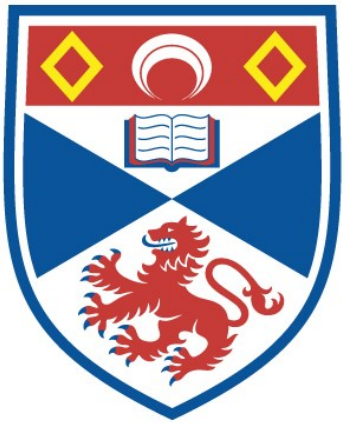
THE THYROTROPHIC AND GONADOTROPHIC FUNCTIONS OF THE ELASMOBRANCH PITUITARY

C. K. Goddard

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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THE THYROTROPHIC AND GONADOTROPHIC FUNCTIONS OF THE ELASMOBRANCH PITUITARY.

by

C.K. Goddard, B.Sc.

Thesis presented for the Degree of Doctor of Philosophy in the Faculty of Science of the University of St. Andrews

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DECLARATION

I hereby declare that the work recorded in this Thesis has been carried out by me, except where otherwise stated, and that the Thesis is of my own composition. I further declare that it has not been submitted in any previous application for a higher degree.

RESEARCH CAREER

The research work recorded in this Thesis was carried out between the years 1953 and 1957, at the Gatty Marine Laboratory, St. Andrews. I was enrolled as a research student on 6th November, 1953, as a graduate of the University of Edinburgh.

SUPERVISOR'S CERTIFICATE

I certify that Charles Keith Goddard has fulfilled the conditions laid down in the regulations for the Degree of Ph.D., under Ordinance No. 16 of the University Court of the University of St. Andrews, and that he is accordingly qualified to submit this Thesis for the degree of Doctor of Philosophy.

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PREFACE.

The elasmobranchs are an ancient group of vertebrates; the earliest fossil remains are found in Devonian strata, and forms morphologically similar to those now in existence had been evolved by the Jurassic. The group has undergone little change since the Devonian, and, although highly specialised in some respects, they are generally regarded as "primitive" in the sense that they represent an early stage of vertebrate evolution.

Despite the obvious merit of elasmobranchs as subjects for comparative study, little is known of their endocrine physiology. The present work attempts to determine whether the thyroid gland and gonads are under pituitary control, as has been demonstrated in other vertebrate groups, and, if so, which portion of the pituitary gland is responsible for such control.

Experimental study of the elasmobranch pituitary is facilitated by the cartilaginous skeleton, which allows ready access to the cranial cavity. However, hypophysectomy in elasmobranchs is not a simple operation, as will appear in due course. A major disadvantage to the use of elasmobranchs for experimental work is the difficulty of husbandry. Most workers have reported survival periods of four to six weeks after hypophysectomy; only Vivien (1940, 1941) has reported longer survival periods.

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 of a large number of fish none survived 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 physiological processes in elasmobranchs, and should yield further information on the inter-relationships between the various endocrine glands of these fish.

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THE ELASMOBRANCH PITUITARY GLAND

Introduction.

The elasmobranch pituitary gland consists of three main portions or "lobes". These are (see Fig.1, p.3):-

(1) The rostral lobe, a delicate tongue of tissue lying between the lobi inferiores of the hypothalamus, posterior to the optic chiasma. This lobe has been variously referred to as the "juxta-neural portion" (Tilney, 1911), the "superior sac" (Charipper, 1937), and the "pars anterior" or "anterior lobe" (most workers). The term "rostral lobe" was proposed by Stersi (1909).

(2) The neuro-intermediate lobe, a large and somewhat bulbous lobe attached postero-dorsally to the rostral lobe. The saccci vasculosi lie above and overlap this lobe on either side. The neuro-intermediate lobe has been referred to as "the posterior part" (Herring, 1911), the "distal epitholial portion" (Tilney, 1911) and the "superior lobe" (Baumgartner, 1915). The terminology used here has been accepted by most workers.

(3) The ventral lobe, lying ventral to the other two lobes and embedded in the floor of the cranium. This lobe is a unique feature of the elasmobranch pituitary. It is attached by the "interhypophysial stalk" to the caudal end of the rostral lobe (Baumgartner, 1915; Norris, 1941), with which it shares a common lumen. It has been referred to as the "inferior lobes" (Baumgartner, 1915), the "pars

ventralis" (Howes, 1936; Hogben, 1936), and the "inferior sac" (Charipper, 1937). The terminology used here has been accepted by most workers.

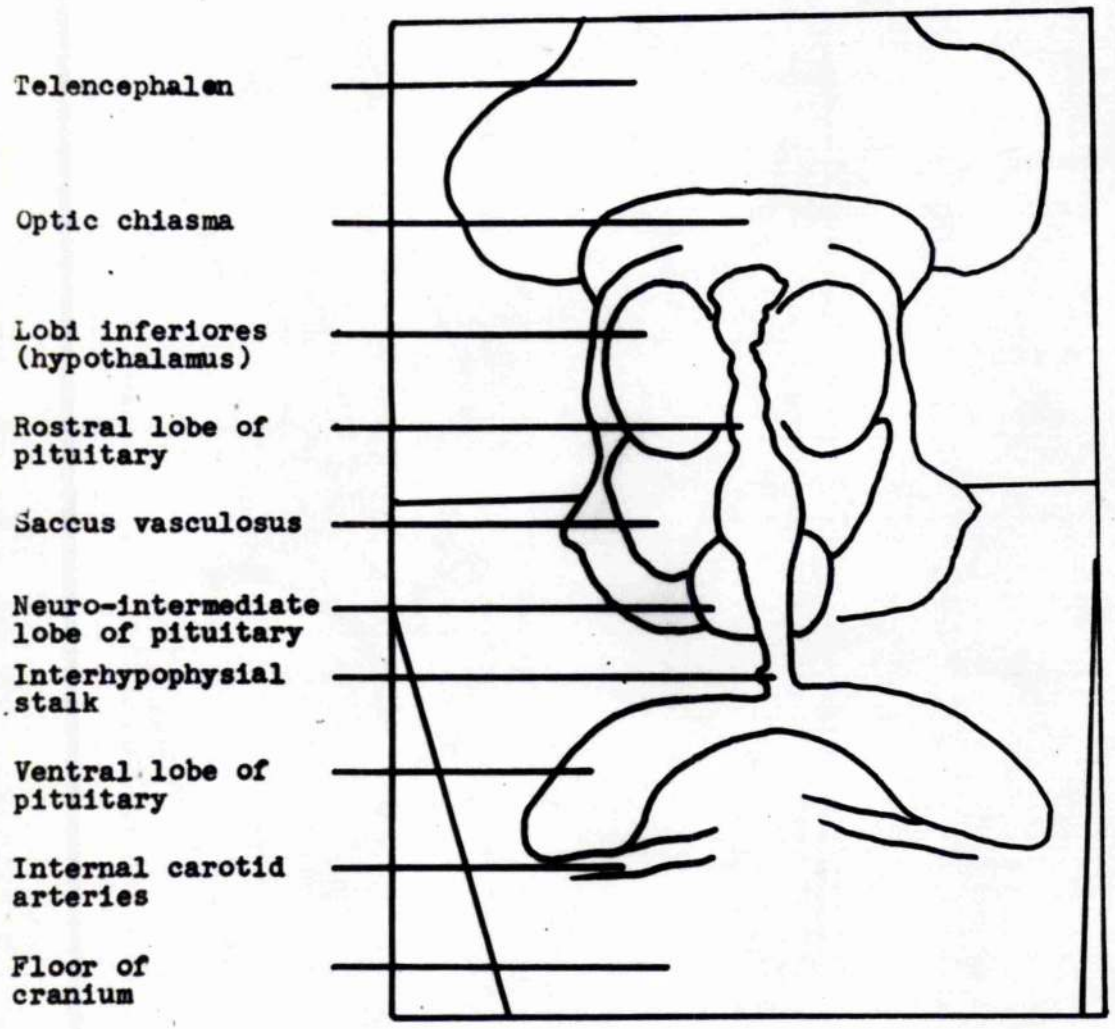


Fig.1. Pituitary region of Raia clavata, adult male. Brain deflected upward and backward to show ventral lobe of pituitary attached to floor of cranium.

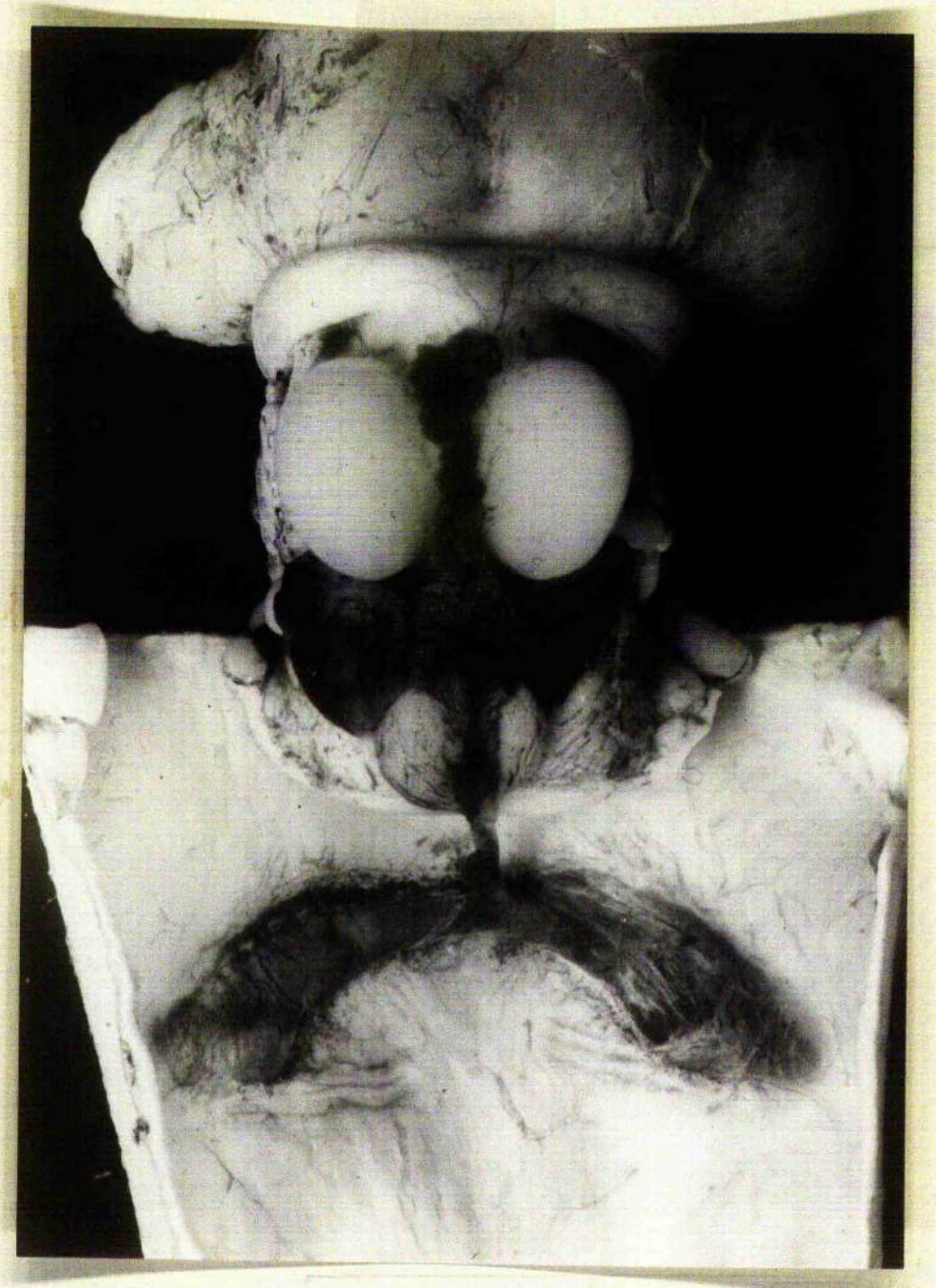


Fig. 1.

Critical Review of the Literature.

The literature prior to 1915 has been extensively reviewed by Baumgartner (1915). I have not attempted to cover this early work completely, and in a few cases Baumgartner's reviews have been adopted. The reviews in question are noted as taken from Baumgartner.

The ventral lobe of the pituitary has been inadequately treated or ignored by most workers. In this thesis it has assumed some importance, and will be given particular attention in the review of the literature.

(A) Development. Refer to diagrams, fig. 2, p. 13.

Rabl-Rückhard, (1880, reviewed by Baumgartner), stated that in the "Acanthias" embryo of 60 mm. length there is a ventral outgrowth from the hypophysis; this is presumably the anlage from which the ventral lobe develops, and Rabl-Rückhard appears to have been the earliest worker to describe the origin of this lobe.

Haller, (1896, reviewed by Baumgartner), gave an account of the development of the hypophysis in Mustelus. By the time the embryo has reached a length of 90 mm. the hypophysis has lost its connection with the mouth, and from the floor of the caudal end two lateral evaginations have developed; from these evaginations the ventral lobe is formed. In the fully-developed pituitary of a 20 cm. Mustelus

Haller described the interhypophysial canal joining the ventral lobe to the rostral lobe.

Chiarugi, (1898, reviewed by Baumgartner), dealt briefly with the hypophysis of Torpedo. He found a connection between the premandibular somites and the hypophysis. This connection was later described and figured by de Beer (1926), who regarded it as an important clue to the homology of the ventral lobe.

