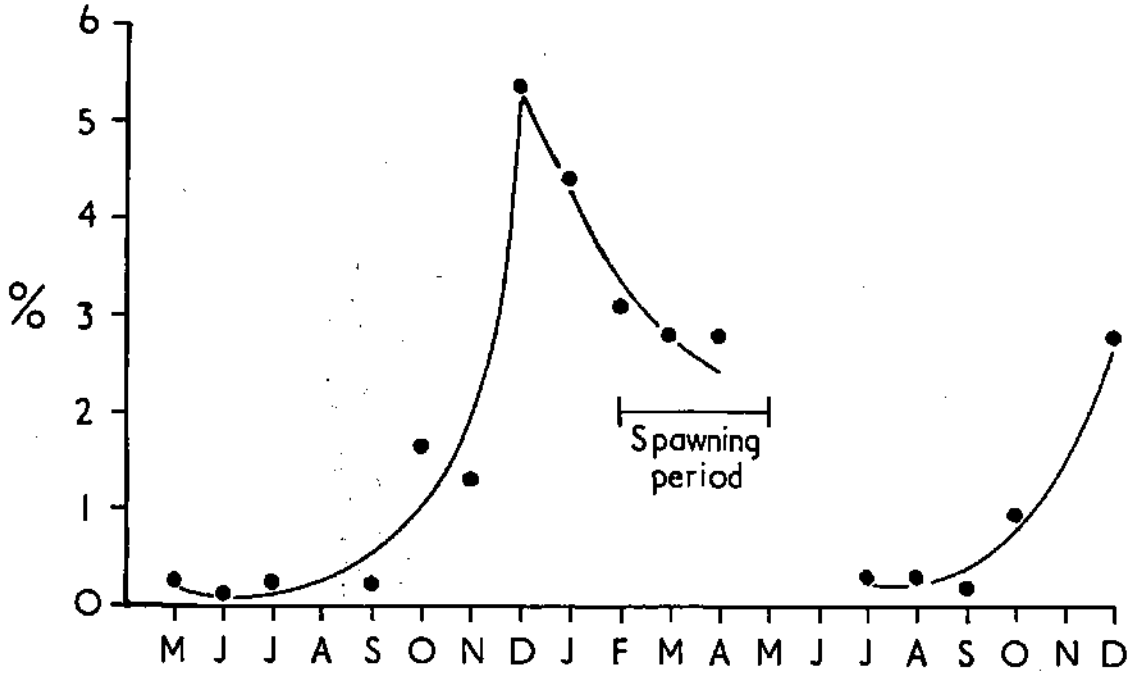
Effect of hypophysectomy on Oocyte proportions in immature P. PLATESSA.

Fig. 7



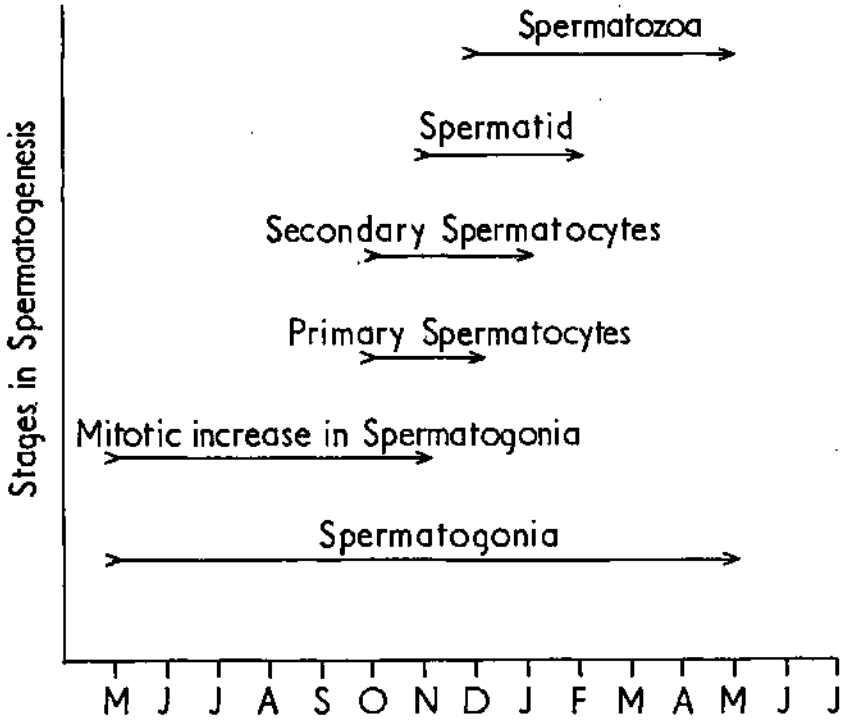
Oocyte proportions after injection of plaice pituitary glands into hypophysectomised plaice.

Fig. 8



Variation in testis weight, expressed as a percentage of total body weight.

Fig. 9



Diagrammatic representation of stages in spermatogenesis.

Fig. 10

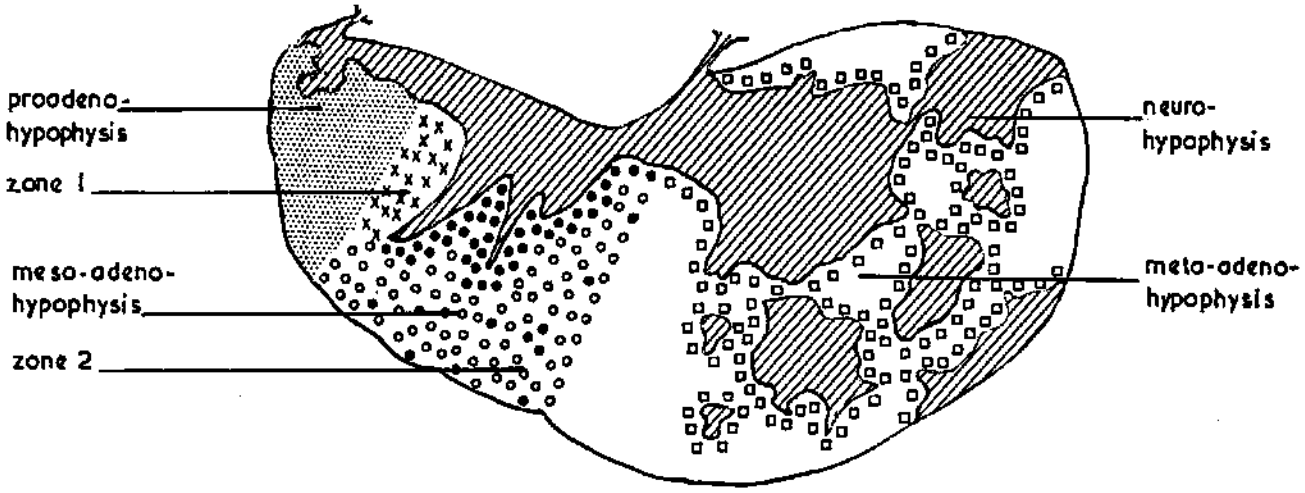


Fig. IIa

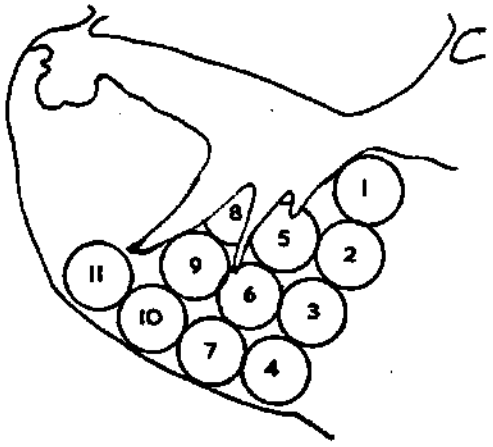


Fig. IIb

Fig. 11a. Diagrammatic L.S. pituitary. Acidophils of meso-adenohypophysis represented by solid circles and basophils by empty circles. Basophils of meta-adenohypophysis represented by empty squares and chromophobes left blank.

Fig. 11b. Fields of meso-adenohypophysis counted. (see page 156).

Name	b	λ
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
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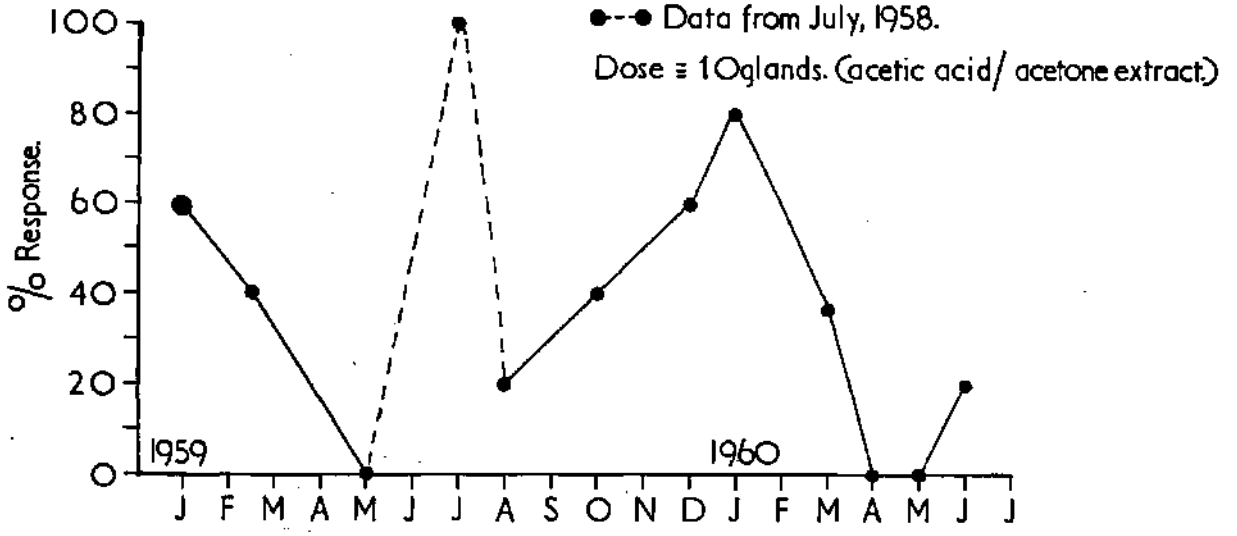
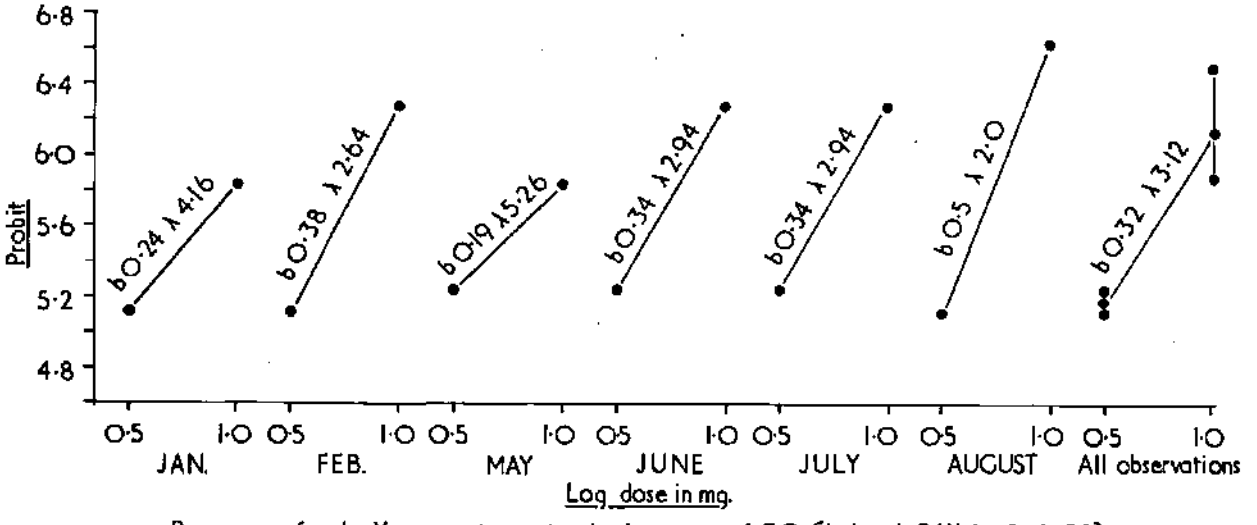
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

Correction to values of b and λ in Fig. 1.



Response of male Xenopus to plaice pituitary material from different months.

Fig. 12



Response of male Xenopus to a standard amount of C.G. (Lab. std. F.W. 1. 2:4:59)

Fig. 13

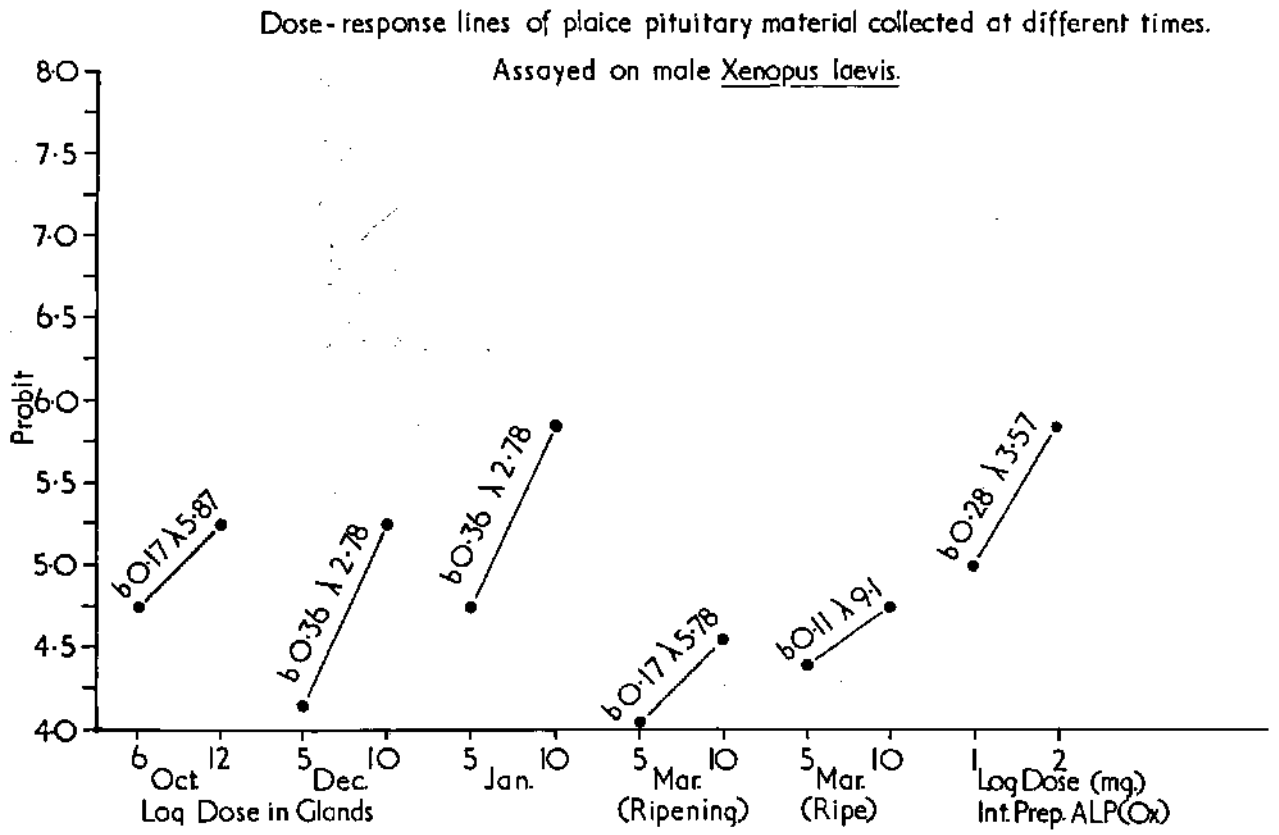


Fig. 14

Date	b	λ
Oct.	1·7	0·587
Dec.	3·6	0·278
Jan.	3·6	0·278
March (ripe.)	1·7	0·588
March (ripe)	1·1	0·91
Int. Prep.	2·8	0·357

Correction on values of b and λ in Fig. 14.