Rossi (1902) described and figured various stages in development of the pituitary of Torpedo, with particular reference to the lateral lobes. The latter first appear in embryos of about 11 mm. length as paired diverticulae from the ventro-lateral walls of the hypophysis. They are separated by the carotid arteries from a second pair of diverticulae which later fuse to form the ventral lobe.

It is clear from the work of de Beer (1926) and Norris (1941) that in Torpedo the lateral lobes consist of a single pair of diverticulae which are subdivided at an early stage of development by the carotid arteries. The two pairs of diverticulae described by Rossi should therefore be interpreted as together constituting the lateral lobes. This subdivision of the lateral lobes in Torpedo has caused much confusion in the literature. The origin of the ventral lobe is best seen in those forms (e.g. Scyalus) in which such subdivision does not occur.

Gentes (1908a) speaks of the superior and inferior sacs of the pituitary, evidently referring to the rostral and ventral lobes. Working on Torpedo marmorata Riss., he came to the conclusion that the inferior sac does not arise from the superior sac. In the 22 mm. embryo the hypophysis shows an annular constriction which gradually deepens; in this way the hypophysis is divided into two portions, one dorsal and the other ventral. These are the superior and inferior sacs; they are contemporary in origin, and represent subdivisions of Rathke's pouch. From each side of the ventral sac two prolongations grow outward laterally, and these are the lateral lobes. The constricted portion between the two sacs becomes an oblique, elongated canal which persists in the adult. Gentes apparently did not recognise the neuro-intermediate lobe as a separate entity, since he makes no mention of it.

In another paper (1908b) Gentes described the lateral lobes as blind outgrowths from the inferior sac, two on each side. He briefly referred to previous work on the lateral lobes of higher ver: :tebrates, and it was apparently his intention to trace their homo: :logues in elasmobranchs.

It is clear from the work of Rossi (1902) de Beer (1926) and others that Gentes is incorrect, both in his description of the manner in which the ventral lobe arises and in his interpretation of

what constitutes the lateral lobes. The ventral lobe arises as paired evaginations of Rathke's pouch; moreover these evaginations are the homologues of the lateral lobes of higher vertebrates. The outgrowths mentioned by Gentes are subdivisions of the ventral lobe. Norris (1941) found that in many genera the lateral portions of the ventral lobe tended to subdivide, and expressed the view that the internal carotids were responsible: "It has been suggested by Sterzi that the notch which causes this division in each lateral part of the (ventral) lobe is the result of the growth of the embryonic lobe against a blood vessel. This is undoubtedly the true explanation and the blood vessel is the internal carotid artery or its immediate branches" (Norris, 1941, p. 17).

Herring (1911) described development of the pituitary in Scyllium (Scyliorhinus) canicula and Raia batis. He failed to mention the ventral lobe, although the anlage from which it develops is clearly shown in his figures of embryo dogfish.

Sterzi (1912, reviewed by Baumgartner) described the development of the hypophysis in "Acanthias". From the hypophyseal pouch two lateral diverticulae arise; these later form the endocranial portion (ventral lobe). The dorsal lobe (neuro-intermediate) develops at the superior end of the hypophysial pouch, and Sterzi mentioned the presence of blood vessels and nerve fibres between the epithelial

cords of this lobe.

Woerdeman (1915) studied development of the pituitary in Torpedo. He found that after the formation of Rathke's pouch two further ecto: dermal invaginations occur. The hypophysis then consists of a small Rathke's pouch somewhat constricted from an anterior "mittelraum" and anterior to the latter a "vorraum". No other worker has described three separate invaginations of oral ectoderm.

According to Woerdeman, in 20 mm. embryos the mittelraum becomes divided, by a horizontal constriction, into a dorsal and a ventral portion. Woerdeman described the lateral lobes as growing outward from the ventral portion, which also carries the stalk connecting the hypophysis with the oral epithelium.

Woerdeman's work was commented on by Baumgartner (1915), whose findings on Squalus differed from Woerdeman's in the following respects:- Baumgartner reported two ectodermal invaginations, one of which is Rathke's pouch; he found that the ventral lobe arises as a pair of outgrowths from Rathke's pouch, not by constriction of Rathke's pouch as described by Woerdeman. Finally, Baumgartner found that the stalk connecting the hypophysis with the oral epithelium remains attached to the rostral lobe until it disappears; at no time is it attached to the ventral lobe, which arises posterior to the stalk in question.

Baumgartner suggested that the ventral sacs and lateral lobes of Woerdeman probably represent the ventral lobe, and in this he is undoubtedly correct. Woerdeman's study adds little to our informat: :ion on the elasmobranch pituitary; it adds appreciably to the confus: :ion surrounding development of the gland in Torpedo.

Baumgartner (1915) studied development of the hypophysis of Squalus acanthias, and gave a detailed description of its morphology and histology. He found that Rathke's pouch gives rise to the posterior part of the rostral lobe, the remainder of this lobe being formed from a later ectodermal invagination. Rathke's pouch also gives rise to the intermediate component of the neuro-intermediate lobe postero-dorsally, and to the ventral lobe postero-ventrally.

The ventral lobe develops from a pair of evaginations which arise on either side of Rathke's pouch. They are first noticeable in embryos of about 22 mm. length, posterior to the connection be: :tween the hypophysis and the oral epithelium. As development proceeds they migrate posteriorly and ventrally. From an early stage the two evaginations are connected by a "ridge" across the hypophysial anlage; in the adult S. acanthias this connection is no more than a constriction separating the ventral lobe into right and left halves.

The neuro-intermediate lobe is first indicated in the 28 mm.

embryo by two slight "outpouchings" at the posterior end of Rathke's pouch. In this region the epithelial cells proliferate towards the overlying floor of the saccus vasculosus and give rise to the cell columns which constitute the hypophysial component of this lobe.

Development of the rostral lobe involves differentiation of the epithelium of Rathke's pouch and the anterior invagination. The result is a folded epithelium with gland-like diverticulae, surrounding a persistent hypophysial cavity.

De Beer (1926), in his monograph on the vertebrate pituitary, described Rathke's pouch in selachians as a hollow ectodermal invagination, similar to that of amniotes but unlike the solid ingrowth found in amphibians and teleosts. He confirmed that the ventral lobe develops from paired outgrowths of Rathke's pouch, and homologised these outgrowths with the lateral lobes of higher vertebrates.

In the early Torpedo embryo de Beer described and figured a pair of "proboscis pores" by which Rathke's pouch communicates with the two premandibular somites. After connection with the premandibular somites has been severed the lateral lobes arise at the place where the proboscis pores opened. According to de Beer, this condition is "precisely comparable" with that seen in the duck. Since the lateral lobes give rise to the pars tuberalis in higher vertebrates, de Beer argued that the ventral lobe of elasmobranchs is homologous with the pars tuberalis.

De Beer noted that in the older Torpedo embryo the developing ventral lobe consists of two processes on each side; these two pairs of processes are separated by a transverse blood vessel (the internal carotid anastomosis). This is the condition that has caused so much confusion in the literature; it requires no further comment.

Van de Kamer and Schuurmans (1953) studied development of the saccus vasculosus of S. canicula. The saccus evagination arises from the floor of the third ventricle, between the optic chiasma and the recessus posterior. At an early stage in development the caudal part of the hypophysial anlage makes contact with the ventral wall of the saccus evagination, and the latter proceeds to differentiate as neurohypophysial tissue. This differentiation takes place throughout the region of contact with Rathke's pouch. Van de Kamer and Schuurmans suggest that differentiation is "by a process of continuing induction with the orohypophysis as inductor". Dorsally the saccus evagination differentiates as characteristic saccus vasculosus tissue.

Van de Kamer and Schuurmans regard the Saccus vasculosus as the homologue of the processus infundibuli of mammals, and this view is shared by Norris (1941). Norris argues that the elasmobranch "infundibulum", stretching from the optic chiasma to the mammillary body, could not be homologous with the mammalian infundibulum. It may be suggested here that these workers are only partially correct: the homologue of the processus infundibuli of mammals appears to be

the embryonic saccus evagination, not the saccus vasculosus. The embryonic evagination gives rise to the saccus vasculosus dorsally and to neurohypophysial tissue ventrally, so that both of these tissues together evidently constitute the homologue of the mammalian infundibulum.

Fig. 2.

Diagrammatic representation of development of elasmobranch pituitary.

(In all figures the anterior is to the left.)

A-F. Elasmobranchs. (A-E. sagittal sections; F. ventral view.)

G. Mammal.

A-D. Successive stages in embryonic development, from various authors.

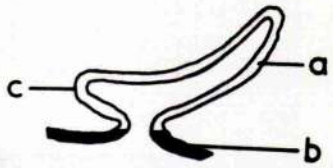
E. Adult skate (Raia radiata Don.), from serial sections.

F. Adult dogfish (S. canicula), from Norris.

G. Embryonic rabbit, from de Beer.

(a). Rathke's pouch.
(b). Oral epithelium.
(c). Anterior diverticulum of Rathke's pouch.
(d). Saccus evagination from floor of dienecephalon.
(e). Optic chiasma.
(f). Neuro-intermediate lobe.
(g). Lateral lobes of hypophysis.
(h). Saccus vasculosus.
(k). Interhypophysial stalk.
(m). Rostral lobe.
(n). Ventral lobe.
(o). Lobi inferiores of the hypothalamus.
(p). Pars anterior.
(q). Lateral lobes growing upward toward tuber cinereum.
(r). Pars intermedia.
(s). Hypophyseal cleft.
(t). Infundibular process.

A



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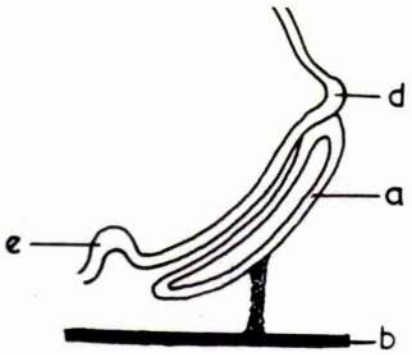

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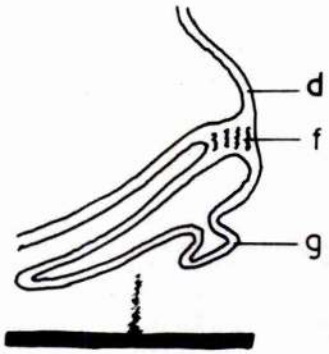

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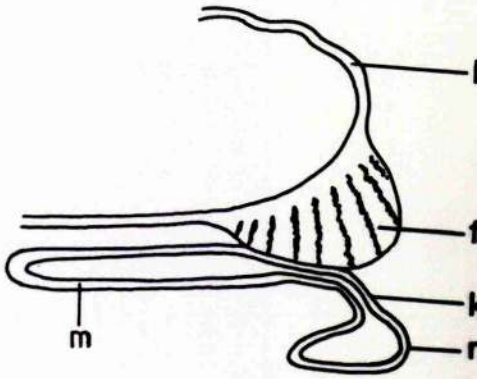

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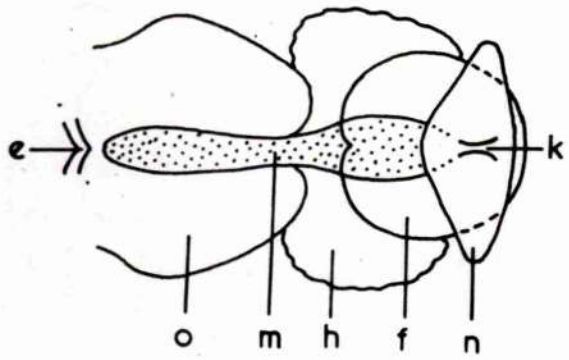

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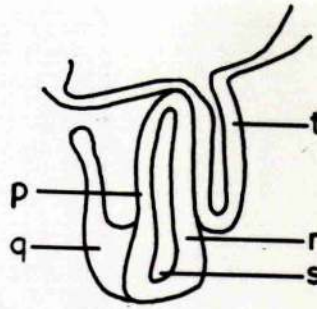


G

Fig. 2

(B) Morphology and Histology.

The earliest description of the elasmobranch hypophysis appears to be that of Miklucho-Maclay (1870). Following Rathke, he defined the hypophysis as the oral component of the pituitary. However, from his description and figures it is clear that he con: :sidered the ventral lobe to be the hypophysis. He mistook the remainder of the pituitary (i.e. the rostral and neuro-intermediate lobes) for part of the saccus vasculosus, and described the lumen of this portion as communicating with that of the saccus in some selachians. It may be noted that a connection between these two lumina has not been found by other workers.

Herring (1908b) took the view that the skate pituitary is not differentiated into anterior and posterior lobes. However, he noted an intermixture of nervous and epithelial tissue in the "main body" of the pituitary (neuro-intermediate lobe), and was prepared to concede that a posterior lobe might be represented. Herring described a fine strand of connective tissue connecting the pituitary with the cartilaginous floor of the cranium, and interpreted this as the remnant of the neck of Rathke's pouch: in this Herring was quite mistaken. No trace of the neck of Rathke's pouch remains after embryonic life. The strand in question is the interhypophysial stalk of Norris (1941); it consists of epithelial pituitary tissue, and connects the ventral lobe (which is attached to the floor of the

cranium) with the remainder of the pituitary. Herring did not mention the ventral lobe, and it must be concluded that he was unaware of its existence.

A later paper by Herring (1911) on development of the elasmobranch pituitary has already been mentioned. In this paper he expressed the view that the rostral lobe is the homologue of the pars intermedia of the mammalian pituitary, but this view is not shared by other workers.

Tilney (1911), in a paper on the comparative histology of the pituitary, did not mention the ventral lobe. Working on Squalus acanthias, he found that the lumen of the juxta-neural portion (rostral lobe) showed "no evidence of any contained substance whatever". This conflicts with Baumgartner (1915), who described the presence of colloid in this lumen. According to Tilney, the cells of the juxta-neural portion are basophil and irregular, tending to form acini, whereas those of the distal epithelial portion (neuro-intermediate lobe) are acidophil, with scanty cytoplasm. He also stated that the blood spaces of the distal epithelial portion drain into the vessels of the saccus vasculosus, but gave no evidence in support of this conclusion.

Herring (1913) in a contribution on the comparative anatomy and physiology of the pituitary, stated definitely that there is neither

a pars nervosa nor a pars intermedia in the elasmobranch pituitary. It is difficult to reconcile this with his earlier views on the subject.

In the rostral lobe Herring found evidence of centrifugal secretion by the cells bordering the lumen; the secretion is in the form of deeply-staining granules which pass into the surrounding connective tissue, en route to the neighbouring blood vessels.

By the following year Herring (1914) had again modified his views on the histology of the skate pituitary: he now thought it probable that "part of the chromophobe portion is true pars inter: :media ....", apparently referring to the neuro-intermediate lobe.

Baumgartner's work (1915) on the hypophysis of Squalus acanthias has already been mentioned. Part of this work deals with morphology and histology of the gland in the adult. Baumgartner found that the rostral lobe is separated into anterior and posterior portions by a slight constriction of the middle. The neuro-inter: :mediate lobe is considerably wider than the rostral lobe and is attached dorsally to the saccus vasculosus. A slight constriction separates the ventral lobe into right and left halves; its width is somewhat less than that of the neuro-intermediate, and it is connected to the caudal end of the rostral lobe by the interhypophysial canal.

Both the rostral and ventral lobes are essentially sac-like,

with their lumina in communication by way of the interhypophysial canal. The walls of both lobes tend to form tubule-like structures, and in the rostral lobe the tubules may anastomose extensively among themselves. Baumgartner found a cavity in the neuro-intermediate lobe of the embryo, presumably part of the original cavity of Rathke's pouch, but in the adult this lobe shows no sign of a lumen.

Baumgartner described a colloidal secretion in the lumina of the rostral and ventral lobes, and also in the lumina of the tubules of these lobes. He found a similar secretion occupying spaces among the cell columns of the neuro-intermediate lobe. He noted the presence of nerve fibres between the cell columns of the neuro-intermediate lobe, and referred to the work of Stersi who had described these fibres as coming from the lobi inferiores of the brain.

Baumgartner described the cells of the rostral and ventral lobes as acidophils, but the staining reaction of the neuro-intermediate lobe is not so clear-cut: the cells may behave as acidophils or baso: :phils. The response seems to vary with the stain used.

Parker (1918), describing the structure of the elasmobranch pituitary, adopted a curious terminology. He did not figure a ven: :tral lobe. He divided the "pars buccalis" into an anterior lobe, a superior or dorsal lobe and an inferior or ventral lobe; the anterior lobe corresponds to the rostral, and the other two lobes appear to be

subdivisons of the neuro-intermediate. Following Tilney, he regarded the rostral lobe as the homologue of the pars tuberalis.

Hogben and de Beer (1925) were the first workers to identify in the selachian pituitary a definite pars nervosa, although Herring (1908b), Baumgartner (1915) and others had reported nerve fibres in the neuro-intermediate lobe. According to Hogben and de Beer, the pars nervosa consists of a mass of neuroglia fibres "which leave the floor of the infundibular cavity and ramify among cells which it is permissable to identify with pars intermedia of other forms".

De Beer's monograph (1926) on the vertebrate pituitary has already been mentioned. In the elasmobranch pituitary he described the presence of blood vessels outside the epithelium of the rostral lobe; those epithelial cells which are orientated towards the blood vessels are eosinophils, whereas the others are basophils. In the blood vessels he found a substance which he identified as the product of the eosinophils. De Beer took the view that both eosinophils and basophils belong to the same cell type, and the difference in staining reaction indicates different stages in a functional cycle.

In the neuro-intermediate lobe he noted the extensive vascularisation of the intermediate component; this lobe is composed of basophils and penetrated by neuroglia of the pars nervosa. De Beer

described the ventral lobe as composed of basophils and large eosino: :phils, the latter showing evidence of secretory activity.

According to de Beer the ventral lobe is attached dorsally to the neuro-intermediate lobe; in this de Beer was mistaken. He also stated that in rays the ventral lobe is free; if this means, as it appears to mean from the context, that in rays (Rajidae?) the ventral lobe is not attached to the cranium, it is certainly incorrect.

This error was repeated by Hogben (1936) and by Waring (1938), both of whom described the ventral lobe in Raia as "free". Morris (1941) found the ventral lobe attached to the floor of the cranium in all forms examined, including Raia, though the attachment is less tena: :cious in the batoids than in the sharks. Fig. 1, p. 3, shows clearly that in Raia the ventral lobe is attached to the floor of the cranium.

Comes (1935), working on the female Torpedo, described the tubules of the rostral lobe as composed of chromophobe and eosinophil cells. Comes held that the colloid found in the lumina of the tubules is secreted by the chromophobes which border on the lumina. He noted that the amount of colloid increases at gestation, and concluded that the chromophobes are concerned with production of gonadotrophin.

According to Comes the eosinophils lie peripherally to the chromo: :phobes, and they produce a secretion which passes into the surrounding sinusoids. Comes found this secretion to be particularly abundant in

young fish, and concluded that the eosinophils are concerned with production of growth hormone.

The correlations reported by Comes are interesting, but they hardly warrant the firm conclusions drawn.

Ranzi (1936a) recorded histological changes in the pituitaries of female Torpedo, Trygon (both ovoviviparous) and Mustelus (vivi:parous) during gestation. He noted that as gestation proceeds the rostral and neuro-intermediate lobes show a general hyperaemia; he did not mention the ventral lobe. There is an increase in the amount of colloid in the tubules of the rostral lobe, while the peri:pheral cells of the walls of the tubules show decreased eosinophilia. The cells of the intermediate lobe enlarge and show increased eosino:philia.

In a later paper Ranzi (1936b) extended his observations to cover several other species.

Howes (1936) described the morphology and histology of the pit:uitary in three species of Raia. He divided the rostral lobe into three regions on the basis of staining reaction: an anterior, strongly basophil region; a middle, faintly basophil region; and a posterior, mainly acidophil region. He suggested that these are the homologues of the pars tuberalis and the basophil and oxyphil areas respectively of the mammalian pars anterior. He also suggested that the ventral

lobe may be the homologue of part of the mammalian pars tuberalis. His system of homologies is based largely on the staining reactions of the various regions of the pituitary, and should perhaps be treated with reserve. Norris (1941) has pointed out that Howes' figure of the pituitary in ventral view is incorrect.

Butcher (1936) separated the rostral lobe of the dogfish into anterior and posterior portions, which he referred to respectively as pars distalis and pars medialis; the two portions are separated by a constriction with smooth walls and a small lumen. He noted that in the skate the lumen of the ventral lobe communicates with the hypophysial cavity of the rostral lobe. Butcher's statement that the dogfish possesses a "disintegrated and inconstant occurring ventral lobe" is not confirmed by other workers.

Lewis and Butcher (1936a) working on Squalus and Raia, claimed that the pituitary could be separated into six lobes, one of which is the saccus vasculosus. The others are pars distalis and pars medialis (rostral lobe), pars intermedia and pars neuralis (neuro-intermediate), and the pars ventralis (ventral lobe).

Charipper (1937) briefly reviewed the literature on the mor:phology and cytology of the pituitary of lower vertebrates. His view that the selachian pituitary is "atypical in most respects" was later challenged by Norris (1941). Charipper pointed out some

of the numerous contradictions in the literature on the subject of cytology, and remarked on the confusion in terminology and homology of the various parts of the gland.

Charipper regarded the rostral and ventral lobes together as homologous with the anterior lobe of higher vertebrates; he also claimed that the lumina of these two lobes represent the original cavity of Rathke's pouch. These views do not stand up to close examination. De Beer (1926) and others have suggested that the ventral lobe is probably homologous with the pars tuberalis. Moreover, the ventral lobe develops from paired outgrowths of Rathke's pouch, so that its lumen could not be part of the original hypophysial cavity; the latter is represented, in the adult, by the lumen of the rostral lobe.

Waring (1938) stated that the ventral lobe arises from the neuro-intermediate lobe, and that in Raia the ventral lobe is "free". Both statements are incorrect.

Norris (1941) published an excellent monograph on the Plagiostome hypophysis.* Although chiefly concerned with morphology, the author gave some attention to histology and homology. His work covered 51 genera, among which he distinguished two main types of hypophysis, a selachoid and a batoid, as well as some transition forms connecting these two types. A major point of difference is that in the selachoid

* Plagiostomes defined as all elasmobranchs except the Holocephali.

type the rostral and ventral lobes contain a lumen, whereas in the batoid type the entire hypophysis is solid.

Norris found the ventral lobe to be highly variable in form, size and position. It is attached to the rostral lobe by the interhypophysial stalk, and is usually located ventral to the posterior third of the neuro-intermediate lobe. It lies embedded in the tough connective tissue of the endocranium, on the floor of the cranial cavity. The sharks are provided with a depression in the cartilage, the hypophysial fossa, in which the ventral lobe is accommodated. Norris was able to trace the gradual fading out of the hypophysial fossa among the squaloid and galeoid sharks; in the batoids the fossa has disappeared, leaving the ventral lobe attached more or less loosely to the floor of the cranium.

According to Norris the ventral lobe "is frequently functionless and in some instances reduced to little more than a thin membranous sac. But in other forms its size, vesicular histological structure and abundant blood supply indicate an important function" (p. 16). The statement that it is "frequently functionless" is open to question; moreover, Norris does not appear to have allowed for the marked increase in size of the ventral lobe at sexual maturity.

Over the last 4 years a considerable number of pituitary dissections have been carried out at the Catty for the purpose of making

extracts of the various lobes. It has been noted that the ventral lobe increases considerably in size at sexual maturity, and that the size increase is not proportional to that shown by the remainder of the pituitary. It is reasonable to suggest that the variations in size of the ventral lobe reported by Norris are characteristics of age rather than of species.

Norris divided the rostral lobe into an anterior pars distalis and a posterior pars medialis. He found that the rostral lobe and the pars intermedia (of the neuro-intermediate) will react to both basic and acid stains, but the cells of the rostral lobe "are pre: :dominantly acidophil". He noted that in both lobes the blood vessels are bordered by acidophils. The staining reaction of the ventral lobe is similar to that of the rostral lobe.

Scharrer (1952) studied the neural component of the pituitary and its relationship with the hypothalamus. Working on Scyllium stellare, he found that the fibres composing the pars nervosa orig: :inate in the nucleus praeopticus of the hypothalamus. The axons of the cells comprising the nucleus pass backward in the floor of the third ventricle as the tractus praeoptico-hypophyseus and enter the neuro-intermediate lobe of the pituitary. They do not form a discrete pars nervosa as in other vertebrate groups, but become dis: :tributed among the cells of the pars intermedia. Scharrer maintained

that the sum total of these nerve endings within the neuro-intermediate lobe constitutes the pars nervosa of Scyllium. This interpretation agrees with that of Hogben and de Beer (1925).

The cells of the nucleus praeopticus produce a secretion in the form of granules which stain blue-black with Gomori's chrome-haema: toxylin. They show cycles of secretory activity, and the neurosecre: tion is conveyed along the fibres of the tractus praeoptico-hypophyseus to the pituitary, where it is stored in the nerve endings of the neuro-intermediate lobe.

Scharrer argued against the traditional view of the pars nervosa as an endocrine gland, and favoured the more recent one that the hormones of the pars nervosa are produced by the hypothalamus. Accord: ing to this theory the pars nervosa acts as an organ of storage for these hormones, and Scharrer concluded that the condition found in Scyllium supports the theory.

In the same year Nozzi (1952) carried out a similar study on S. canicula. He found that the fibres of the hypothalamo-hypophyseal tract (tractus praeoptico-hypophyseus of Scharrer) originate in the nucleus magnocellularis praeopticus. The cells forming this nucleus are located in the walls of the preoptic recess. It appears that neuro-secretory material, stainable by Gomori's method, is conveyed outward from the cells of the nucleus along dendrites as well as axons.

The secretion travelling along the dendrites passes through the ependyma and enters the ventricular liquor. That passing along the axons finally reaches the neural component of the pituitary.

Van de Kamer and Verhagen (1954) studied the cytology of the neurohypophysis, the saccus vasculosus, and the recessus posterior in S. canicula. They described the neurohypophysis as built up mainly of nerve fibres that form a narrow layer within the neuro-intermediate lobe; the layer of fibres lies immediately beneath the ependymal cells bordering the ventricle. The fibres penetrate between the cells of the intermediate tissue in the form of strands. There are blood capillaries among the fibres and between the tissues of the pars nervosa and the pars intermedia.