PLATE I

Fig.1

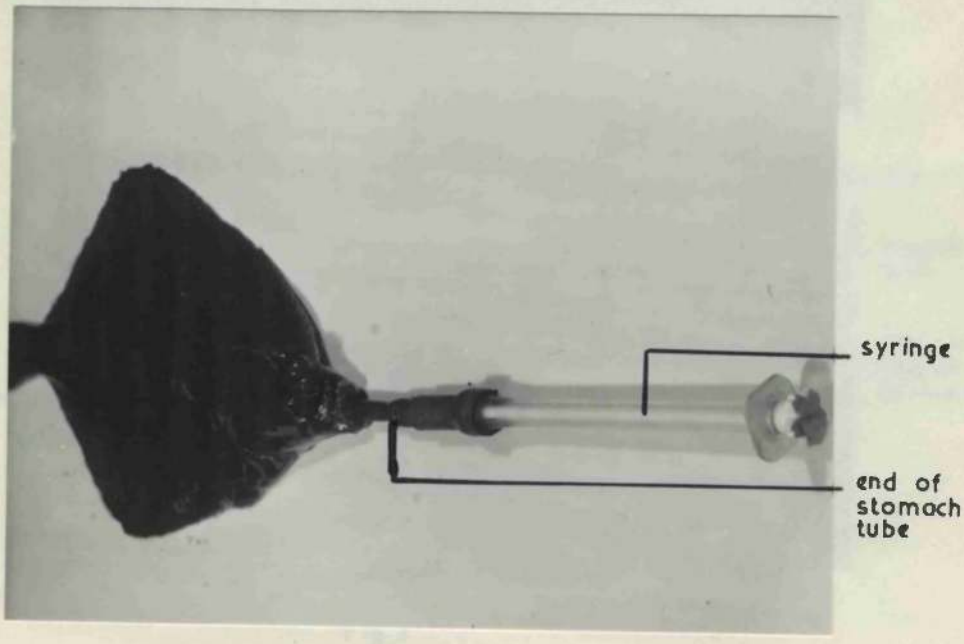


Fig.2

PLATE 2

Fig. 1

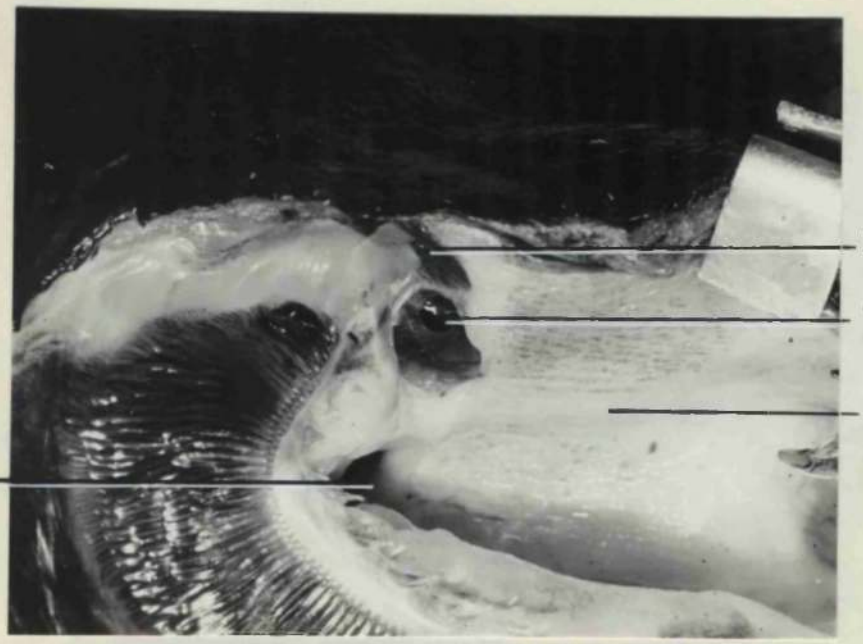


Fig. 2

PLATE 3

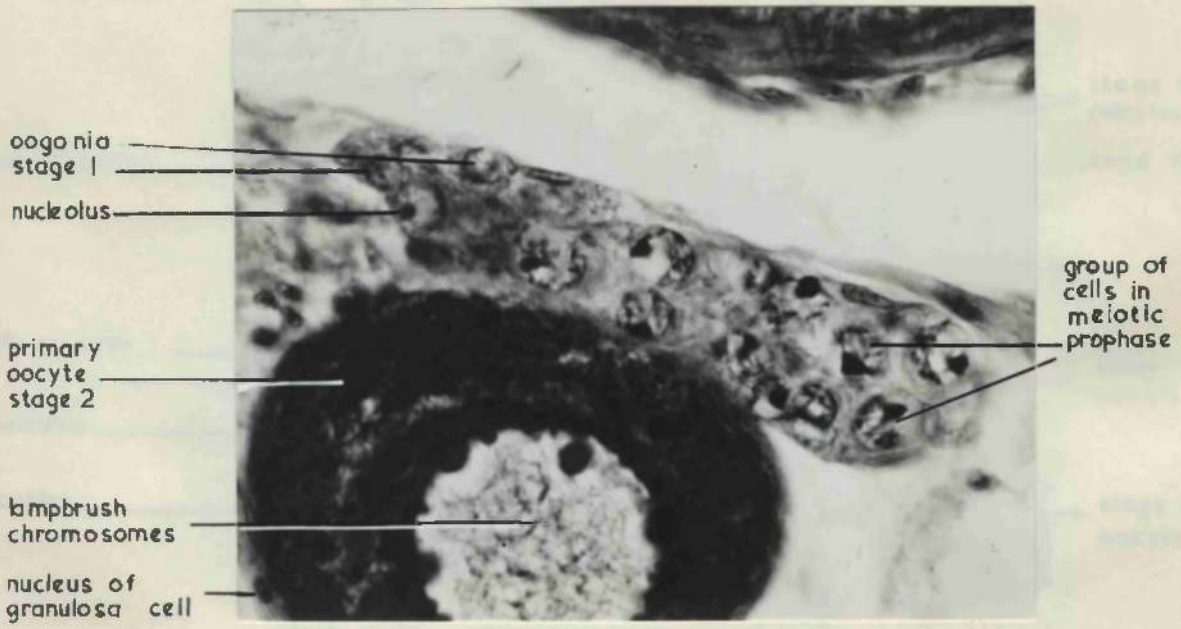


Fig. 1

Fig. 2

PLATE 4

Fig. 1

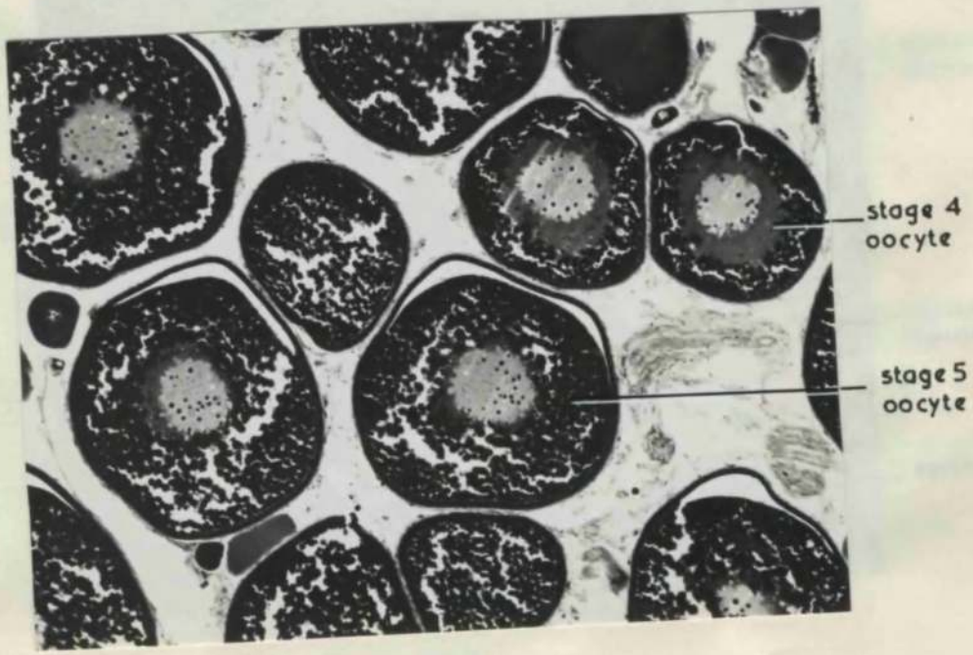


Fig. 2

PLATE 5

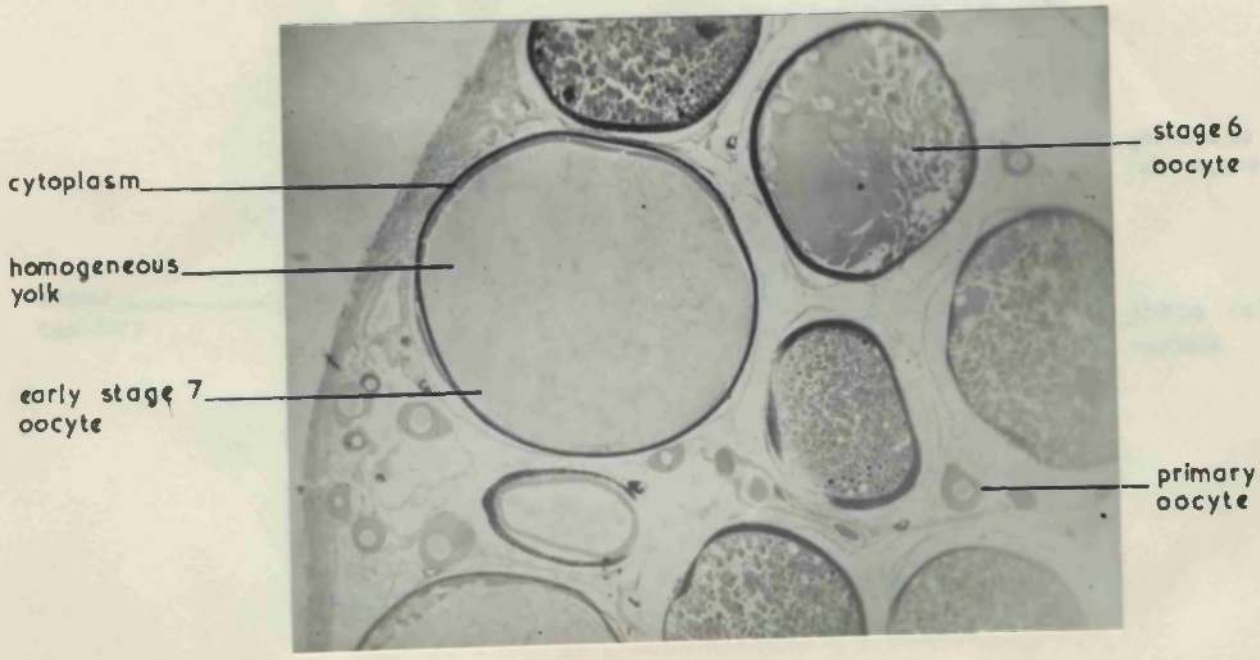


Fig. 1

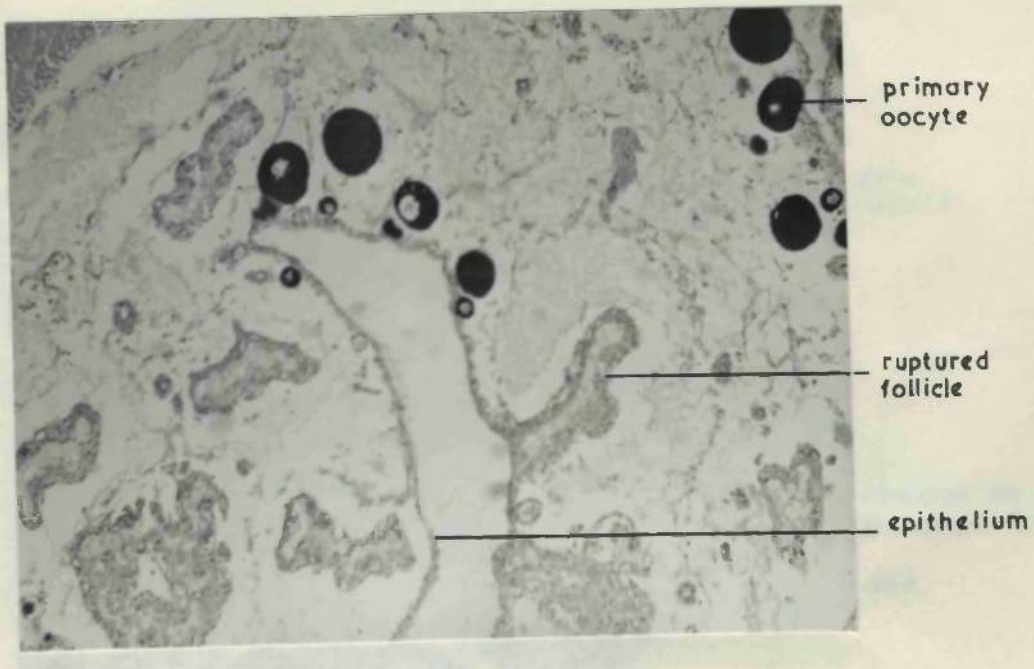


Fig. 2

PLATE 6

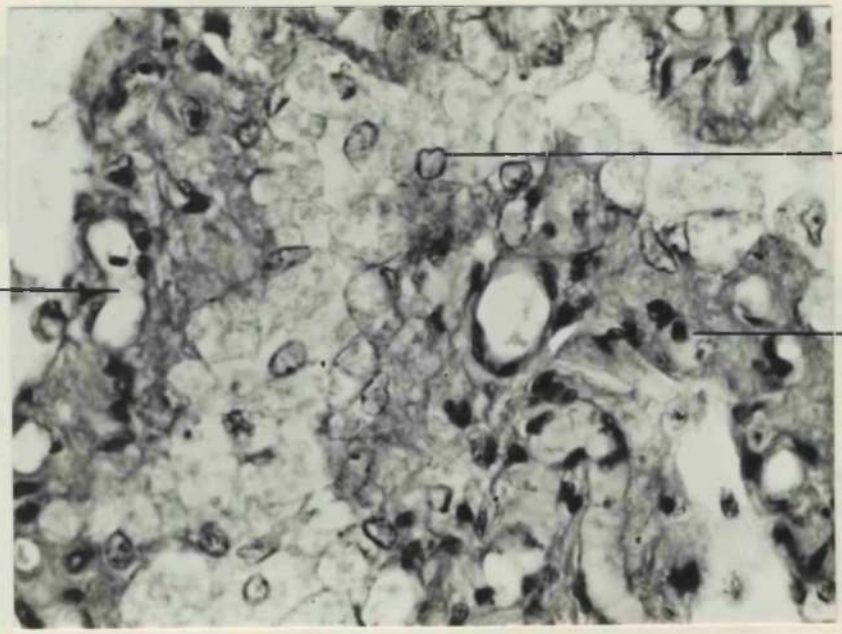


Fig. 1

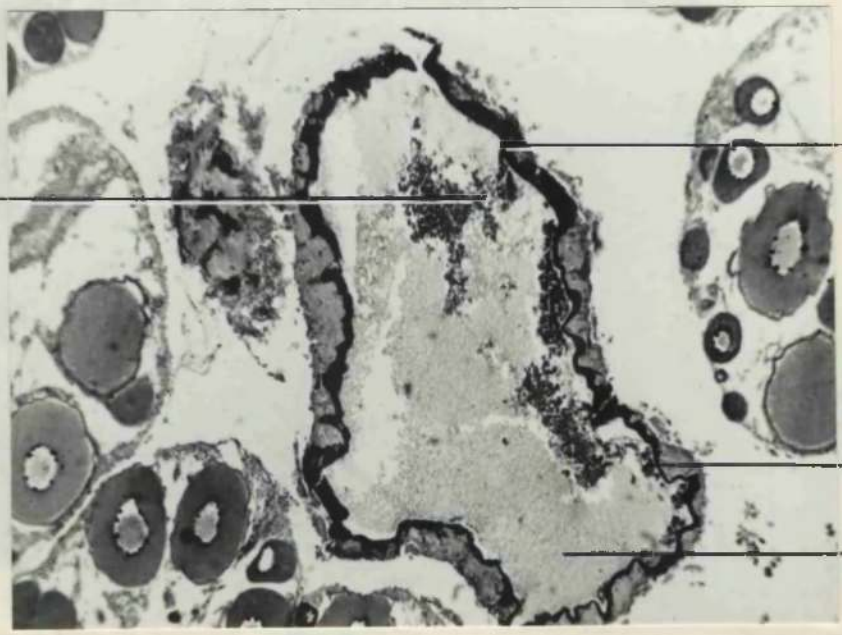


Fig. 2

PLATE 7

Fig. 1

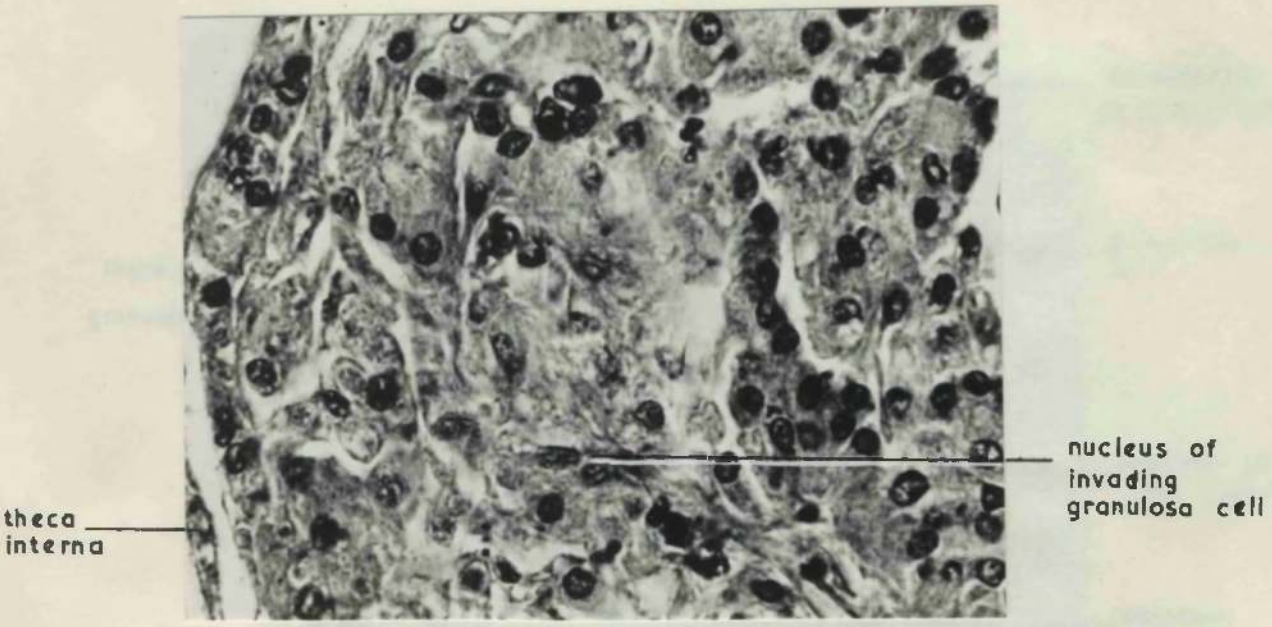


Fig. 2

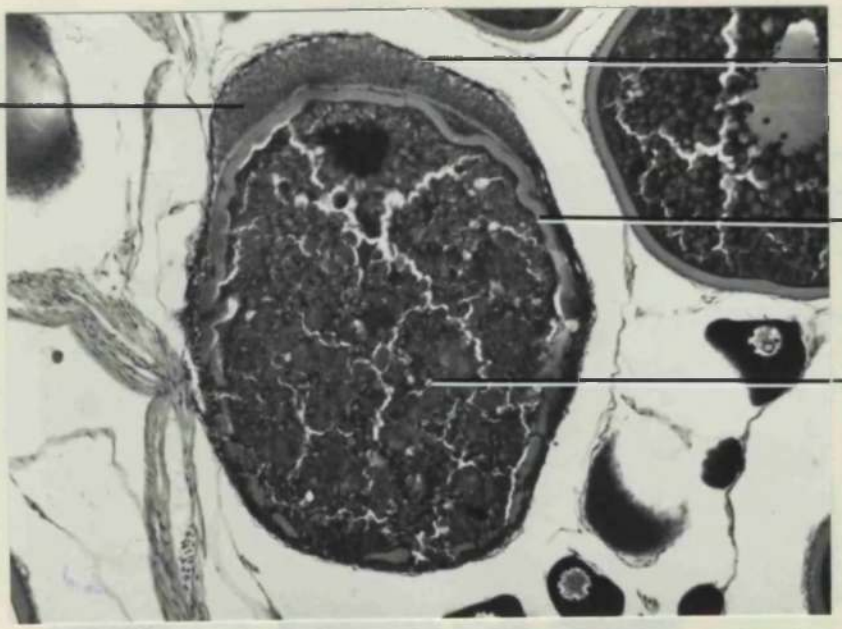

ooplasm
follicular membranes
zona radiata
yolk globules

Fig. 1


theca
granulosa
multiplication of theca cells
granulosa cells
remnant of zona radiata
ooplasm outside zona

Fig. 2

PLATE 9

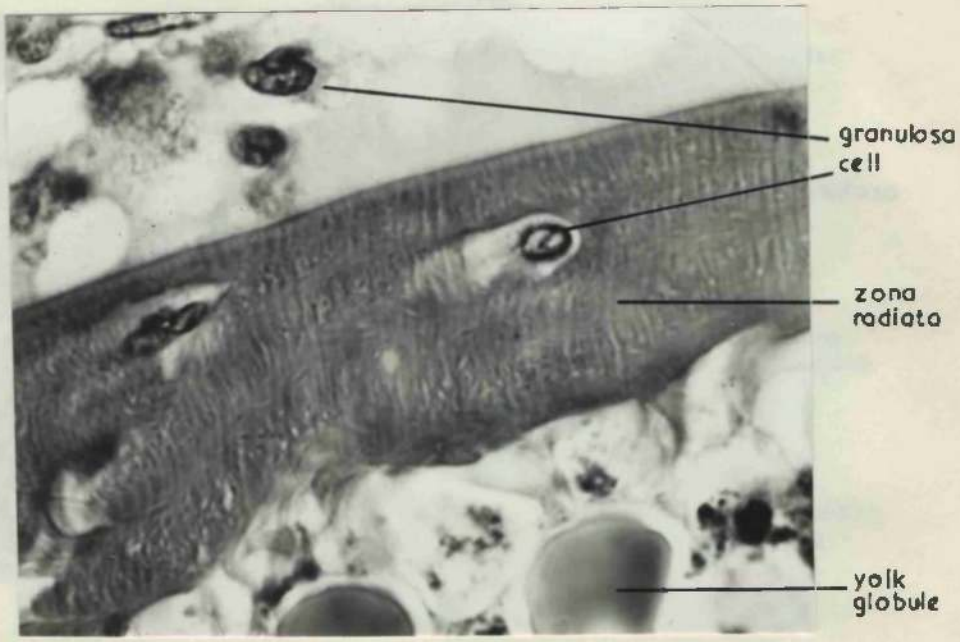


Fig. 1

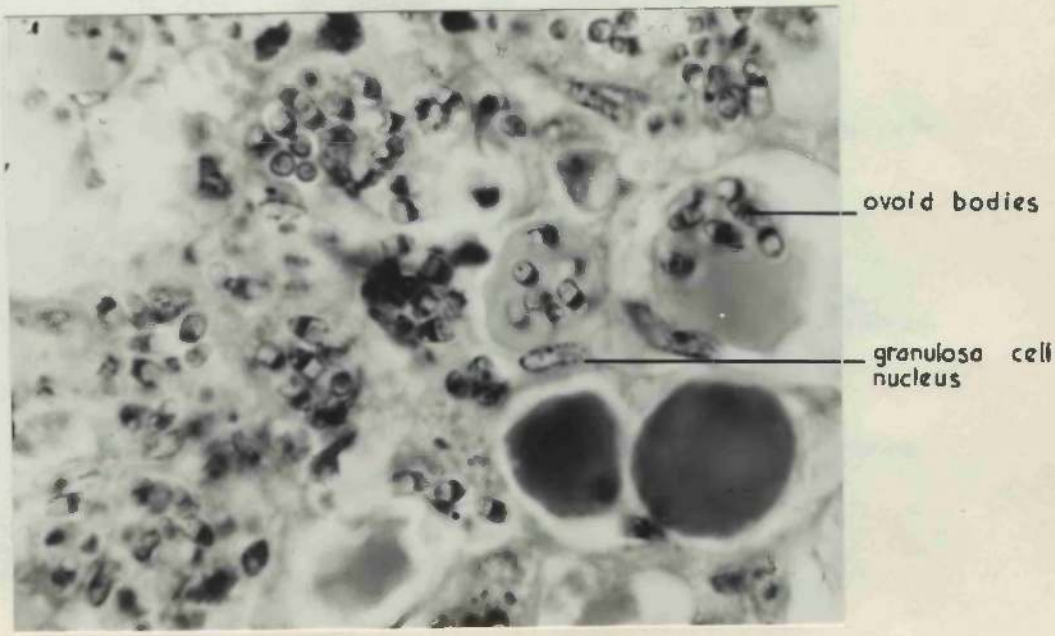


Fig. 2

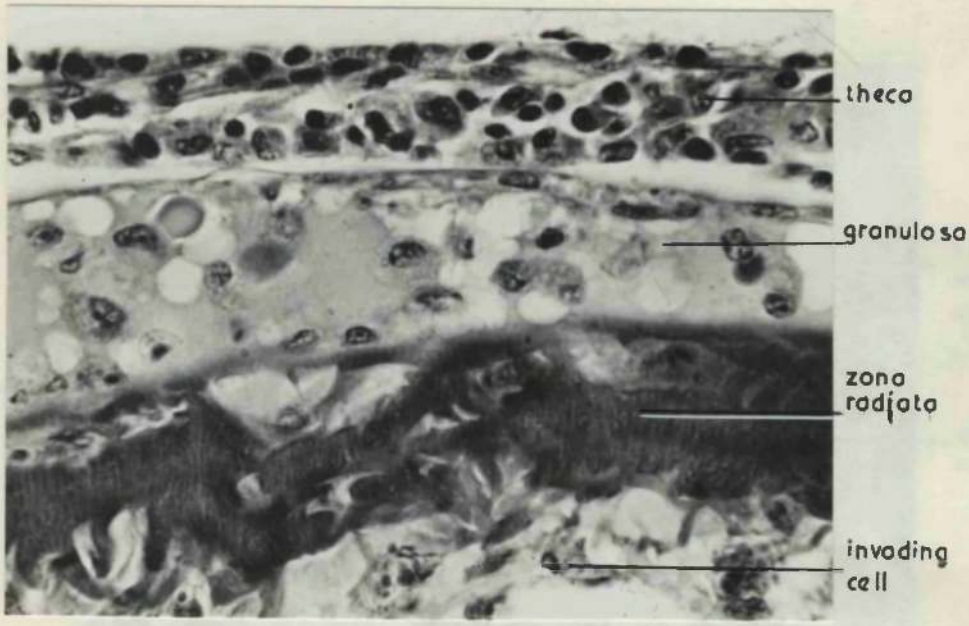

theca
granulosa
zona radiata
invading cell

Fig. I

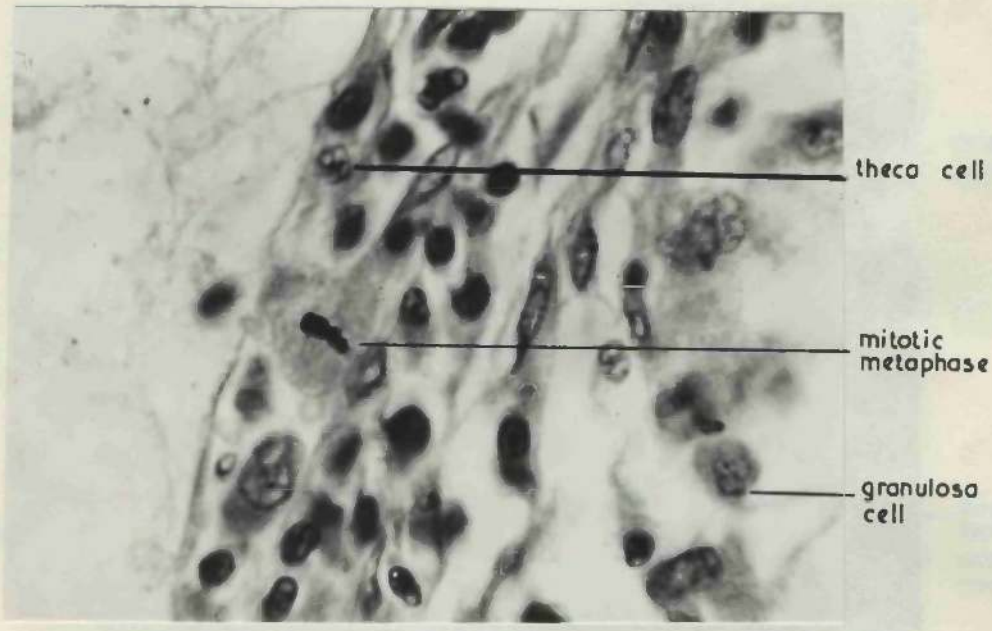

theca cell
mitotic metaphase
granulosa cell

Fig.2

PLATE II

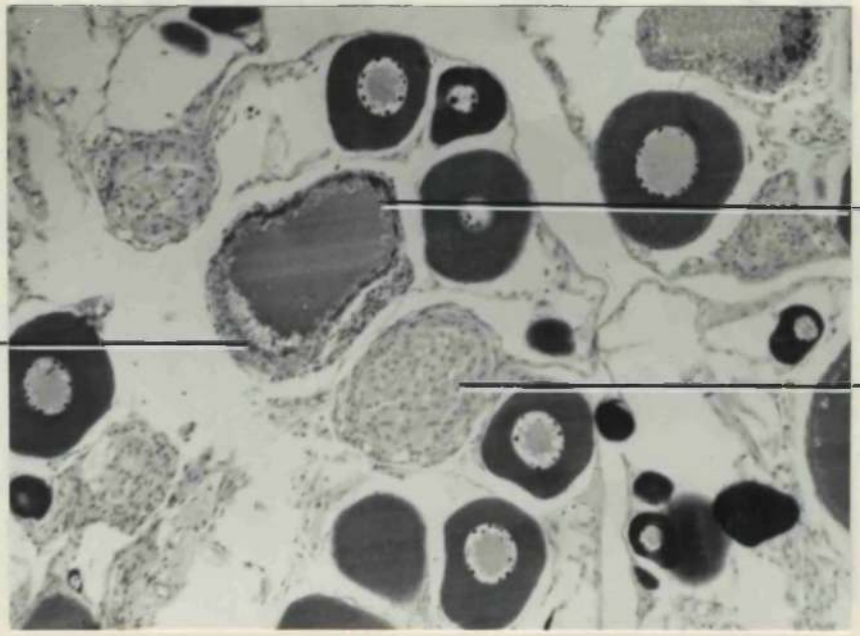


Fig. 1

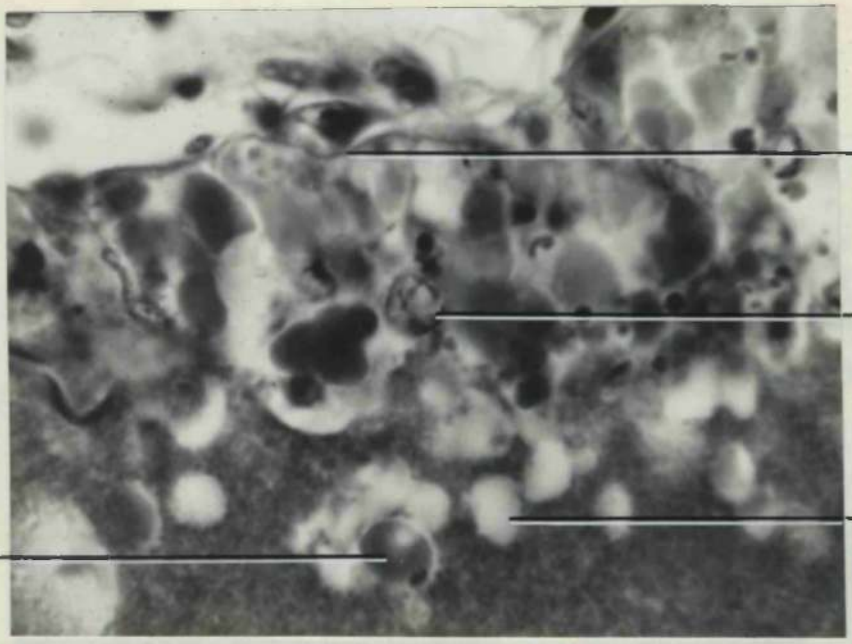


Fig. 2

PLATE 12

Fig. 1