Their description of the saccus vasculosus may be noted. The epithelium consists of several cell types; the "coronet-cell" is the main type, but they also found ciliated cells, supporting cells, and cells possessing a threadlike protrusion with a globule on top. The coronet cells are described in detail; the authors think it probable that they are identical with the "parenchymatous pituicytes" (see below) of the pars nervosa, and that both are transformed ependymal cells.

In a later paper (Van de Kamer and Verhagen, 1955) the same workers described two types of pituicyte scattered among the fibres

of the pars nervosa: large, vacuolated cells which they designated "parenchymatous pituicytes", and small irregular cells with branching cytoplasmic processes. They expressed the view that the latter type corresponds to the pituicytes found in the posterior lobe of other vertebrates.

They came to the conclusion that the parenchymatous pituicytes originate from the ependymal layer and possess secretory activity, their products being extruded into the spaces between the nerve fibres. These cells stain heavily with Gomori's chrome-haematoxylin, and the authors took the view that they actually produce the neuro-secretory material they contain.

Van de Kamer and Verhagen also noted migration of intermediate cells into the tissue of the pars nervosa, discontinuity of the epen:ymal layer of the pars nervosa, and secretory activity by the ependy::mocytes. They concluded that histologically and cytologically the pars nervosa of the elasmobranch pituitary is similar to that of other vertebrates.

(c) Physiology.

(a) Pressor, antidiuretic and oxytocic activity.

Herring (1908a, 1908b) was the first worker to attempt an investigation of the physiological activity of the elasmobranch pituitary. He reported that Ringer extracts of skate pituitary and saccus vasculosus produced no pressor effect when assayed on the cat.

In a later paper Herring (1913) reported an "immediate and well-marked" effect on mammary secretion when a Ringer extract of skate pituitary material was injected into the lactating cat. The effect reported by Herring has the appearance of a milk-ejection response. It is now known that milk ejection in the rabbit is controlled by the neurohypophysis, and there is evidence that oxytocin is the factor responsible (Cross and Harris, 1952; Cross and Van Dyke, 1953). Unpublished work carried out in this laboratory has confirmed Herring's report; extracts of dogfish neuro-intermediate lobe have produced the milk-ejection response in lactating rabbits.

The following year (Herring, 1914) the same worker reported that "a .25 per cent. extract of skate's pituitary" caused a definite contraction of the rat's uterus. This is clearly an oxytocic effect; taken in conjunction with his earlier results, it indicates the presence of an oxytocin-like substance.

Hogben (1925) assayed Ringer extract of the skate pituitary for

"avine depressor substance". He found that both skate and cod pituit: :ary material caused a drop in blood pressure in the duck, but the response was considerably smaller for skate than for an equal weight of cod material. It has since been shown that the avian depressor effect is a property of oxytocin (Gaddum, 1928).

Hogben and de Beer (1925) assayed Ringer extracts of skate and cod pituitary material for pressor activity. They found that 10 mgm. cod pituitary elicited a definite response when assayed on the cat, but there was no response to 50 mgm. skate pituitary.

Hogben and de Beer also assayed for oxytocic activity using the guinea pig uterus. The skate material elicited a response, but this was small compared with the response to cod material: the latter was estimated to be twenty to thirty times more active than the skate material. The guinea pig uterus is sensitive to histamine, and this result could have been due to histamine contamination; however, the authors point out that Herring had obtained a response to skate mater: rial using the rat uterus, and the latter is relaxed by histamine.

Geiling and Le Messurier (1936) assayed dogfish and skate pituit: :ary extracts for pressor, antidiuretic and oxytocic activity, but gave no information concerning the methods used. They reported "a suggestion of slight pressor activity" in the neuro-intermediate lobe and the saccus; the antidiuretic assay gave inconclusive results. They mentioned no results for the oxytocin assay. It may be noted

that their methods recorded both pressor and antidiuretic activity in the pituitary of the sculpin (Teleostei). However, the lack of information makes it difficult to judge the value of these results.

Heller (1941) recorded the presence of antidiuretic principle in pituitaries of elasmobranchs assayed on rabbits. He noted Herring's failure to find a pressor principle, but explained the apparent contradiction by the "greater sensitivity" of his own method. The same worker (Heller, 1945) reported the presence of "water- balance principle" in the elasmobranch pituitary.

Hogben (1936) suggested that the ventral lobe might be respon: :sible for production of a pressor substance. This suggestion was based on the observation that skate from which the ventral lobe had been removed showed a pink flush. Hogben's suggestion was later questioned by Parker (1937), who pointed out that elasmobranchs which have blanched normally also take on a pink flush, possibly because the superficial blood vessels become more readily visible when the chromatophores are 'contracted'.

In any case it is doubtful whether Hogben succeeded in removing the ventral lobe. His description of its morphology is equivocal: "in skates . . . . . the pars ventralis is free" (p. 146) and: "in skates the ventral lobe adheres to the membranous floor of the brain case" (p. 151). It is difficult to reconcile these two statements.

Hogben's figure suggests that the portion he took to be the ventral lobe is in fact the interhypophysial stalk; moreover, the interhypo:physial stalk could be removed by suction (the method used by Hogben), but it is doubtful whether the ventral lobe of skate or dogfish could be removed by that method.

(b) Chromatophorotrophic activity.

Lundstrom and Bard (1932) reported the first attempt at hypophy:sectomy in elasmobranchs. Working on Mustelus canis, they showed that the neuro-intermediate lobe controls chromatophore expansion. However, no attempt was made to remove the ventral lobe. The authors' assumption that it was removed along with the neuro-intermediate lobe is not valid (see p. 54 et seq).

The work of Lundstrom and Bard appears to have quickened interest in the physiology of the elasmobranch pituitary, for the following decade produced a number of papers on the subject. All subsequent attempts at hypophysectomy were based on the technique of these two workers, but, with the possible exception of Vivien's work, none of the operations appears to have included ventral lobectomy.

Waring (1935) investigated pituitary control of the chromato:phores in S. canicula. He concluded that melanophore expansion is caused by a water-soluble hormone produced by the neuro-intermediate lobe; the mechanism for contraction is not so clear: "it appears that

the anterior (rostral) lobe in Scyllium is certainly involved in the control of melanophore contraction, but it seems that the weight of evidence is against this control being exercised by the production of a contracting hormone".

In a later paper Waring (1936) tried to standardise the melano:phore-expanding potency of the dogfish pituitary against that of commercial "pitressin" and frog pituitary extract. His attempt met with only limited success. In a series of subsequent papers (Waring, 1938; Waring, Landgrebe and Bruce, 1942; Waring, 1942) Waring and co-workers added little to his earlier work on chromatophore control. They brought forward some evidence for the production of a chromato:phore-contracting hormone by the rostral lobe, but this evidence is no more than suggestive.

Wykes (1936) worked on chromatophore control in a number of elasmobranchs. She found that neither section of spinal nerves nor electrical stimulation had any effect on the chromatophores. On the other hand ligation of the aorta had a marked effect. Her results support the view that the chromatophores are under hormonal control.

Hogben (1936) investigated chromatophore function after hypophy:sectomy in several species of skate and dogfish. He confirmed that the neuro-intermediate lobe is responsible for chromatophore expansion, and his work shows that pressor or oxytocic factors are not responsible

for this effect. He noted that the pallor response is abolished when the rostral lobe alone is removed.

Lewis and Butcher (1936b) assayed extracts of elasmobranch pituitary for the chromatophore-expanding factor. The extracts were injected into "bleached frogs"; those prepared from the pars intermedia caused darkening of the frogs. Extracts prepared from the pars distalis of the rostral lobe also caused darkening, but extracts of the other lobes tested did not.

Lewis (1936) was able to grow cells from various parts of the selachian pituitary in tissue culture. She found that extracts prepared from cultures of the pars distalis and the pars intermedia caused darkening when injected into bleached frogs.

Parker (1937) discussed the results of previous work on chromatophore control in elasmobranchs and reported the results of further work. He agreed with other workers that pigment dispersal (chromatophore expansion) is caused by a substance produced in the neuro-intermediate lobe of the pituitary.

On the subject of pigment concentration (chromatophore contraction) Parker appears to hold the view that three separate mechanisms have been evolved among elasmobranchs: direct nervous control, found in Mustelus; a concentrating hormone produced by the rostral lobe of the pituitary, found in Scyliorhinus and others; and mere absence of

an expanding factor, found in Raia erinacea. Parker would have been safer in concluding that the evidence does not yet warrant a firm statement on the mechanism of chromatophore contraction in elasmobranchs.

Abramowitz (1939) reviewed previous work on pituitary control of the chromatophores and reported the results of further work. His experimental work included hypophysectomy (total and partial), section of the optic nerves, and brain lesions in Mustelus canis. He confirmed that the neuro-intermediate lobe is responsible for chromatophore expansion. He also found that the chromatophores do not respond directly to light, and that rostral lobectomy results in loss of the white background response. On the last point his work contradicts that of Parker (1937), who stated that rostral lobectomy in this species did not affect the colour response. Abramowitz further demonstrated that certain superficial lesions of the hypothalamus, such as could occur in rostral lobectomy, also result in loss of the white background response. He argued that the result of rostral lobectomy could be explained by postulating interruption of the mechanism inhibiting secretion by the pars intermedia. He concluded in favour of the "unihormonal theory", according to which contraction is due to diminution or cessation of intermedin secretion by the pars intermedia.

Parker (1948) reviewed the work on chromatophore control in elas-

:nobranchs. His review is somewhat biased in favour of nervous control for which there is no adequate evidence, and sheds no further light on the role of the pituitary in chromatophore control.

(c) Diabetogenic activity.

Orias (1932) found that pancreatectomy caused a diabetic condit: :ion in Mustelus canis: this was alleviated by hypophysectomy, but hypophysectomy alone did not alter the blood sugar level.

Abramowitz, Hisaw, Boettiger and Papandrea (1940) confirmed the finding of Orias (1932) that hypophysectomy will alleviate pancreatic diabetes in M. canis. They produced evidence that the rostral lobe is responsible for this effect, and that the "diabetogenic hormone" is not identical with intermedin.

(d) Gonadotrophic activity.

Creaser and Gorbman (1939) assayed the pituitary of Squalus sucklayi for gonadotrophic activity. They found that "doses" of four to sixteen pituitaries had no effect on Rana pipiens, but give no further details.

Vivien (1940) published some preliminary results on the effects of hypophysectomy in Scyliorhinus canicula. Expressing gonad weight as a fraction of the total weight of the fish, he presented a list of figures which showed a progressive decrease in gonad weight following hypophysectomy. His best post-operative survival periods were 300 days (male) and 306 days (female).

In the following year (Vivien, 1941) the same worker published a lengthy treatise on his work. This paper leaves much to be desired, but the results are of sufficient importance to be worth careful attention.

According to Vivien, hypophysectomy blocks pubertal development in the young dogfish; in mature fish it causes involution of the gonads. The latter is a slow process, requiring at least two months before the symptoms become clear. Pituitary extracts and implants cause resumption of genital activity during the resting period of the sexual cycle, which shows a reproductive phase from February to July; however, in the female treatment must be administered near to the time when genital activity would normally be resumed. Mammalian pituitary extracts give similar results, but very large doses are required: about two hundred to three hundred times the weight of fish pituitary material required. Vivien wrote this paper while a prisoner of war in Germany. It appears to have been written from memory, since he mentioned the total destruction of his documents and material by enemy action. While sympathising with the circumstances under which Vivien wrote, it seems advisable to await further evidence before accepting his conclusions.

The work of Carlisle (1954) may be mentioned here, since it bears on the subject of gonad control in elasmobranchs. Carlisle injected

mammalian gonadotrophic preparations into starved male dogfish (species not stated). His findings suggest that chorionic gonadotrophin, lacto:
:genic hormone and "Praephyson" (anterior pituitary gonadotrophin) are capable of maintaining spermatogenesis.

Carlisle's criterion of spermatogenic activity was presence of sperm in the cloacal fluid after stroking of the abdomen. This tech:
:nique has long been used at the Gatty, with very uncertain results. With S. canicula it has been found that such stroking may or may not cause discharge of sperm, regardless of whether or not the animals are fed. Moreover, since a negative result could not establish the absence of spermatozoa, it seems advisable to treat these findings with some reserve.

Witschi (1955) assayed the elasmobranch pituitary for gonadotrophic activity. He found that the "shark" pituitary gave both follicle-stimulating (FSH) and luteinising (LH) reactions. The FSH assay was based on vaginal cornification in rats, and the incomplete assay data given shows that 50 mg. pituitary material is larger than the "unit dose". The LH assay was based on feather reaction of the weaver finch, and 3 mg. pituitary material elicited the unit reaction.

Hisaw and Albert, in a personal communication quoted by Dodd (1955), stated that in Mustelus canis ovulation is suppressed by hypophysectomy, but it can be re-initiated by implanting pituitary glands of the same

species. They reported that in this species the corpora lutea are not dependent on the presence of the pituitary for their formation or maintenance, and that the first three months of gestation are also independent of pituitary influence; on the last point their observations did not cover the remaining eight months of gestation.

STATEMENT OF THE PROBLEM.