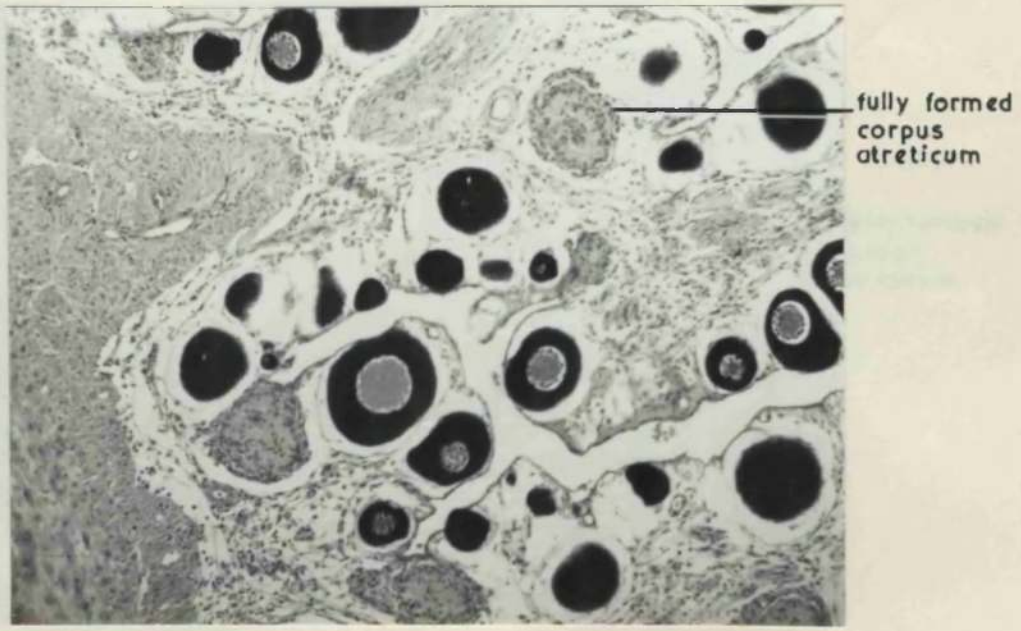


Fig. 2

PLATE 13

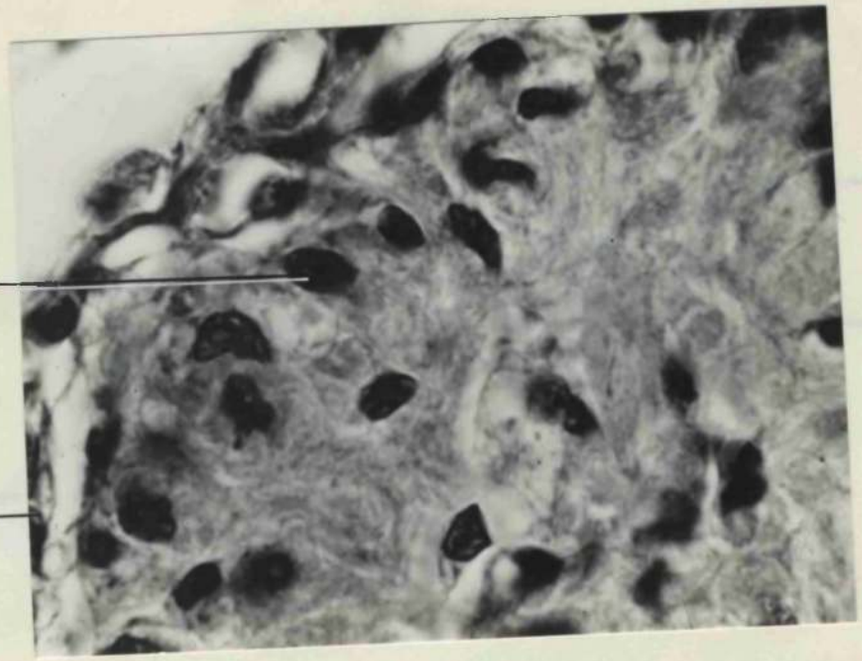


Fig. 1

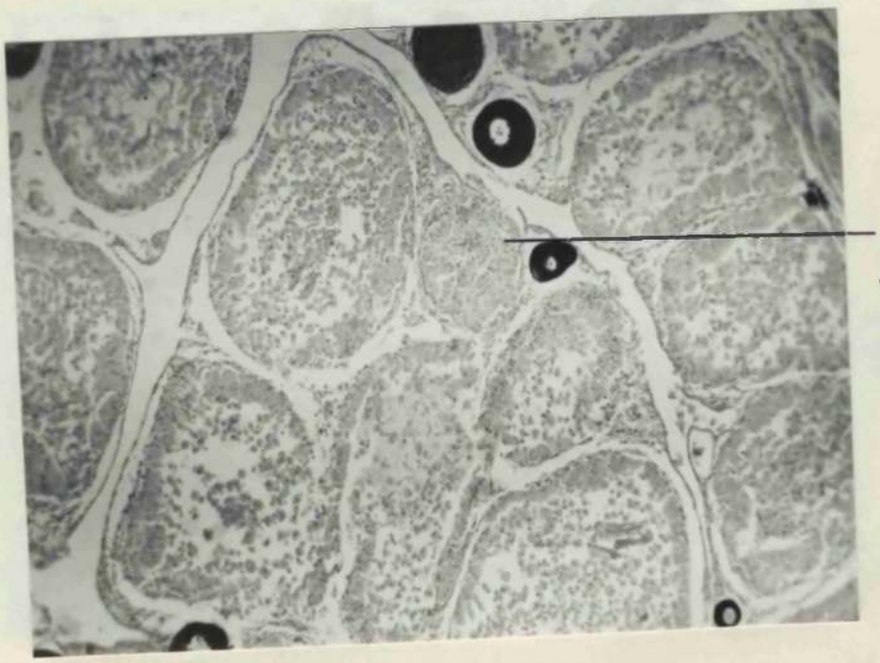


Fig. 2

PLATE 14

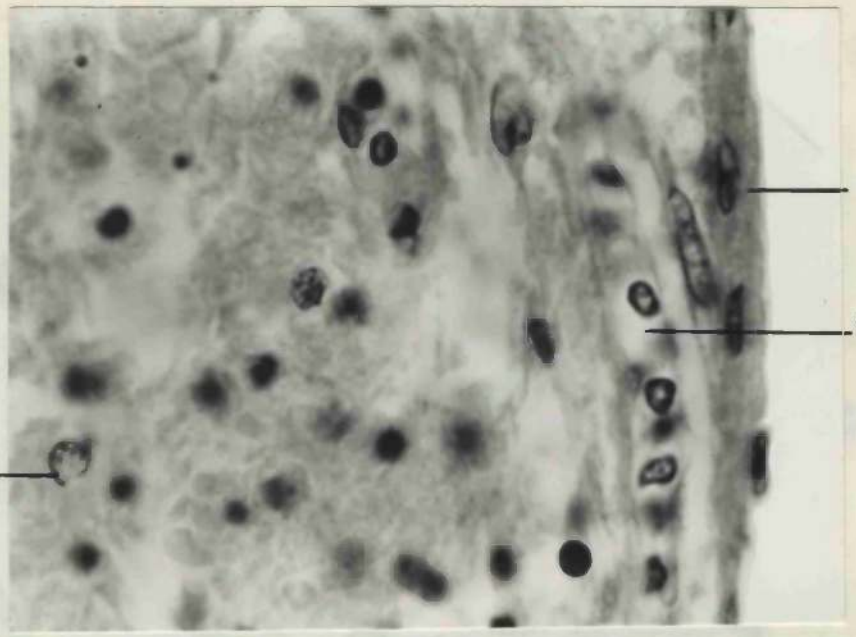


Fig. 1

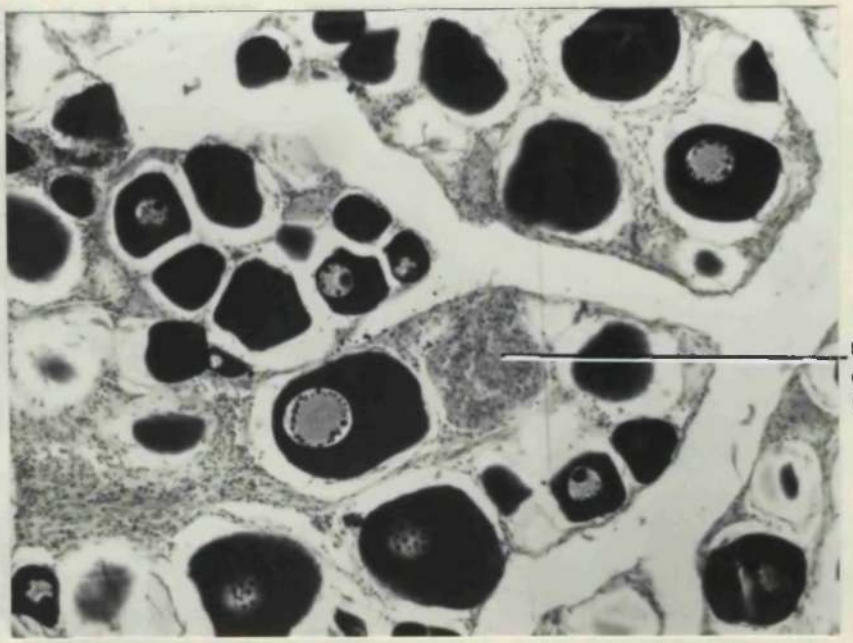


Fig 2

PLATE 15

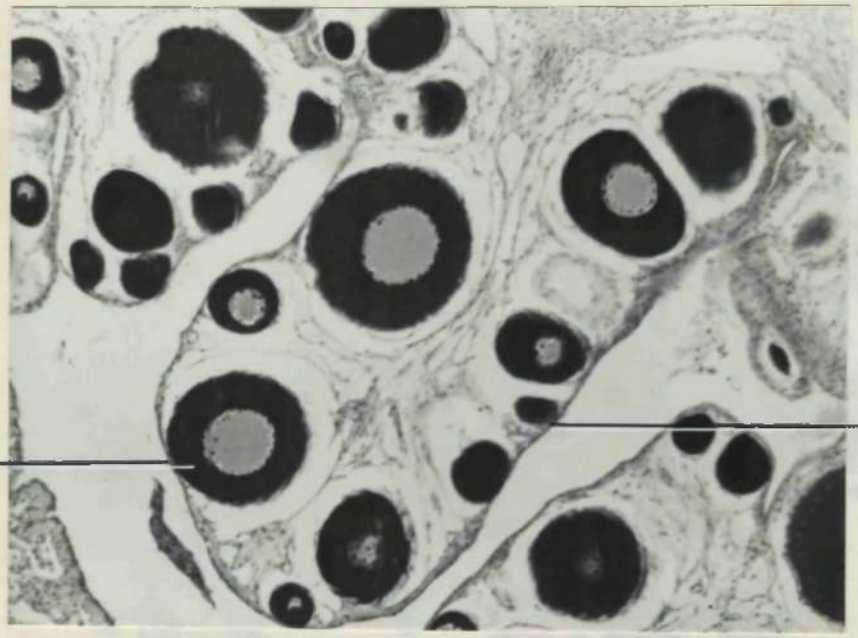


Fig. 1

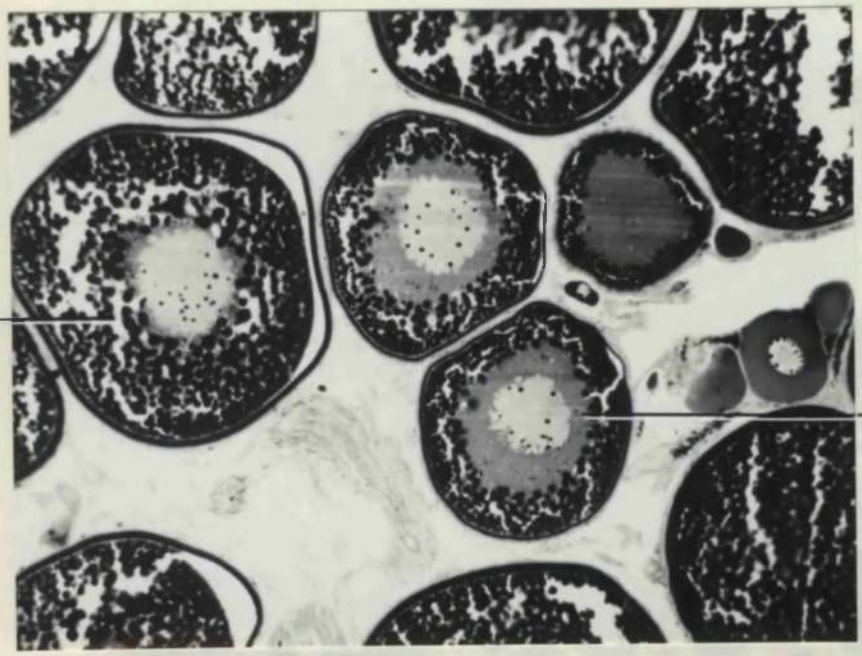


Fig. 2

PLATE 16

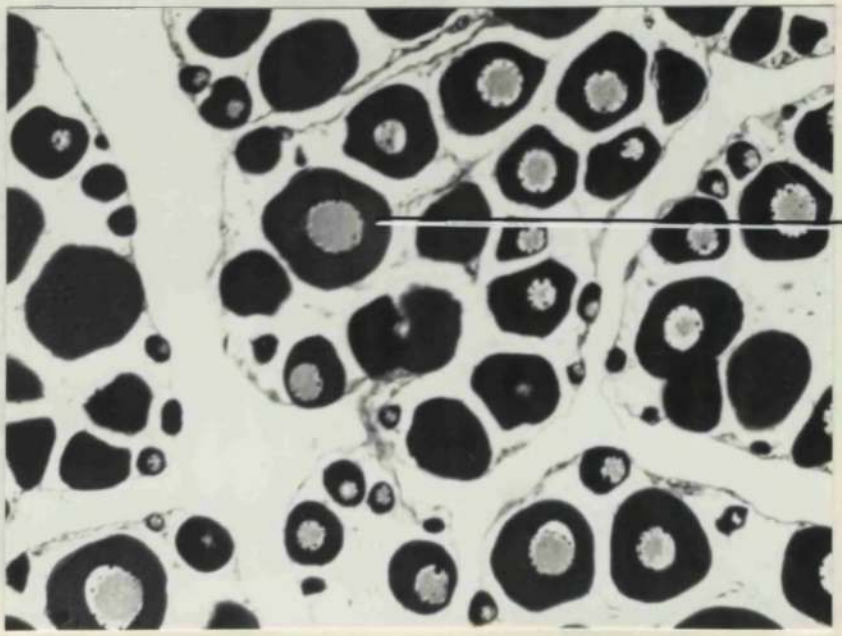


Fig. 1

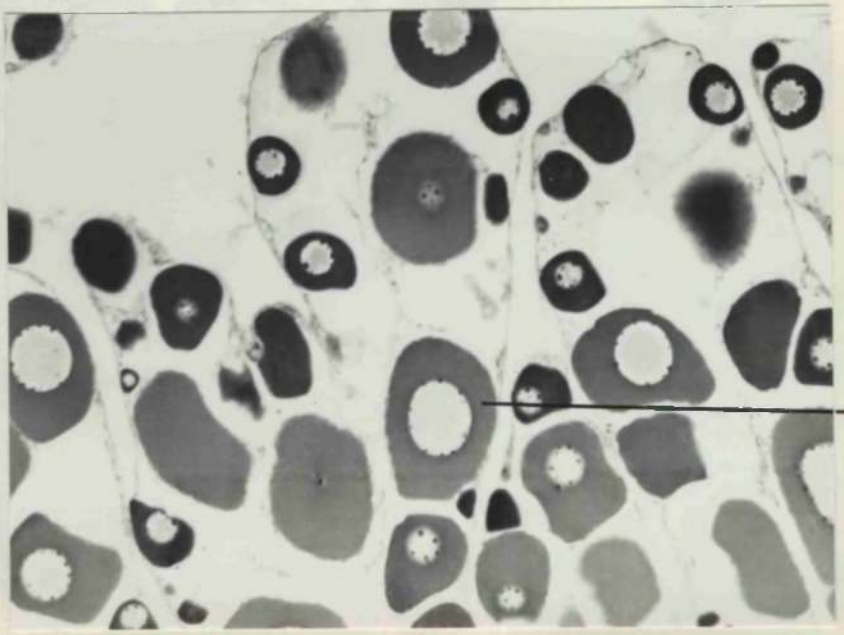


Fig. 2

PLATE 17

Fig. 1

Fig. 2

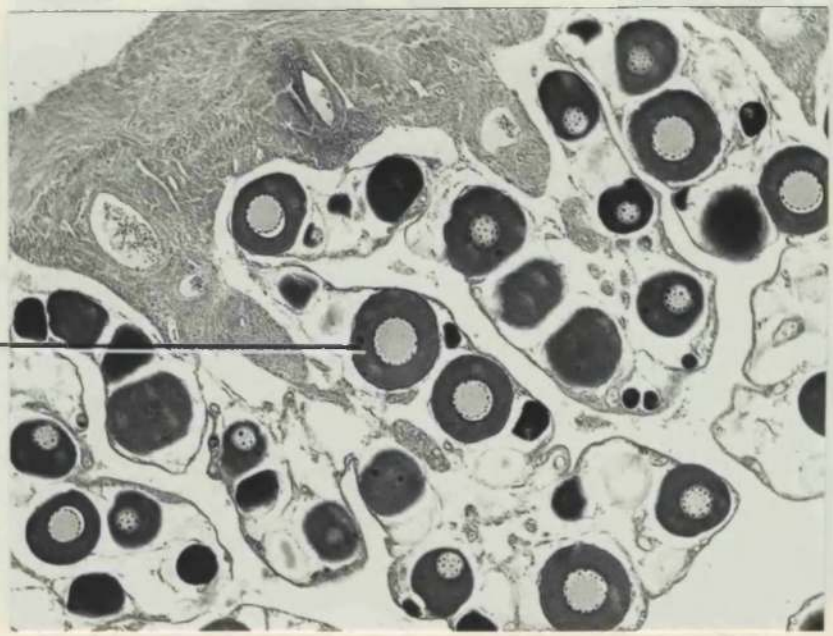


Fig. 1

Fig. 2


stage 4 oocyte
primary oocyte

Fig.1

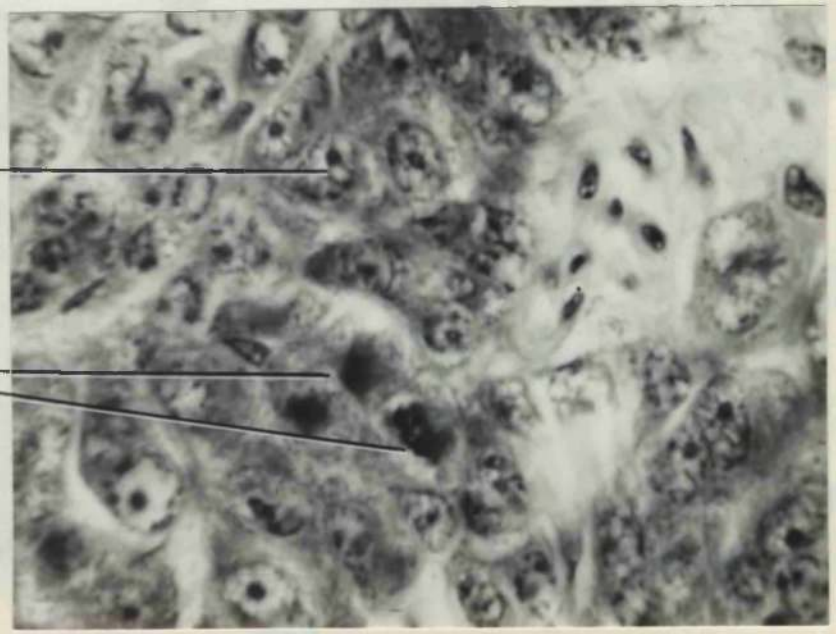

spermatogonia
mitotic metaphase

Fig.2

PLATE 20

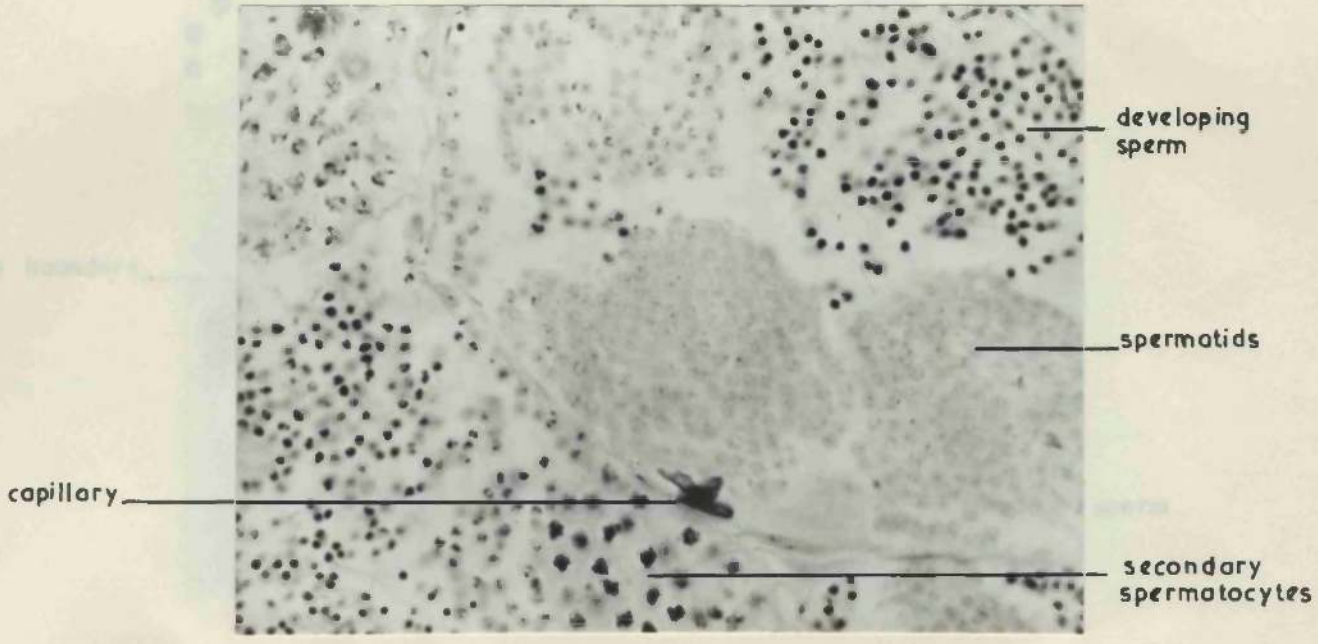


Fig. 1

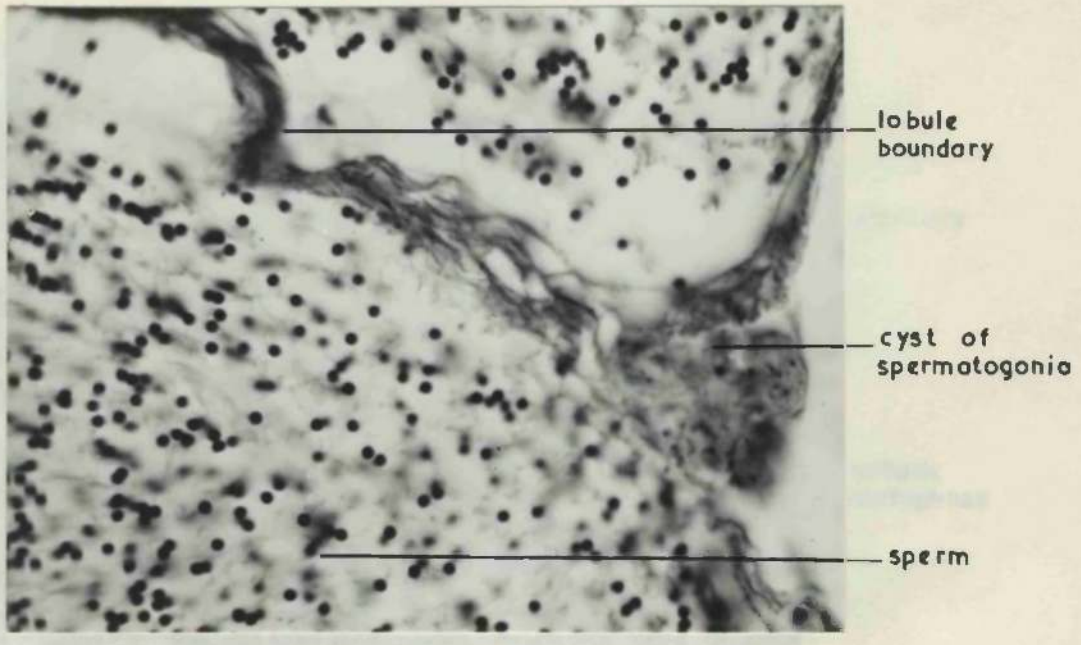


Fig. 2

PLATE 21

Fig. 1

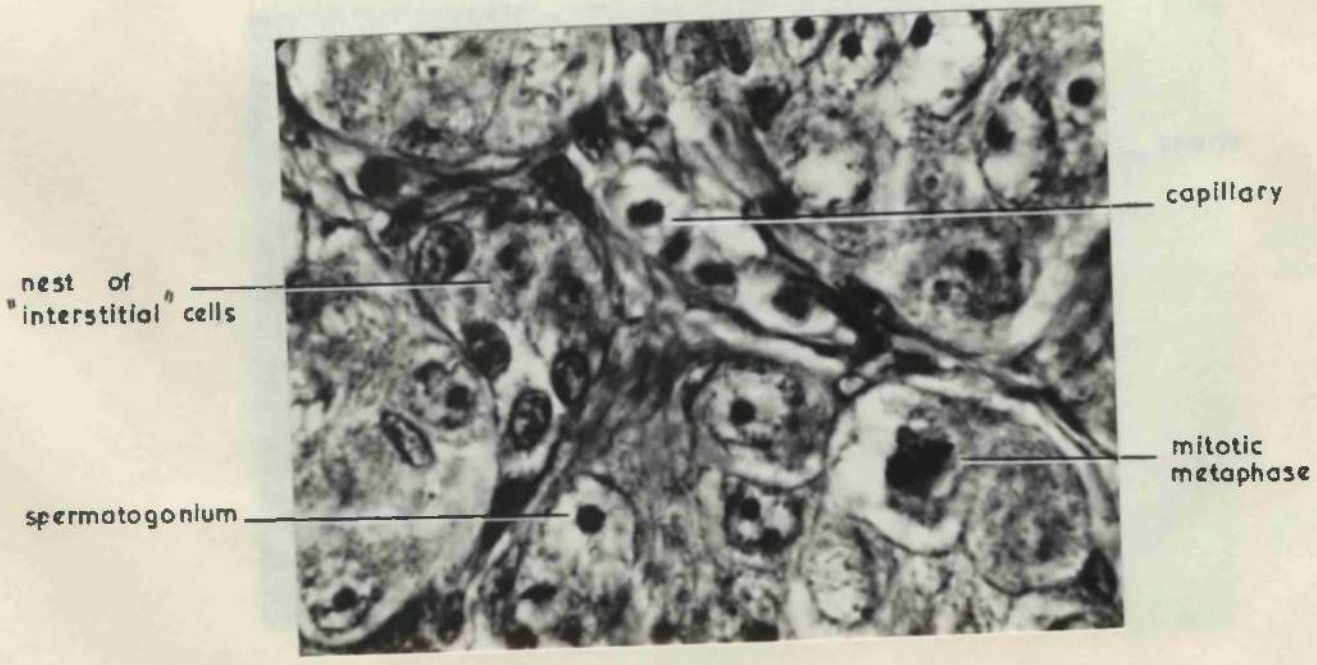


Fig. 2

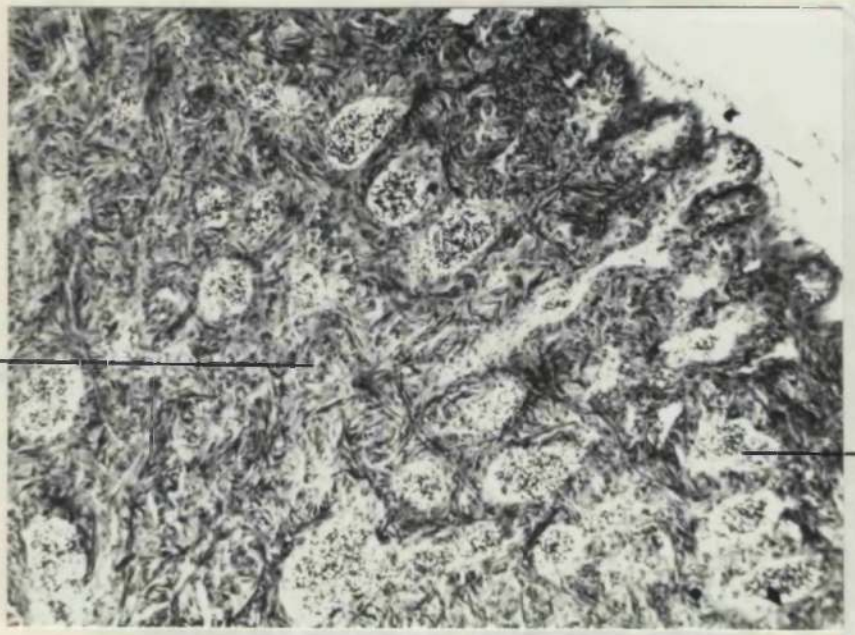


Fig. 1