1. Pituitary control of the thyroid.

It has not been demonstrated that in elasmobranchs the thyroid is under pituitary control. Waring et al. (1942) found that a month after hypophysectomy the thyroid of S. canicula shows no change. Oliverecu (1950) treated Scyllium stellare and S. canicula with various 'goitrogens', but was unable to detect any significant histological effect on the thyroids. Both workers suggested that the elasmobranch thyroid might not be under pituitary control. Waring mentioned an: :other possibility: that thyroid involution might be a slower process in elasmobranchs than in other vertebrates. Oliverecu put forward a third possibility: after reporting that elasmobranch pituitary extract will result in thyroid hyperactivity when injected into dogfish, she suggested that the elasmobranch pituitary might be incapable of react: :ing swiftly to changes in the amount of thyroid hormone in circulation.

The brief report by Oliverecu (1950), on the effect of pituitary extract, is the only evidence for the existence of a thyrotrophic hormone in elasmobranchs. In the face of the negative results mention: :ed above, further evidence is clearly desirable. Hypophysectomy was expected to provide such evidence; however, the post-operative survival periods must be sufficiently long for any effect on the thyroid to be: :come clear, or for the absence of effect to be placed beyond question.

2. Pituitary control of the gonads.

Direct evidence for pituitary control of the gonads in elasmobranchs in furnished by Vivien (1940, 1941) and by Hisaw and Albert (Dodd, 1955). Their work supports the view that the gonads are under pituitary control, but that of Vivien is open to severe criticism, and that of Hisaw and Albert reveals marked differences between the type of control found in mammals and that in elasmobranchs.

The elasmobranch pituitary has been assayed for gonadotrophic activity on other vertebrates (Creaser & Gorbman, 1939; Mitsehi, 1955), but the results are conflicting. Carlisle (1954) found that mammalian gonadotrophic preparations will cause spermiation when injected into male dogfish, but Carlisle's assay is based on an observation that is not supported by our own work (see review of literature).

Thus, there is some evidence, both direct and indirect, for pituitary control of the gonads in elasmobranchs, but much of this evidence is unsatisfactory. Two aspects of the subject were selected for attention: it was decided to re-investigate the effects of hypophysectomy on the testis, and to determine which lobe (or lobes) of the pituitary is responsible for gonadotrophic activity.

Programme.

This programme therefore covers two projects: (a) an investigation of the thyrotrophic, and (b) an investigation of the gonadotrophic

activities of the elasmobranch pituitary. Both projects are based on hypophysectomy, and the same fish were used for both investiga:tions.

Sources of Supply and Methods of Transportation.

This work was carried out on the dogfish Scyliorhinus canicula L. Most of the fish were supplied by the Millport Marine Biological Station; one consignment was received from the Port Erin Marine Biological Station, Isle of Man.

The fish received from Millport were usually dispatched in "live cans" by ferry and rail. The trip required twelve to twenty four hours, and each live can contained about sixty litres of water on arrival at St. Andrews. It was found that the optimum number of fish that could be transported in this way was four to each can.

One of the live cans was eventually fitted with a bracket to carry a small oxygen cylinder. The oxygen was fed as a feeble jet to a diffuser, through which it passed to the water. After this method of oxygenation was adopted there were fewer deaths in transit, and the fish appeared in better condition on arrival.

On two occasions large consignments of fish were received from Millport. The fish were transported by boat to the mainland in open baths, and were then transferred to similar baths mounted on a lorry and equipped with an oxygen supply. The journey took about nine hours, and most of the fish arrived in good condition.

The fish received from Millport were operated on at the Gatty after

a period of acclimatization (1 - 2 weeks) in the aquarium tanks. The consignment received from Port Erin consisted of fish which had been operated on before leaving the Isle of Man. These fish travelled to St. Andrews by air and lorry. Aeration on the journey was provided by air pump and oxygen. Of sixty fish which made the journey, two were dead on arrival.

Certain tentative conclusions may be drawn from this experience in transporting live dogfish: it is apparent that Scyliorhinus travels well under conditions that must involve some stress. Casualties tend to be fewer in winter than in summer; presumably a lower metabolic rate in winter requires smaller supplies of oxygen. There is reason to think that aeration helps by removing some of the mucous that accumulates in the water; the mucous is brought to the surface by bubbles, and tends to form a surface froth. Each fish seems to require about fifteen litres of water; with less they show signs of stress.

HUSBANDRY.

Aquarium facilities.

The fish were kept in large indoor tanks, each with a capacity of about two cubic metres. Vivien (1941) found they lived well when kept ten or fifteen to two or three cubic metres of water. The conditions at the Catty were similar, with about fifteen fish to two cubic metres. The tanks were well aerated, and a supply of fresh sea water was piped to each tank daily. The water temperature varied from about 6° C. in winter to about 18° C. in summer (see graph, fig. 3 A).

Feeding.

Problems encountered. In early attempts at feeding the fish were offered lugworm, which was placed in the tanks once or twice weekly. A few fish were seen to take the lugworm, but it soon became apparent that most were not eating. Mortality was high among both operated and unoperated fish. Most deaths occurred within two months of their arrival at the laboratory. Post mortem examinations revealed loss of weight in all cases, and the stomachs were usually found to be empty. It became evident that, if survival periods of the order of a year (see Vivien, 1941) were to be achieved, a more successful method of feeding would have to be devised.

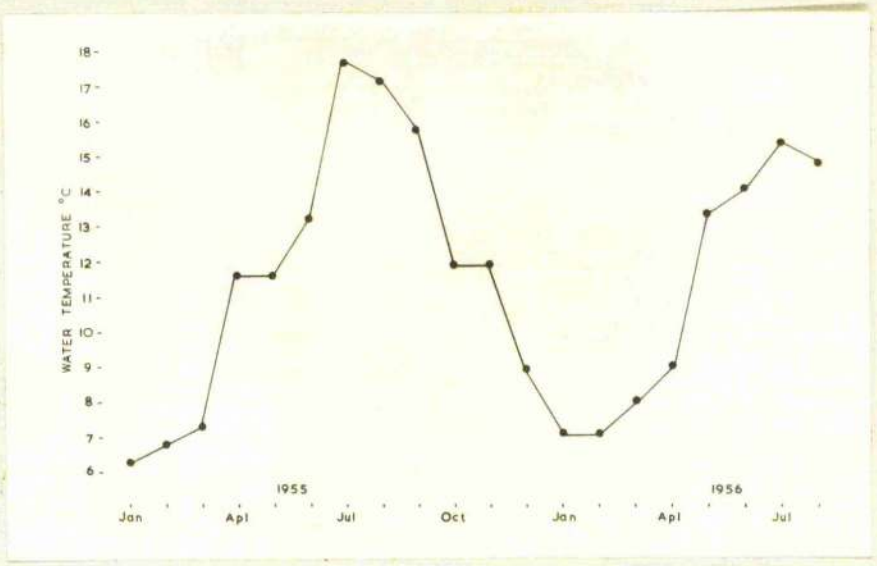
Review of the literature. The natural diet of S. canicula is

Fig. 3.

A. Water temperatures in aquarium. Monthly averages for period Jan., 1955, to Aug., 1956.

B. Syringes used in force-feeding. 'Ortho' syringe above and icing syringe below.

C. Force-feeding procedure, showing ortho syringe being inserted into stomach.


WATER TEMPERATURE °C
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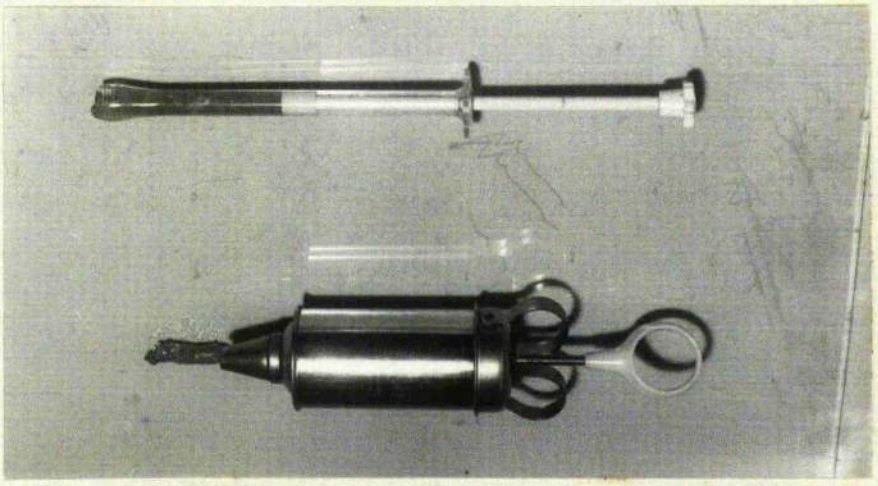
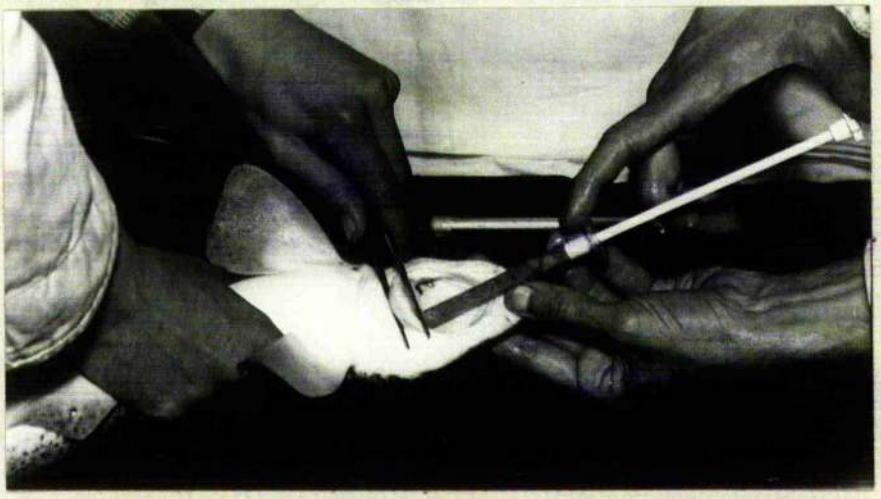


B



C

Fig. 3.

known. Ford (1920) and Bales (1949) examined the stomach contents of a large number of specimens, and recorded the presence of various species of fish, crustacea and molluscs. However, there are con: :flicting reports on the feeding habits of this species under labor: :atory conditions.

Waring (1935) offered his fish herring and "coal fish", and the use of herring was again reported by Waring et al. (1942). The food was placed in the aquarium tanks, and its acceptance by the dogfish is implied in both papers.

Vivien (1941) stated that in captivity S. canicula will not feed of their own accord, even when presented with their usual diet of whelk, crab, etc. He had to use, both on controls and on hypophy: :sectomised fish, a method of force-feeding which required a glass tube fitted with a plunger. The tube was filled with a mixture of whelk, patella and crab, inserted into the stomach, and the food ejected by means of the plunger. Each fish received 50 - 60 cc. of this mixture per week. Vivien recorded post-operative survival periods of more than a year.

Matty (1954) carried out thyroidectomy on S. canicula, and reported that within a few days of the operation "all the operated animals began to feed upon strips of fresh squid muscle". Matty obtained post-operative survival periods of "at least seventy two days". This appears to be the only case in which S. canicula is

explicitly recorded as taking food of its own accord in captivity.

This review of the literature indicated that Scyliorhinus could be induced to take food, provided the right diet was offered. Matty's diet of fresh squid muscle seemed to be favoured, but fresh squid are not readily available at the Catty. It was therefore de: :cided to offer the fish a wide choice of food, including the items most frequently mentioned in the literature.

Accordingly, the fish were offered molluscs (whelk, limpet, mussel, cockle), crustacea (crab, prawn) and fish (cod, herring). The results were as before: a few fish were seen to take the food, but mortality continued to be high, and the evidence indicated that most were not eating. This finding has since been confirmed by Lowenstein (personal communication), who has reported that in his experience S. canicula does not feed consistently after surgical pro: :cedures; he has had to discontinue experiments involving operated fish, since they usually died of inanition before they were ready for use.

Force-feeding. After numerous attempts, a method of force-feeding was devised, based on that of Vivien. The apparatus adopted was a plastic prophylactic syringe of about 1 cm. internal diameter and 14 cm. long. These are syringes of a standard size, produced by the Ortho Pharmaceutical Ltd.; the barrel is of uniform bore through: :out its length and a fitted piston traverses its entire length. The

capacity is 6 gm. of minced herring.

The "ortho syringe" is used in the same way as Vivien's glass tube: the tip is inserted past the cardiac sphincter into the stomach and the contents ejected by means of the piston (fig. 3 C). The food material must be delivered into the stomach; if placed in the oesophagus it is regurgitated.

Filling the ortho syringe with food material presented a problem. At first the finely-minced food was packed into the barrel by hand; this proved a long and laborious process. The problem was eventually solved by using a confectioner's icing syringe. The latter can be fitted with nozzles of various shapes; for our purpose a rounded nozzle was selected and cut short so as to give an aperture of 1 cm. diameter. When this syringe was charged with minced food material it proved capable of expressing a "jet" of the required diameter. The aperture was then held up against the tip of the ortho syringe and a jet of food material injected into the latter.

This combination of ortho syringe for force-feeding and icing syringe for charging the ortho proved very effective. Filling the icing syringe presents no difficulty: the barrel is large and holds enough at one charging to fill fifteen orthos. The two syringes are shown in fig. 3 B.