Fig. 2

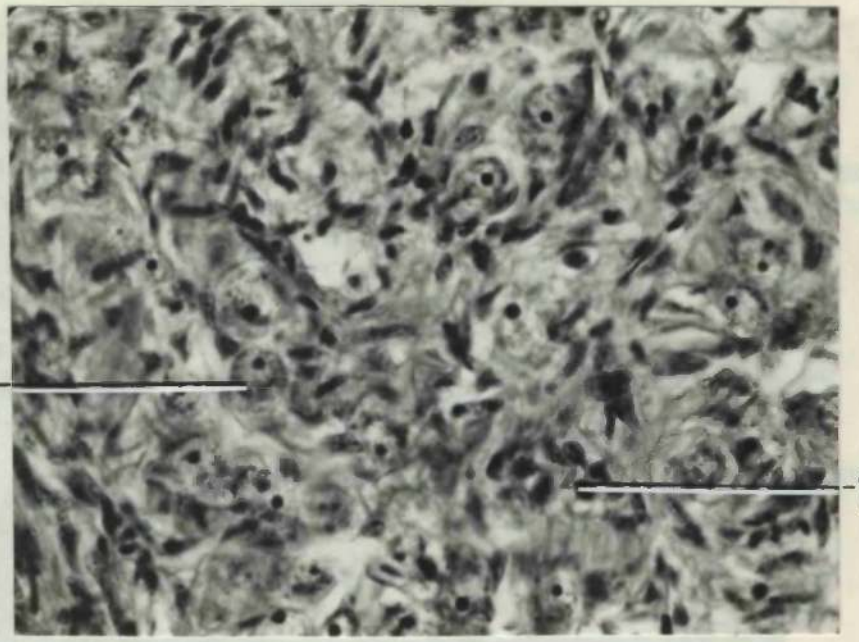

spermatogonium
connective tissue

Fig. 1

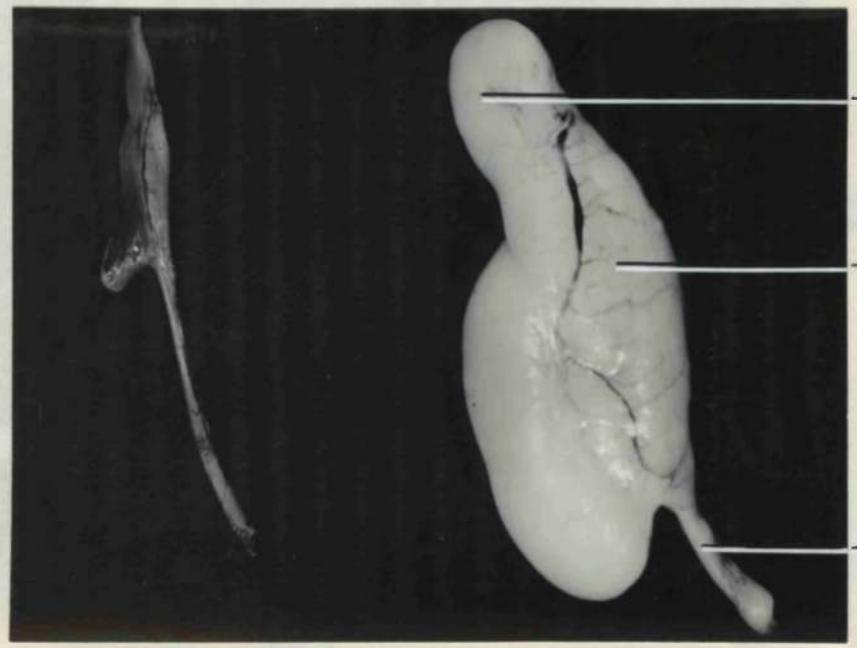

testis
collecting ducts
testis duct

Fig. 2

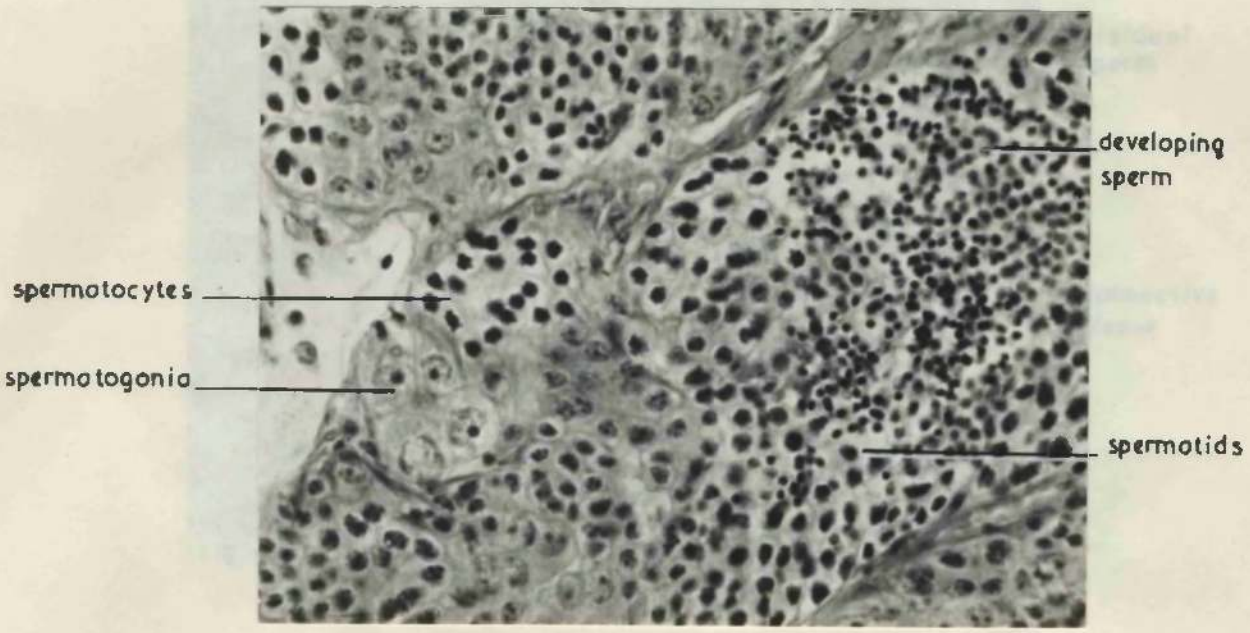

developing sperm
spermotocytes
spermatogonia
spermotids

Fig. 1


residual sperm
connective tissue

Fig. 2

PLATE 25

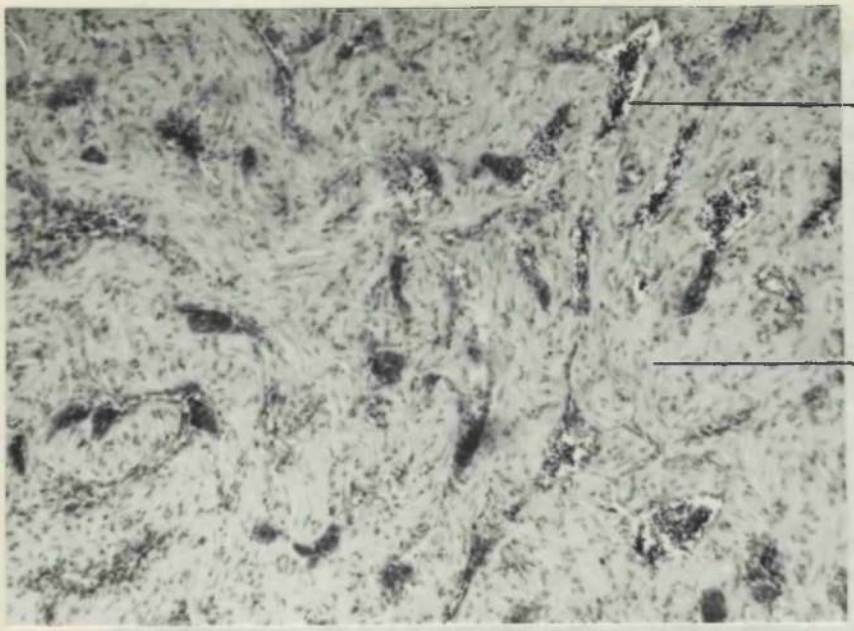


Fig. 1

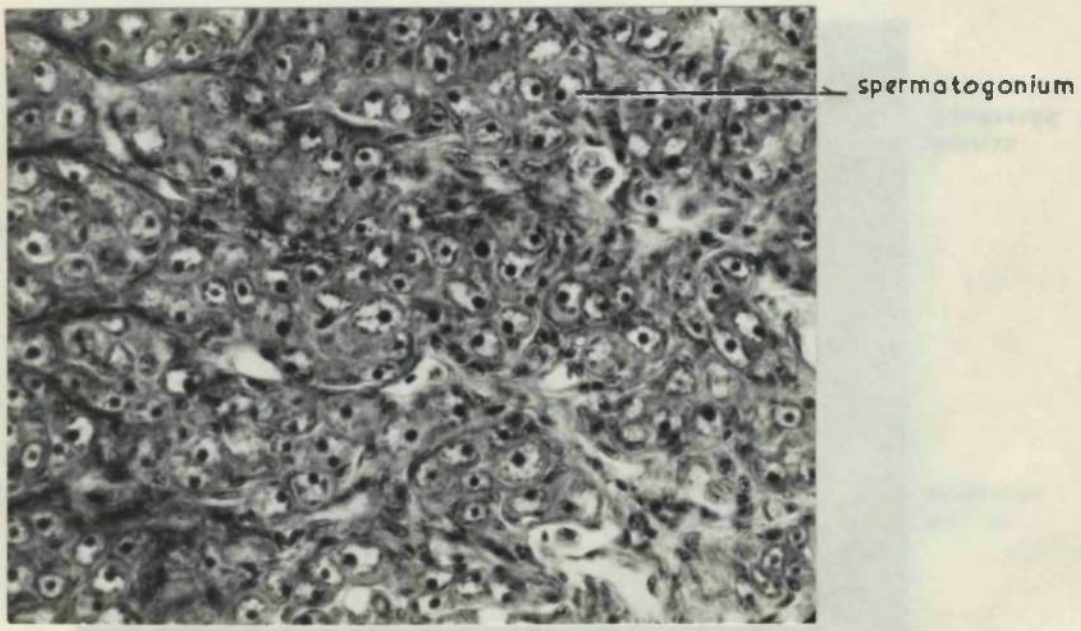


Fig. 2

PLATE 26