Diet. In deciding upon the diet, it was necessary to consider

whether the proposed food was acceptable to the fish, i.e. whether it formed part of the natural diet, and whether it was readily available. A survey of the literature shows that herring is mentioned by most workers: it is recorded by Ford and Hales as a common constituent of the stomach contents, and it was used by Waring (1935) and by Waring et al. (1942). It is also available at St. Andrews throughout the year. Herring was therefore adopted as the article of diet; it must be finely minced for use in the syringes.

Amount of food and frequency of feeding. It was found that six "ortho" syringes (36 g. herring) comfortably filled the stomach of an adult dogfish. Two days after feeding the stomach contents had been reduced to a fluid-like chyle, and four days after feeding the stomach was usually empty. It therefore appeared that the fish should have been force-fed about every fifth day. However, the adverse effects of handling had to be considered; it was thought advisable to reduce this to a minimum. Dogfish struggle violently when handled, and there is some evidence that mortality rises with excessive handling. It was therefore decided to force-feed once weekly.

Adequacy of diet. Experience has shown that thirty six g. herring per week will maintain an adult dogfish in good condition. Some experimental fish show a loss of weight, but others record a gain, and it seems fair to conclude that the diet is adequate.

It is worth noting that the only fish to give survival periods of the order of a year were those that had been consistently force-fed. One such female (control) has survived operation by twenty eight months at time of writing, and continues to produce egg purses; this is clear evidence that the diet used was capable of maintaining reproductive activity.

Force-feeding has an obvious advantage: it ensures that all the experimental animals get the same amount of food. It follows that any inanition effects could be expected to appear - if at all - in controls as well as hypophysectomised fish.

HYPOPHYSECTOMY.

Type and Number of Operations.

The operations performed were total hypophysectomies, partial hypophysectomies and control operations. In the latter the procedure was identical to that used for hypophysectomy, but the pituitaries were left in situ.

Over the period covered by this research a total of two hundred and forty such operations were carried out. The results reported here were obtained from seventy one fish (58 , 13 ♀). A further ten are still alive; these have been earmarked for dissection at eighteen months after operation and will be used to supplement the results reported here for purposes of publication.

Of the seventy one fish on which this report is based, thirty one were dissected post-mortem; these are indicated in the Appendix. The latter were dissected immediately after death, and in no case did the thyroids or testes show any histological characteristics that 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 that were alive at dissection, with supporting evidence provided by the post-mortem material.

Review of the Literature.

Hypophysectomy has been carried out on elasmobranchs, with

varying degrees of success, by a number of workers: Lundstom and Bard (1932), Orias (1932), Hogben (1936), Waring (1935, 1938), Abramowitz and co-workers (1940) and Vivien (1941).

The rostral and neuro-intermediate lobes were removed either by forceps or by suction, after cutting a window in the cartilaginous floor of the cranium. On the other hand there appears to be no agreement as to how the ventral lobe should be dealt with; indeed, there appears to be some confusion on the morphology of the ventral lobe (cf. Hogben, 1936; Waring, 1935, 1938; Vivien 1941).

Lundstrom and Bard (1932) carried out "complete" and partial hypophysectomies on Mustelus canis, but state that "the ventral lobe . . . . has not been taken into separate account in our experiments". Orias,(1932), working on the same species, used the technique of Lundstrom and Bard. It can be taken that none of these workers succeeded in removing the ventral lobe.

Hogben (1936) operated on several species of skate and two species of dogfish, including S. canicula. He was aware that in the dogfish the ventral lobe is embedded in the floor of the cranium, but was clearly uncertain as to its morphology in the skate (see review, p. 30). It is very doubtful whether his method of ventral lobectomy, involving suction, could have been effective in removing this lobe, either in skate or dogfish.

Waring (1935) carried out hypophysectomy on S. canicula, but his uncertainty with regard to the ventral lobe is reflected in the statement that "In all operations the ventral lobe was presumably severed or removed".

In a later paper Waring (1938) reported a number of operations on S. Canicula and Squalus acanthias. According to Waring "The ventral lobe arises from the neuro-intermediate lobe at its junction with the pars anterior (rostral lobe). In Raia it is free". Neither of these statements is correct. However, he correctly described the ventral lobe of dogfish as attached to the floor of the cranium. He also stated that when removing the ventral lobe in dogfish the cartilage incisions must be carried far backward, severing the carotoids and inter-orbital vessel; from this it may be inferred that his method of ventral lobectomy involved cutting out the block of cartilage containing this lobe.

Abramowitz, Hisaw, Boettiger and Papandrea (1940) working on Mustelus canis, did not attempt to remove the ventral lobe. Vivien (1941) performed hypophysectomy on S. canicula, and checked his work by histological sections of the pituitary region after dissection. However, Vivien's sketch of a sagittal section through the pituitary region is incorrect in several points, and it is difficult to see how he could have removed the ventral lobe by the method he described (using suction).

The Ventral Lobe.

In Scyliorhinus the ventral lobe is firmly embedded in the tough connective tissue endocranium (fig. 4 B, C). It lies just anterior to the inter-orbital ridge of cartilage. The inter-orbital vein lies within the inter-orbital ridge, and is separated from the ventral lobe by a thin lamina of cartilage. Immediately beneath the ventral lobe lie the endocranial portions of the internal carotid arteries.

Inspection of fig. 4 suggests that there is little chance of dissecting out the ventral lobe without severe damage to the neighbouring blood vessels. This is borne out by the work of Baumgartner (1915), de Beer (1926), and by our own experience; even under the ideal conditions of routine dissection it proved almost impossible to remove the ventral lobe without damage to the adjacent blood-vessels. Moreover, these attempts at dissection convinced us that the ventral lobe could not be removed by suction: it is necessary to use forceps to tear the tough connective tissue in which it is embedded.

The problem presented by the ventral lobe becomes much more acute when operating on live dogfish. The incisions in the cartilage must not be carried so far back as to damage the carotids and inter-orbital vein; this means that the attempt on the ventral lobe must be made through a window in the cartilage located anterior to the lobe (fig. 5 D). The site of the ventral lobe is not exposed to view, and it

Fig. 4.

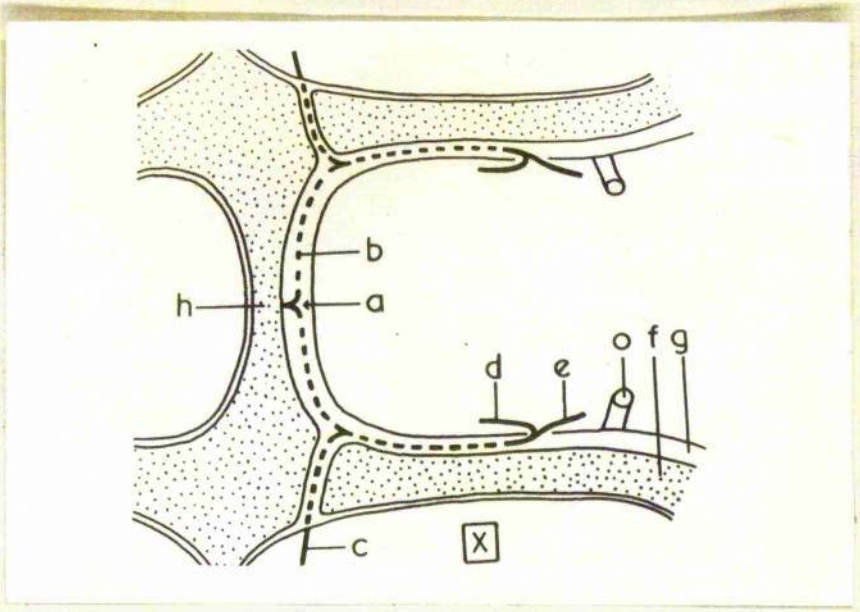
S. Canicula. Relationship between pituitary and floor of cranium.

A. Diagram of floor of cranium as seen from above; anterior to the right. The ventral lobe (not indicated in diagram) lies immediately dorsal to 'a' and 'b'.

B. Diagram of parasagittal section through floor of cranium, anterior to the right.

C. Photograph of pituitary region, anterior to the right, with part of cranium cut away. Blood vessels injected with latex to show carotid (b) lying below ventral lobe (m)

a. Point at which sinus cephalicus emerges from cartilage to branch right and left within the connective-tissue endocranium.
b. Endocranial portions of internal carotids (carotid anastomosis of Norris, 1941).
c. Spiracular epibranchial artery.
d. Posterior cerebral artery.
e. Median cerebral artery.
f. Cartilage of cranium.
g. Connective tissue endocranium.
h. Inter-orbital ridge.
k. Inter-orbital vein.
m. Ventral lobe of pituitary.
n. Mucous membrane of roof of mouth.
o. Optic nerve.
p. Internal carotid before entering cartilage.
s. Saccus vasculosus.
t. Neuro-intermediate lobe of pituitary.
v. Rostral lobe of pituitary.
x. Orbit of eye.



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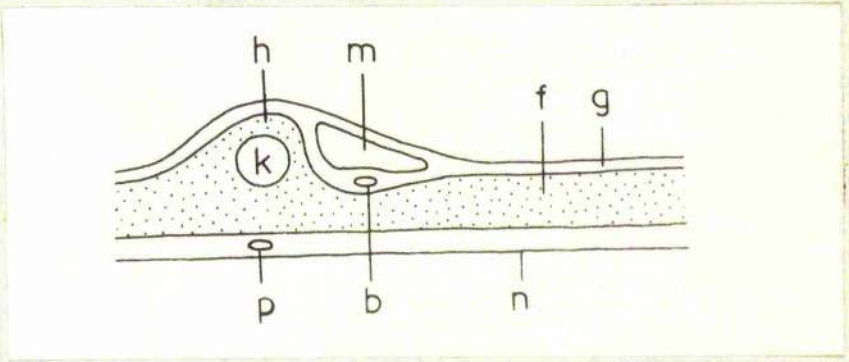

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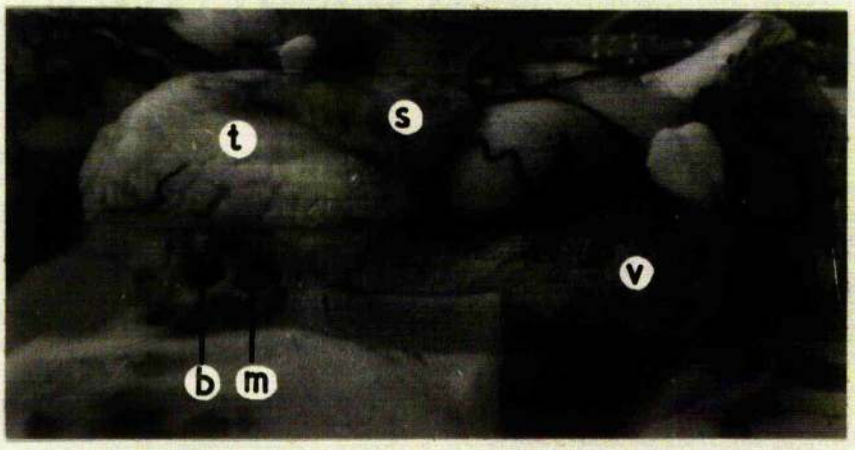


C

Fig. 4.

Fig. 5.

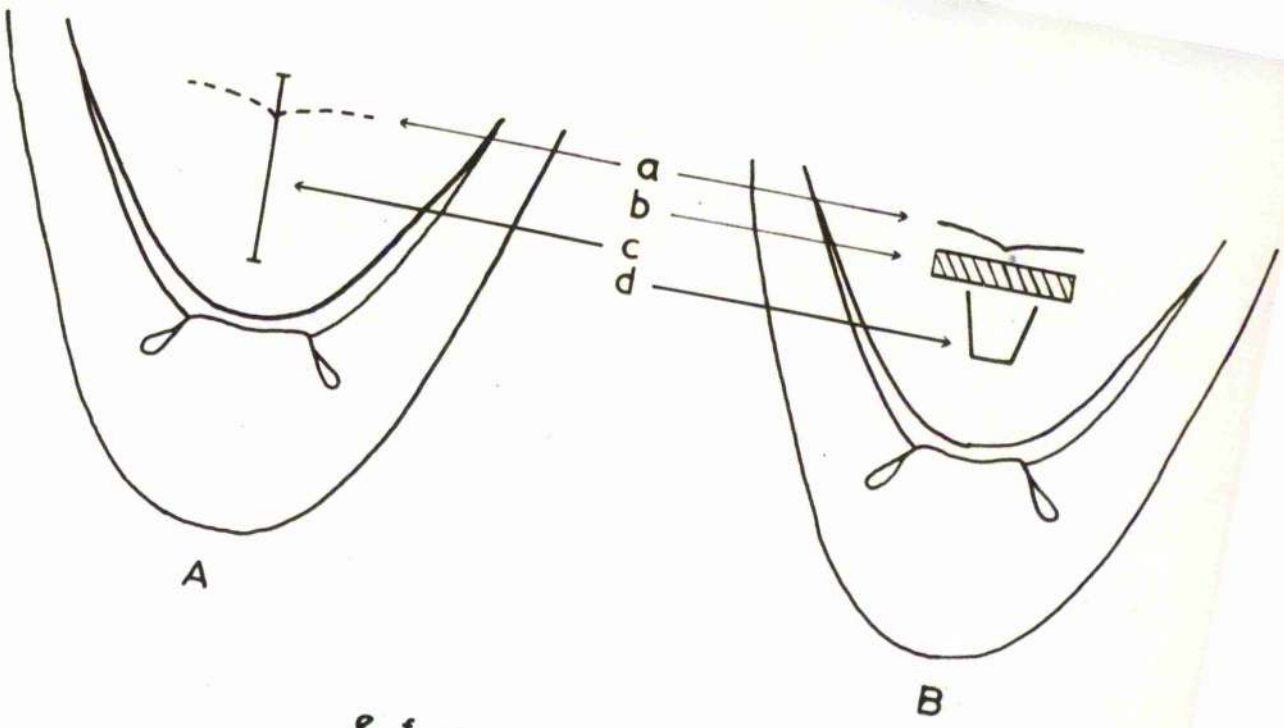
Diagrams illustrating technique of hypophysectomy in *Scyliorhinus canicula*.

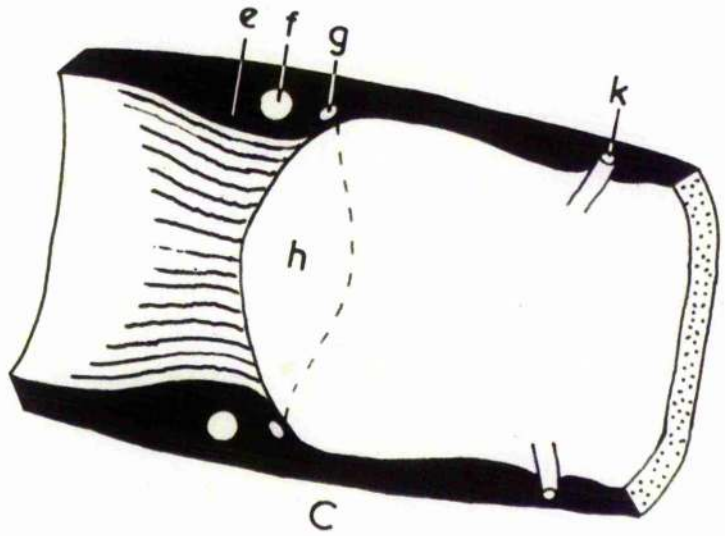
A. Roof of mouth. (a) Internal carotids lying beneath mucous membrane; (c) incision in mucous membrane.

B. Roof of mouth after exposing surface of cartilage - (a) Internal carotids; (b) region occupied by ventral lobe of pituitary and endocranial portion of internal carotids; (d) Incision in cartilage.

C. Dorsal view of floor of cranium, anterior to the right - (c) Inter-orbital ridge; (f) inter-orbital vein; (g) endocranial portion of internal carotids; (h) region occupied by ventral lobe of pituitary; (k) optic nerves.

D. Sagittal section through floor of cranium, anterior to the right. Cartilage flap deflected; seeker about to be applied to ventral lobe region. (a) Internal carotids before entering cartilage; (g) endocranial portion of internal carotids. The sinus cephalicus is shown connecting (a) and (g); (c) Inter-orbital ridge; (f) inter-orbital vein; (h) ventral lobe of pituitary.





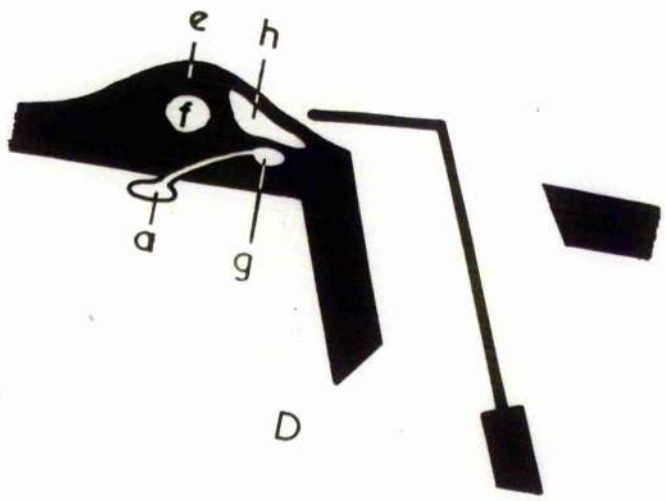


Fig. 5

follows that any attempt at dissection would have to be carried out "in the dark", and from a difficult angle. Under these conditions dissection was ruled out as impracticable.

The possibility of cutting out the block of cartilage containing the ventral lobe was considered, and in a few cases this was actually done, but most of these fish died within three weeks, and none survived more than two months. Moreover this technique is of questionable validity, since destruction of the carotids must disrupt to some extent the blood supply to the brain and pituitary.

There remained one alternative: destruction of the ventral lobe in situ. This was the technique used on all our experimental fish, with the few exceptions mentioned above. Destruction was carried out by seeker and by electric cautery.

Procedure.

Anaesthetic. In the early stages of this work the fish were anaesthetised with an alcoholic solution of 'chloretone' (10 g. in 100 ml. of 95% alcohol). This solution was added to the sea water bath in the proportion of one part to ninety nine parts sea water (Young, 1933).

This anaesthetic did not prove entirely satisfactory. Post-operative mortality was high, and there was some indication that the anaesthetic was one of the factors involved. Post-mortem examination of those fish which succumbed within a few weeks of the operation

revealed a peculiar oedematous condition in many: the peritoneum lining the dorsal body wall in the region of the kidneys was distended with fluid. This condition was found in both hypophysectomised and control fish, and it was thought that the anaesthetic might have been respons: :ible.

It is interesting to note that the skin of Scyliorhinus is extreme: :ly sensitive to alcohol. This was reported by Faure-Fremiet (1942) and confirmed at the Gatty. Small amounts of alcohol will cause extens: :ive dermal necrosis if used as an antiseptic agent. It seems possible that the alcoholic content of the anaesthetic bath could have damaged the gills. Such damage might well have caused failure of the osmore: :gulatory mechanism, resulting in the oedematous condition mentioned above.

Chloretone was eventually abandoned in favour of 'urethane' (see Hogben, 1936; Matty, 1954). The urethane crystals are dissolved in a sea water bath (1 g. in 100 ml.) equipped with an aeration tube. The fish are placed in the bath till all movement ceases; the time required for this varies from twelve to twenty minutes.

This method of anaesthetisation has proved satisfactory. Respir: :atory movements recommence almost immediately after the fish are return: :ed to their tanks, whereas with chloretone some time usually elapsed before they reappeared. Mucous does not accumulate in the bath to the same extent as with chloretone, and there has been no recurrence of the

oedematous condition noted when chloretone was used.

Post-operative mortality has been noticeably lower since urethane was adopted as anaesthetic. There are other factors (e.g. improved feeding technique) which might account for this reduced mortality, but it seems probable that the change of anaesthetic was one of the factors involved.

Preliminary procedure. Lundstrom and Bard (1932) maintained a flow of sea water over the gills while operating on elasmobranchs, but Waring (1935) held that a respiratory current is unnecessary. Our own work supports that view. On occasion we have had dogfish on the oper: :ating table for as long as forty minutes; respiratory movements re: :commenced as usual, shortly after the fish were replaced in the tanks, and we were unable to detect any subsequent ill effects. Since the normal operating time is about ten minutes and maintenance of a respir: :atory current would further complicate the procedure, we decided against this refinement. For similar reasons it was decided not to operate under water.

An operating board was roughly padded to the shape of the fish and covered with chamois leather. After being removed from the anaesthetic bath the fish is placed ventral side uppermost on the board and strapped in position by means of a pair of belts. The latter were equipped with sliding buckles for easy adjustment; one is placed just posterior

to the cloaca and the other across the pectoral fins.

The lower jaw is then held open and back by a retractor, exposing the roof of the mouth. At this point the internal carotid arteries can be seen through the mucous membrane covering the roof of the mouth. These arteries are closely associated with the ventral lobe of the pituitary, and a brief description is in order.

The internal carotid arteries. The internal carotids arise from the hyoidean epibranchial arteries. The latter run forward from the first gill clefts and divide, near the spiracles, to form the stapedials and internal carotids (O'Donoghue and Abbott, 1928). The stapedials continue forward; the internal carotids turn toward the midline and run across the roof of the mouth from right and left; in the midline they fuse to form the short "sinus cephalicus" which enters the base of the cranium by a foramen in the cartilage. This foramen is an important landmark in hypophysectomy, and will be referred to again later.

After penetrating the cartilage the sinus cephalicus separates into two vessels which constitute the endocranial portions of the internal carotids. These two vessels run right and left within the connective tissue endocranium; they are located immediately beneath the ventral lobe of the pituitary (figs. 4 A - C). On reaching the lateral wall of the cranium, each internal carotid is joined by a spiracular epibranchial artery; it then turns forward and emerges

from the connective tissue endocranium into the cranial cavity, where it divides to form the cerebral arteries.

The incisions. After exposing the roof of the mouth a median longitudinal incision is made in the mucous membrane. Immediately beneath the latter there is an extensive blood sinus which sometimes bleeds profusely.

After making the incision the edges of the mucous membrane are pulled aside, the wound is swabbed till bleeding stops, and the conn: :ective tissue cleared from the cartilage. By this time the carotids will have been exposed.

The next step is to cut a flap of cartilage from the floor of the cranium; this flap is retracted to provide a window through which the pituitary can be reached (fig. 5 D).

The incisions in the cartilage must be made so as to avoid damage to the ventral lobe and the associated blood vessels. (Damage to the ventral lobe can only be tolerated in cases of ventral lobectomy or total hypophysectomy). To avoid such damage it is necessary to conn: :ence the incisions 6 mm. anterior to the foramen by which the sinus cephalicus enters the cartilage (figs. 5B, 5D). A flap of convenient size is about 8 mm. long by 8 mm. at its widest point, tapering slightly anteriorly. While making the incisions it is advisable to hold the scalpel at an angle so as to give the flap a bevelled edge.

This bevelling prevents the flap from slipping inwards when it is pressed back into position at the end of the operation.

The flap of cartilage is then deflected backwards so as to reveal the base of the brain and most of the pituitary. Directly under the window will be found the rostral lobe; part of the neuro-intermediate lobe will be visible under the posterior edge.

Removal and Destruction of Pituitary.

In control operations the endocranial space beneath the window is swabbed with cotton wool, the cartilage flap replaced, and the mucous membrane sutured. In the case of total or partial hypophysectomies the various lobes of the pituitary are dealt with as follows:-

(a) Rostral lobectomy. The rostral lobe is removed with fine forceps. Its posterior tip, lying beneath the neuro-intermediate lobe, cannot be seen through the cartilage window; histological sections after dissection of the fish often showed that a bit of this portion had been overlooked.

(b) Neuro-intermediate lobectomy. The portion of the neuro-intermediate lobe which is visible is gripped by fine forceps and pulled forward gently; this brings the remainder of the lobe into view, and another pair of forceps is used to tease the lobe out of position. It is not possible to remove the neuro-intermediate lobe without con: :siderable damage to the sacci vasculosi, so that in most cases the

sacci were removed along with this lobe. On other occasions the damaged remnant of the sacci was left in position.

(c) Ventral lobectomy. The ventral lobe is first separated from the remainder of the pituitary with a blunt seeker. A small swab of cotton wool is then placed over the rostral and neuro-intermediate lobes (i.e. on their ventral surfaces) so as to afford them some pro: :tection during the subsequent operation.

Destruction with seeker. A curved seeker with a blunt tip is next inserted through the window. The ventral lobe lies on the anterior face of the inter-orbital ridge of cartilage, and this ridge must be located with the tip of the seeker. After locating the ridge of cartilage, its anterior face is rubbed vigorously with the tip of the seeker (fig. 5 D).

Destruction with cautery. The procedure here is similar. A blunt cautery needle, bent to the required shape, is used. The ridge of cartilage is located and its anterior face rubbed with the tip of the needle.

In both of these procedures the object is to cause maximum damage to the ventral lobe with minimum damage to the internal carotids and the inter-orbital vein. The latter is protected, to some extent, by the surrounding cartilage (fig. 4 B), but the carotids lie in a most vulnerable position immediately beneath the ventral lobe.

In some operations there was a small amount of bleeding, and it

seems probable that in these cases the carotids were broken. However there was no reason to think that such slight damage could seriously impair the blood supply to the brain. On rare occasions there was profuse bleeding, so much so that the operation had to be abandoned.

Effectiveness of the Operation.

The effectiveness of the procedures described above was checked by serial sections of the pituitary region after dissection of the fish.

Rostral and neuro-intermediate lobes. Remnants of these lobes were sometimes left in situ (see Appendix). Such remnants were attributed to two factors: (a) bleeding, which frequently obscured the site of operation and made it difficult to be sure that the whole lobe had been removed, and (b) the fact that only the anterior portions of both lobes could be seen through the window in the floor of the cranium; the posterior portions were out of sight, and had to be dissected "in the dark".

Ventral lobe. Ventral lobectomy was attempted in twenty seven fish. Twenty two percent of these operations (six fish) resulted in complete destruction of the ventral lobe. In another eleven percent (three fish) it was extensively damaged; in the remainder (sixty seven percent) it was either undamaged or damage was so slight that the ventral lobe was classified as "intact".

Reference to notes made at the time of operation showed that drastic measures were necessary to destroy the ventral lobe; apparently the tough connective tissue of the endocranium affords considerable

protection. Cautery is the more effective of the two methods used; in all cases of complete destruction the operation had been carried out by cautery.