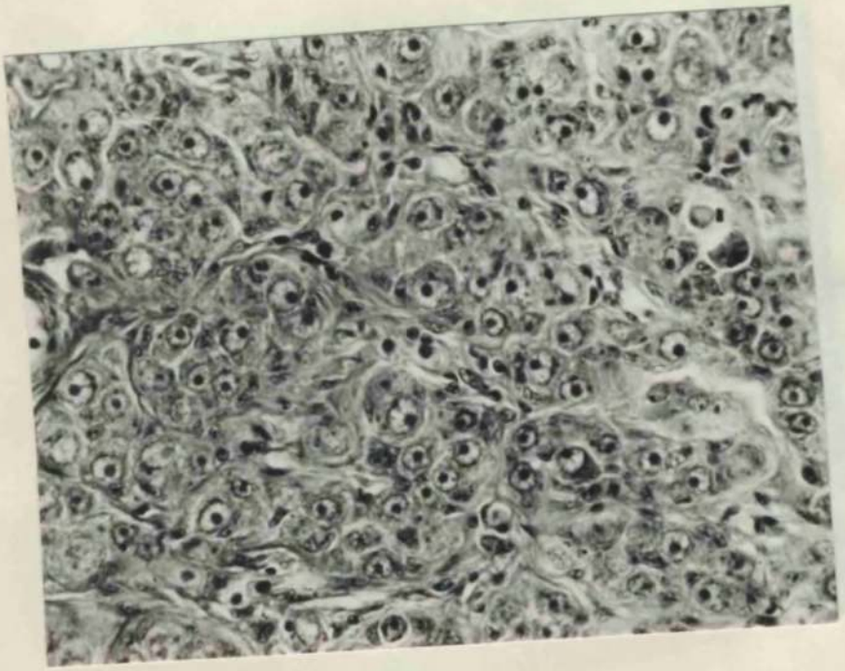


Fig. 1

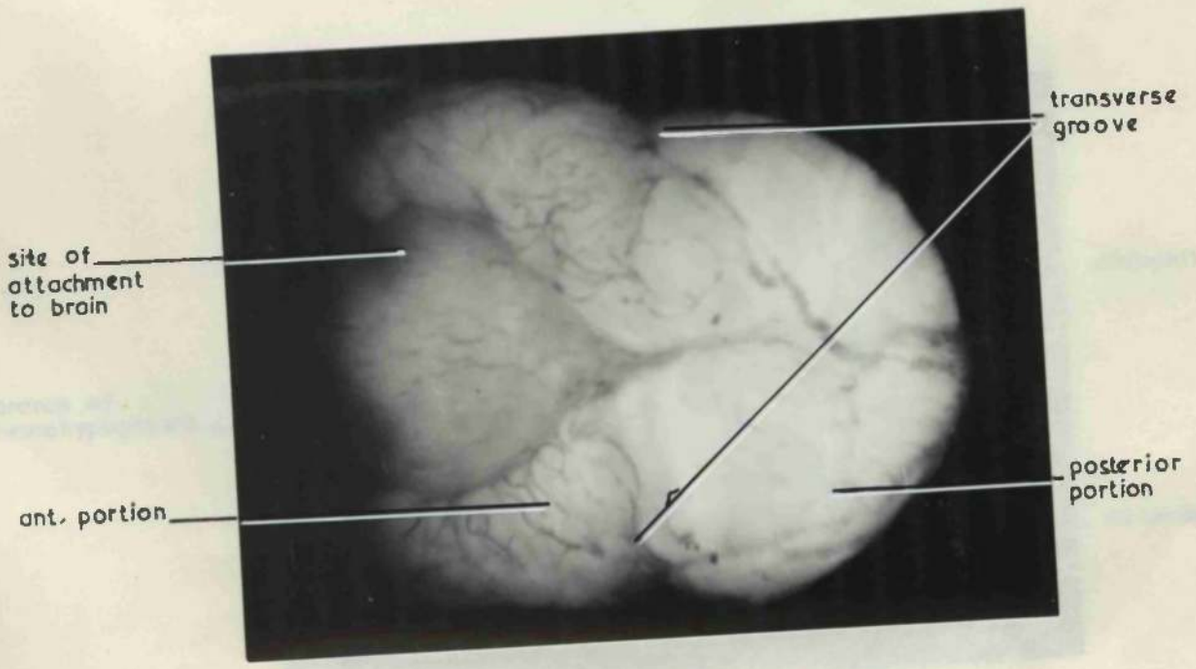


Fig. 2

PLATE 27

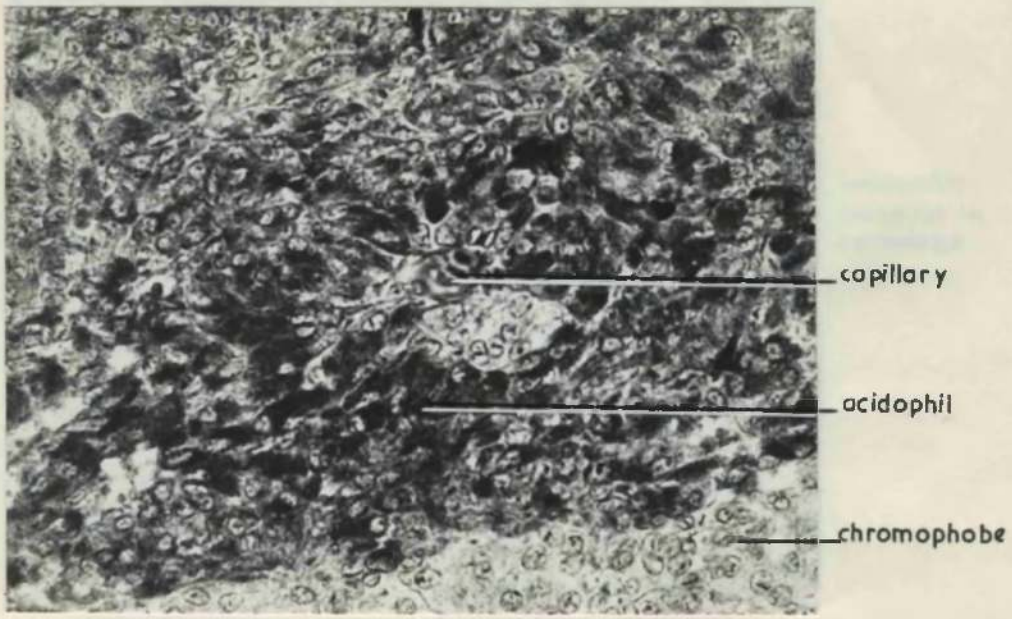


Fig. 1

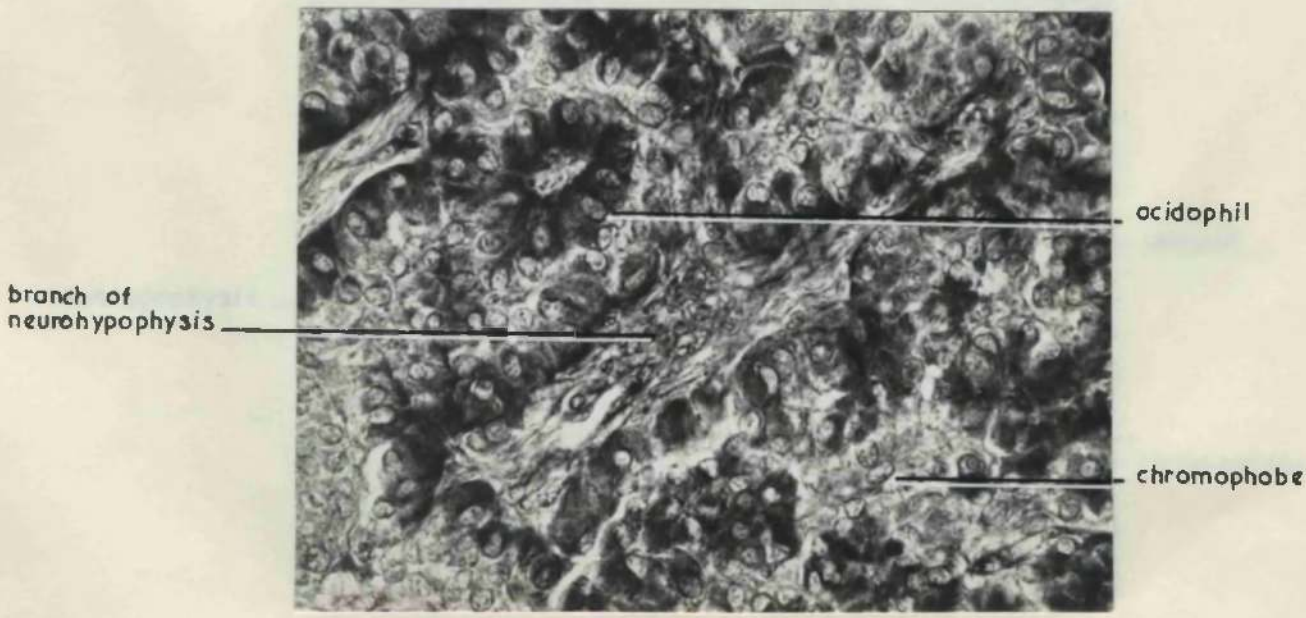


Fig. 2

PLATE 28

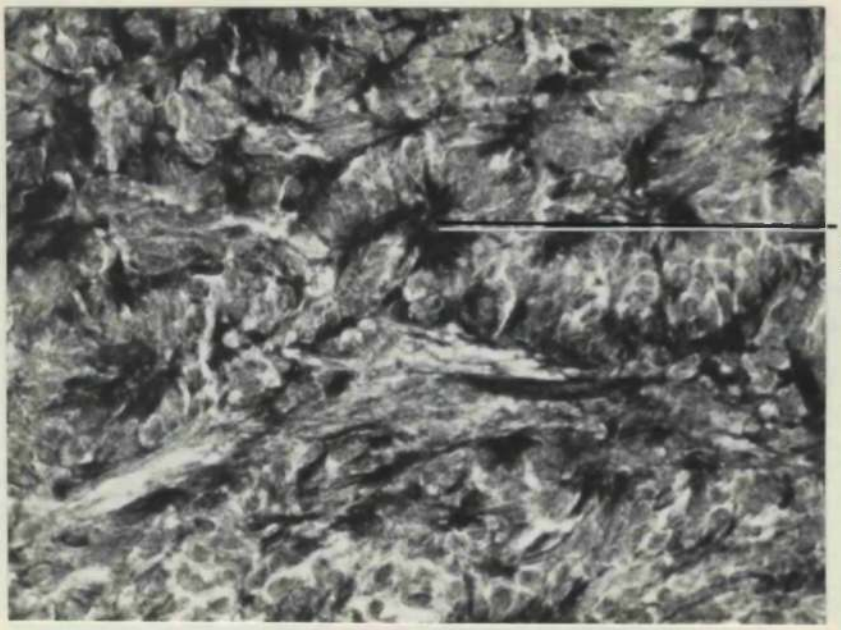


Fig. 1

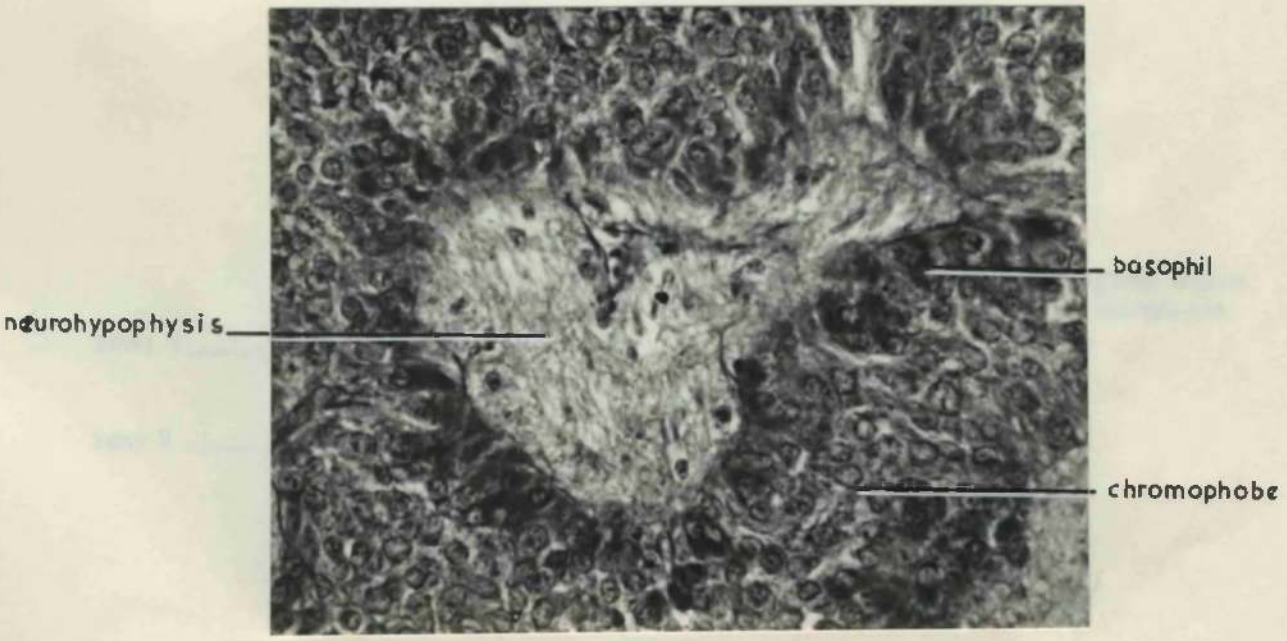


Fig. 2

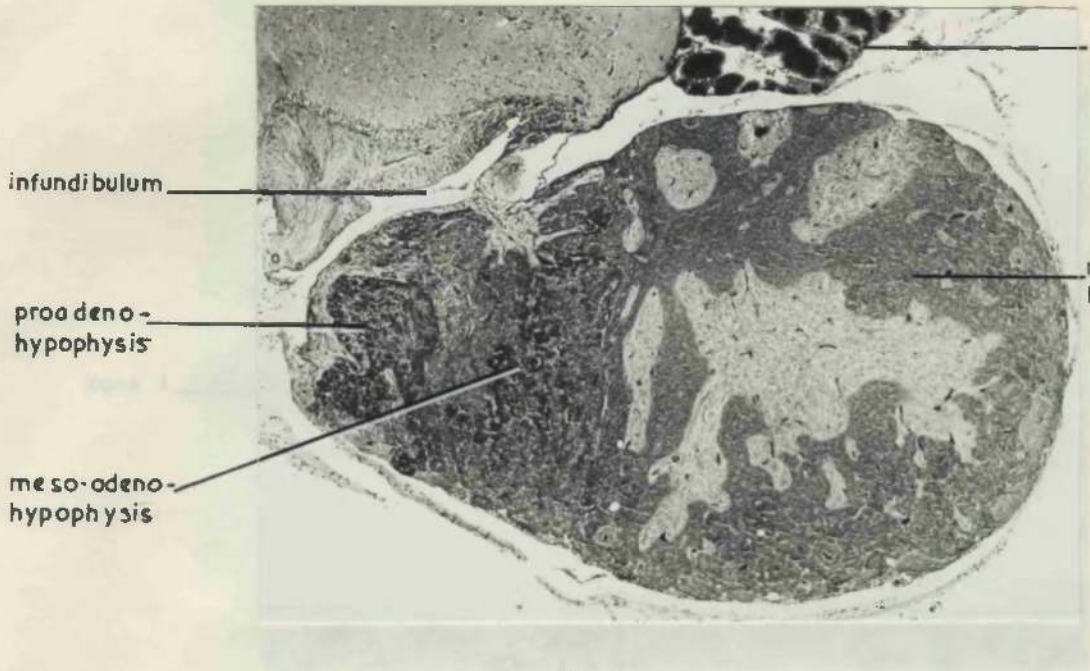

saccus vasculosus
infundibulum
meta-adeno-hypophysis
proadeno-hypophysis
meso-adeno-hypophysis

Fig. 1

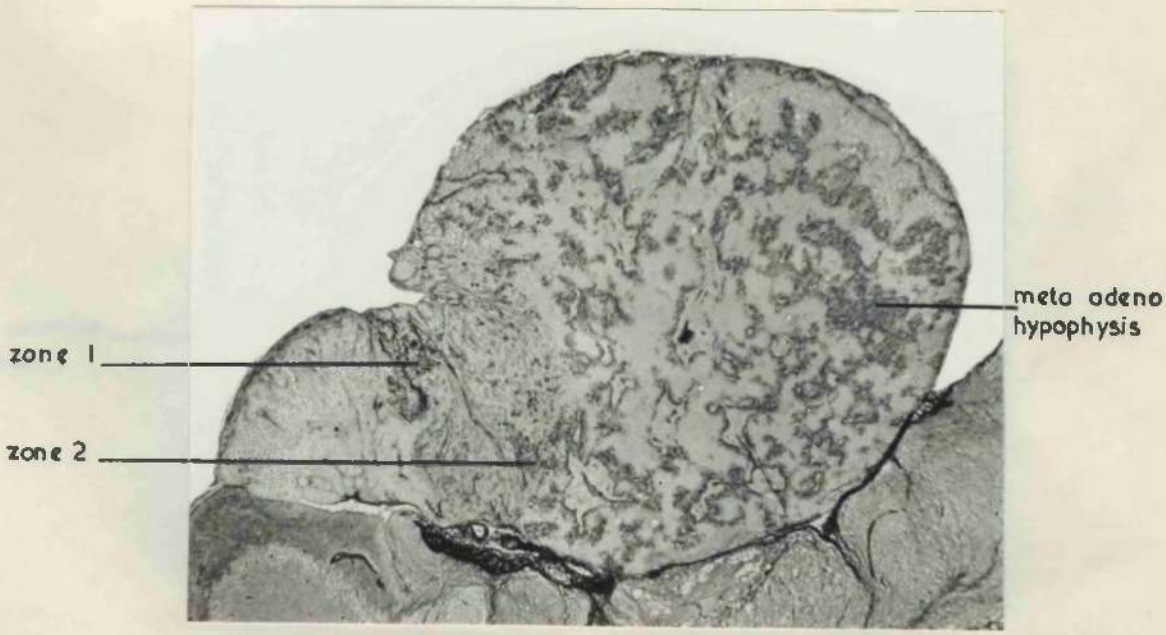

meta adeno hypophysis
zone 1
zone 2

Fig. 2

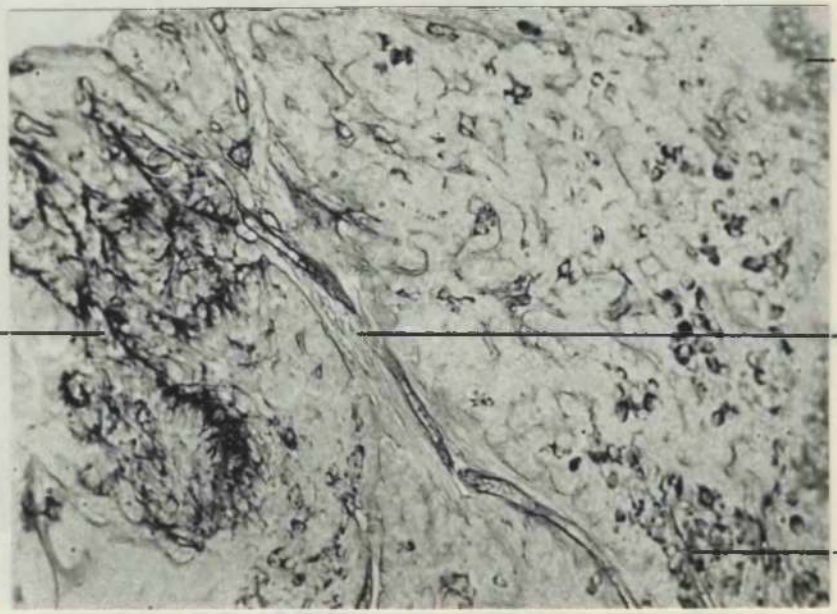

meta
adeno
hypophysis
neuro
hypophysis
zone 1
zone 2

Fig. 1

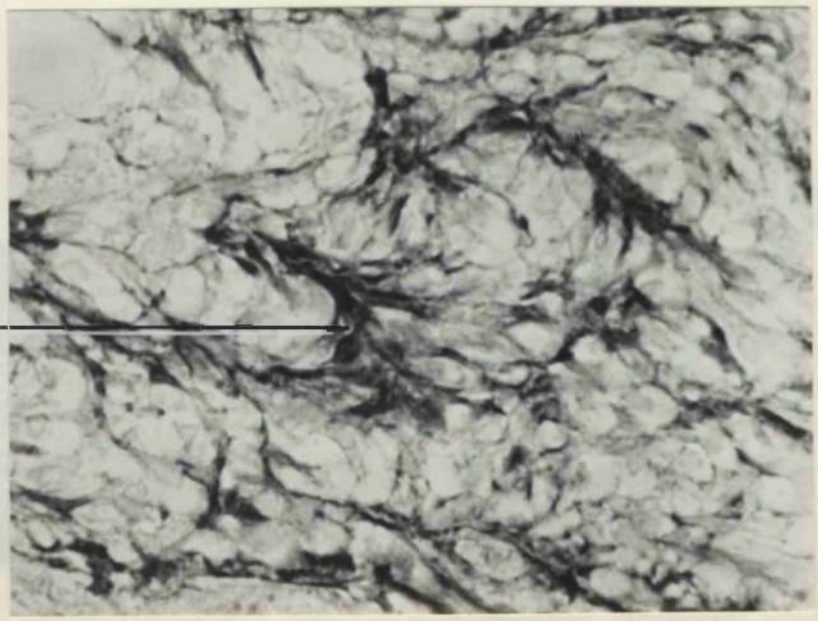

PAS positive
material

Fig. 2

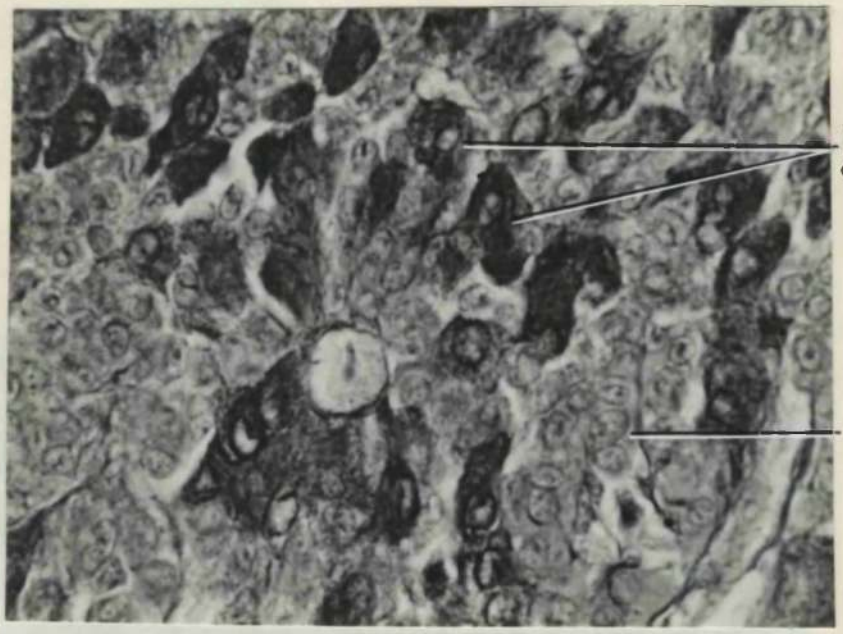

PAS positive
cells
chromophobe

Fig. 1

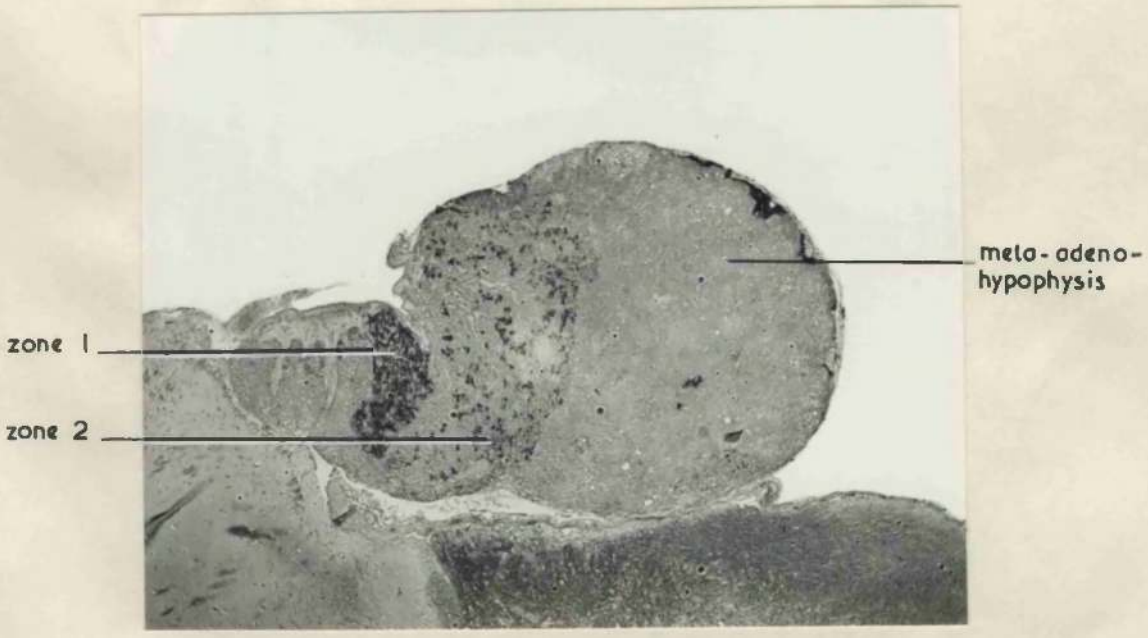

meta-adeno-
hypophysis
zone 1
zone 2

Fig. 2

PLATE 32

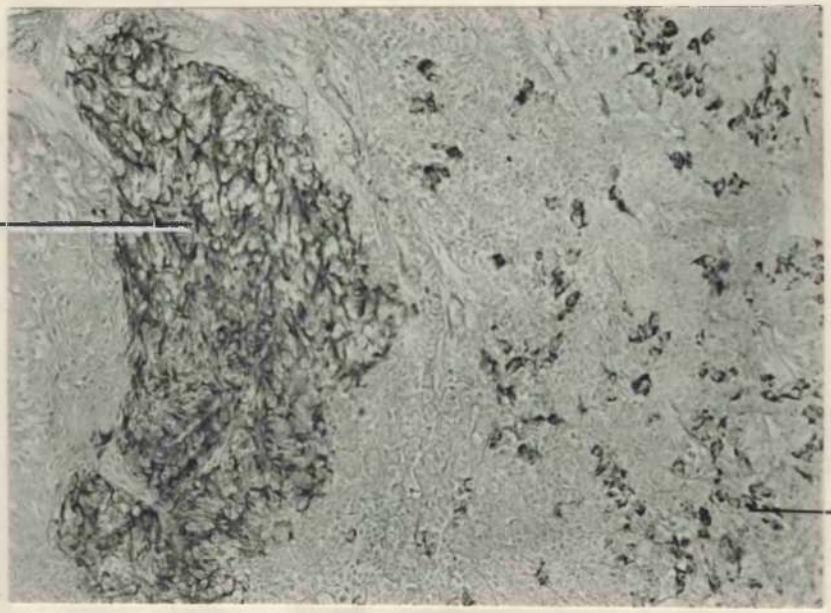


Fig.1

The endocrine control of reproduction in the plaice, Pleuronectes platessa L.

A detailed study has been made of the sexual cycle in the plaice, Pleuronectes platessa. The spawning season is in spring and the liberation of the reproductive products is followed by a period of mitotic activity during which the numbers of primary germ cells is increased. The meiotic division of spermatogonia in the testis begins in autumn and is paralleled by the initiation of vitellogenesis in the ovary. Motile sperm are present in the testis for several weeks before the spawning period, but ripe oocytes are not produced until the spawning season is about to begin.

The cytology of the corpus atreticum and the ruptured follicle or "corpus luteum" is described and their functional significance is discussed. It is concluded that the endocrine nature of these structures has not been demonstrated.

A technique for the removal of the pituitary gland has been developed and post-operative survival of more than one year have been obtained. Much post-operative care is required and all experimental fish had to be force-fed.

The effect of hypophysectomy has been studied on the gonads of plaice at different phases of their annual sexual cycle. In the female, it is clear that the presence of pituitary gonadotrophin is essential for the initiation of vitellogenesis and for the maintenance and continued development of yolked eggs. Gonadotrophin withdrawal

results in the conversion of all yolked eggs into corpora atretica. Although the oviposition of ripe oocytes is inhibited after hypophysectomy, it has not been conclusively demonstrated that this phase of the cycle is under the control of gonadotrophin. Pituitary ablation has no effect on the primary oocytes of the immature fish and these cells in the post-spawning adult female can continue their development up to the stage of yolk deposition, at which point, further development is arrested. The capacity of primary oocytes to enter vitellogenesis is retained in the absence of the pituitary since yolk deposition can be stimulated by the injection of extract of plaice pituitary glands into hypophysectomised animals.

In the male, hypophysectomy results in the inhibition of spermatogenesis, although there is some evidence that this process, once started, can continue in the absence of pituitary gonadotrophin. In contrast to the situation in the female, spermiation is not prevented by pituitary removal. In both mature and immature fish, the spermatogonia are not influenced by pituitary ablation, but there is some evidence that mitotic division of these cells is prevented.

The cytology of the plaice pituitary gland is described and 2 zones of basophils have been identified in the meso-adenohypophysis. The presence of glycoprotein material in these cells has been established by the use of the periodic acid Schiff reaction and the granules of both cell types are aldehyde fuchsin positive. Considerable variation was found in the numbers of zone 2 basophils throughout the year and evidence is presented that these basophils are responsible for the secretion of gonadotrophin.

A technique for the bioassay of plaice gonadotrophin is described using male Xenopus laevis as a test animal. The results indicate that the amount of gonadotrophin present in the pituitary fluctuates during the year, being least in the post-spawning period of the fish and increasing in the autumn and winter during spermatogenesis and oogenesis.

Pituitary glands from several other teleost species have also been assayed on the same test animal and the MED 50 (minimum effective dose producing a 50% response) is of the same order (5 - 10mg.) for all species except the cod where it is about 2.25 mg.

Plaice gonadotrophin is capable of stimulating the uterus of the immature female mouse, but the amount of material required are large and the response is considered to be of a threshold nature. In view of this, mammalian test species are thought to be unsuitable for the assay of teleost gonadotrophin.

The nature of the gonadotrophins of the plaice pituitary is discussed and evidence is presented which indicates that the pituitary contains a hormone, the follicle stimulating properties of which resembles those of mammalian follicle stimulating hormone. The evidence for the presence of a hormone comparable to mammalian luteinising hormone is not conclusive.

The concept of the species specificity of vertebrate gonadotrophin is discussed. A comparison of the MED 50 obtained when plaice and Xenopus laevis pituitary material is assayed on male Xenopus laevis suggests that such a specificity does exist.