Clearly these methods of ventral lobectomy leave much to be desired. For future work it is suggested that better results might be achieved by a technique which does not involve entry into the cranial cavity. Having exposed the internal carotids where they cross the roof of the mouth, a dental burr is used to remove the cartilage anterior to the sinus cephalicus, in the region indicated by 'b' in fig. 5B. The ventral lobe is exposed by careful drilling, and can then be removed partly by dissection and partly by suction.

Preliminary attempts using this method show promise, though bleed: :ing is usually severe since some damage to the endocranial portions of the internal carotids is inevitable. However, recent experiments involving latex injections indicate that the internal carotids might not be so vital to the blood system supplying the brain as has been assumed in this work. Such injections show that the cerebral arteries can be supplied by the spiracular epibranchials when the internal caro: :tids are blocked or severed. It is possible that the volume of blood delivered by the spiracular epibranchials would be inadequate for the normal requirements of the brain, but this possibility could be covered by suitable control operations.

Post-operative History.

After operation the fish were marked with small holes punctured in the fins and returned to the tanks. The incision in the mucous membrane of the roof of the mouth required two to three months to heal. The sutures remained in position for a few weeks, but eventually they dis: :appeared; by that time connective tissue was being laid down between the mucous membrane and the floor of the cranium.

All experimental fish were force-fed once weekly. During the first few weeks following operation the incision was liable to bleed at feeding, and care was necessary when inserting the ortho syringe into the stomach.

Some of the fish contracted an inflammation of the snout and nares. It was eventually attributed to their colliding with the walls of the tanks. Penicillin proved an effective treatment: each fish was given a weekly injection of 40,000 to 120,000 units, depending on the extent of inflammation. The brands used were Distaquaine, Permapen Plus and Penidural.

Some of the fish in one tank developed a skin infection. The symptons were white spots which increased in number and finally erupted as sores. Smears from the latter were examined under the microscope but failed to establish the presence of fungal hyphae or bacteria.

Penicillin injections were found to be ineffective against the infection. One fish was painted with a twenty percent solution of copper sulphate, but it died within forty eight hours and the treatment

was not repeated. Others were painted once weekly with strong solutions of acriflavine or gentian violet. Neither treatment had any marked effect. However, the infection did not spread to the other tanks, and it is possible that it was held in check by the sol: :utions used.

Post-operative Complications.

(a) Paralysis. In a few cases the operation was followed by part: :ial paralysis of the fish. Such paralysis occurred both in hypophy: :sectomies and in control operations, and in most cases was accompanied by loss of 'righting reaction'. It affected either side of the fish, which tended to curl in a circle towards the affected side. When disturbed it swam in a circle towards the same side. Most of these fish died, but a few eventually recovered and regained normal muscular control.

The cause of this paralysis is not apparent. An obvious suggest: :ion is damage to the brain during operation, but if that suggestion is valid paralysis should appear immediately after operation. Such is not the case: onset of paralysis may occur at any time up to some weeks after the operation.

(b) Infection of wound. In a few fish the incision in the roof of the mouth became infected. The mucous membrane became tumid and in: :flamed, and in severe cases there was loss of righting reaction. Penicillin treatment proved effective. Dosage was as described above

for cases of snout inflammation. In most cases all signs of infection had disappeared after three weeks of treatment.

METHODS

Weights.

At dissection the body weights and thyroid weights were recorded. The male gonad weights were also recorded, both testes and the attached epigonal tissue being weighed together. The thyroid and gonad weights were related to size of the fish and expressed as a fraction of the body weight.

Histology.

The pituitary, thyroid and testes were fixed for histological study. The standard fixative used was Bouin's fluid. In the case of the testes both Bouin and Sanfelice were used; the latter gave better nuclear fixation than Bouin, and proved helpful when studying the various stages of maturation. The tissues were embedded in paraffin wax and treated as follows:-

Pituitary. Sections cut at 7 μ and 10 μ; stained with Heidenhain's or Ehrlich's haematoxylin; counterstained with eosin or light green.

Thyroid. Sections cut at 4, 6, 8, 10 and 20 μ; stained with Heidenhain's haematoxylin, Ehrlich's haematoxylin or Mayer's haemalum; counterstained with eosin or light green. Azan and Mallory were also used on occasion.

Testes. Sections cut at 4, 6 and 8 μ; stained with Heidenhain's haematoxylin and eosin.

The pituitary region was removed by the following technique: Two longitudinal and two transverse incisions were made in the head so as to cut out a block of tissue including the cranium. The longitudinal incisions passed through the inner edge of each orbit, and extended anteriorly and posteriorly until they met the transverse incisions. The anterior transverse incision was made in line with the anterior tips of the eyes; the posterior transverse incision was made in line with the posterior tips of the spiracles.

The incisions were extended downward from the dorsal surface to the roof of the mouth. The block of tissue so cut out was placed in fixative for at least twenty four hours. Each block was then trans: :ferred to acid alcohol for decalcification of the cartilage. The decalcifying fluid used was a 4% solution of nitric acid in 70% alcohol: about a week was required for complete decalcification.

After decalcification the block of tissue was washed in tap water and trimmed so as to remove most of the cartilage and brain. The trimming process removed all tissue anterior to the optic chiasma and posterior to the inter-orbital ridge. The lateral wall of cartilage was then cut away on one side of the cranium; this latter incision re: :quired some care, since the neuro-intermediate and ventral lobes of the pituitary extend almost to the lateral wall. The remainder of the block contained part of the brain, the pituitary, and part of the

cranium floor. The block was then dehydrated and embedded in paraffin wax in the usual manner.

After embedding, the block was sectioned longitudinally, commencing at the side from which the lateral wall had been removed. The sections were cut at 7 - 10μ, and usually every fifteenth section was mounted. The latter rule was not invariable: with some practice it became poss: :ible to detect "remnants" (see below) of the various pituitary lobes while cutting, and care was taken to mount those sections containing the largest areas of such remnants. This was helpful when later assess: :ing the size of the remnants.

A persistent difficulty was encountered when mounting the sections. The problem was wrinkling of the cartilage, which often caused local distortion of the section. This wrinkling was never eliminated alto: :gether, although it was considerably reduced by careful trimming of the cartilage before impregnating with wax.

This problem could have been solved by celloidin-embedding, but it was considered that the result would not have justified the extra time and effort involved. The paraffin wax sections were adequate for their purpose, which was to show whether any pituitary remnants had been left in situ and to give some idea of the size of such remnants. (In the case of controls the reverse applies: the object of sectioning the pituitaries was to ensure that all lobes were intact).

The testes and thyroid presented no problems. In the case of the testes complete cross-sections were cut. These pieces of tissue were large (cut surface about 1 cm. square), but they proved to be easily handled. The usual procedure was to select a block of tissue from the middle of one testis for histology.

In seven fish sections were cut from the middle of both testes, and in one of these (D 1) additional sections were cut from the anterior end of one testis and the posterior end of the second testis. These fish showed an identical histological picture in both testes. Among them were controls and hypophysectomised fish; one was P 36, which will be figured later as showing a zone of degeneration in the spermatogonia-primary spermatocyte region. It seems fair to conclude that any cross-section of the testes will give a histological picture that can be taken as representative of the gonad as a whole.

Sudan Black.

Cross sections from the testes of two fish (D 3 and D 4) were embedded in gelatine, cut on the freezing microtome and stained with Sudan Black. Testis D 3 showed degeneration of the spermatid region, and the tubules in question were found to be strongly sudanophil (see below). Time did not allow a lipoid study of the testes of the other experimental fish.

Studies using radioactive iodine (^{131}I).

Sixteen males were injected with radio-iodine (^{131}I)

twenty four hours before dissection. Four of the males were given 500µc each; the remainder were given 440c each. Radiation counts from the thyroids of those given 500c . were corrected, for comparison with the other glands, by multiplying by the factor $44/50$.

After dissection the thyroids were placed in Bouin's fluid for twenty four hours: this served the twofold purpose of fixing the tissue and removing inorganic iodine. The thyroids were then removed from the fixative and the radiation from each gland was measured in a scintillation counter. The results were expressed as counts per unit time per mg. thyroid tissue.

The thyroids were then embedded in wax and sectioned. Representative sections were chosen, from about the middle of each gland, for counting in the end-window counter (10µ) and for autoradiography (10µ and 20µ).

(a) Section counts. Those sections selected for counting were mounted on planchettes (sections not dewaxed) and their radiation recorded in standard end-window counting equipment. There was an interval of four to six days between injection of the fish and counting of the thyroid sections. The half-life of ^{131}I is 8.1 days. On the fourth, fifth and sixth days after injection the original amount injected would have decreased to 71%, 65% and 60% respectively. Since most of the counts were made on the fifth day after injection, it was decided to correct the fourth and sixth day counts; the former were multiplied by $\frac{65}{71}$ and

the latter by $\frac{65}{60}$ to give figures comparable with the fifth day counts.

The areas of the counted sections were determined from the adjacent sections. The latter were projected on to squared paper, on which their outlines were drawn; the number of squares covered was taken as the area, expressed in arbitrary units.

Finally the radiation counts were divided by the area of the relevant contiguous sections to give counts per unit time per unit area.

(b) Autoradiographs. A few sections were selected from about the middle of each thyroid for autoradiography. These were mounted on slides, dewaxed and stained (the best stain was found to be Ehrlich's haematoxylin and alcoholic eosin). In every case sections from control thyroids were mounted, on the same slide, alongside those from hypophysectomised fish. The slides were then covered with Kodak autoradiographic film in the darkroom.

Exposure was commenced from six to nine days after injecting the fish and length of exposure varied from sixteen to twenty six days. Both of these factors affect the autoradiograph and since neither was strictly controlled, it follows that the "degree of blackening" shown by the emulsion (see p.102) must be interpreted with caution.

At the end of the exposure period the autoradiographs were de: :veloped, mounted in Canada balsam, and covered with cover-glasses.

Thyroid cell heights. The height of the thyroid epithelium was measured by means of a micrometer eyepiece equipped with a moveable hair; the objective magnification was X42. The procedure was to select one or two sections from about the middle of each gland, the cells to be measured being chosen at random from these sections. Repetition of measurements was avoided by working to a pattern, e.g. from top to bottom and left to right of the section. The person making the measurements did not know the experimental histories of the fish.

The mean cell height and standard deviation were calculated for each gland. It was found convenient to express these parameters in terms of divisions on the micrometer eyepiece. The figures given may be converted to micra by the factor $\times \frac{1}{4.97}$ ($1\mu = 4.97$ divs.)

Critique of Methods.

(a) Histological and gravimetric studies. In order to establish a case for the existence of pituitary thyrotrophin or gonadotrophin, it is sufficient to show that histological changes in the target organs are correlated with hypophysectomy. This principle is well established, and will be applied here. The same applies to the gravimetric studies. Provided a change in weight of the target organ can be correlated with hypophysectomy, that change can be used as evidence for pituitary con: :trol without reference to its functional significance.

It will be seen that in Scyliorhinus no weight changes could be demonstrated in the thyroid or testes, but histological changes do

appear. Although the present work does not require that those changes be related to function, it does allow certain conclusions as to their functional significance, and these conclusions will be stated in due course.

Only in the case of the pituitary did the question of function assume some importance. The histological sections often showed the presence of pituitary remnants. It is possible that such remnants could significantly alter the level of circulating hormone(s). It was therefore essential to take them into account when interpreting the results.

It was decided to indicate the presence of pituitary remnants wherever possible in the text and in the accompanying tables. The condition of the pituitaries at dissection has also been listed in the Appendix, which serves to supplement the information given in the text.

(b) Studies using radioactive iodine. The dosage levels of ^{131}I used in this work were extremely high. For similar studies on mammals the doses required would be about 20-50 μc. In view of the adverse effects of radiation on the thyroid, the use of 440-500 μc. requires some justifi: :cation.

Gorbman et al. (1952) obtained autoradiographs from the thyroids of S. canicula using 20-50 μc. The injections were made intraperitoneally, and the fish were killed at intervals of one hour to six days after injection. Olivereau (1954), working on the same species, reported

autoradiographs with as little as 8-13 μc. The injections were intra:
:peritoneal, and the fish were dissected two to seven days after inject:
:ion.

At St. Andrews we were unable to repeat these results. A total of sixteen fish were injected with amounts of ^{131}I ranging from 50 to 200 μc. The doses were given in single injections or spread over two to four days. The injections were sometimes intramuscular, sometimes intraper:
:itoneal, and the fish were killed twenty four to forty eight hours after the last injection. The thyroids gave negative or extremely faint auto:
:radiographs, and the radiation counts were not significantly above back:
:ground level.

Satisfactory autoradiographs were finally achieved with doses of 500 μc. This was later reduced to 440 μc; it was considered inadvisable to reduce the dose further, since some of the glands gave low counts at this dosage level. It is worth noting that Teh Ping Lin and Goldberg (1951) used similar doses (400 μc) for autoradiography in Mustelus cali:
:fornicus and two species of ray.

Radiation is capable of causing lesions in the thyroid epithelium, and chemical thyroidectomy by means of radio-iodine has become common practice in clinical and experimental work. Similar lesions were report:
:ed in the elasmobranch thyroid by Teh Ping Lin and Goldberg (1951) and by Gorbman et al. (1952), but Olivereau (1954) was unable to confirm those reports. In this work it was expected that the time interval

between injection and dissection (24 hours) would be too short to allow such lesions to develop. This expectation was justified, for there were no histological changes which could be correlated with the presence of ^{131}I.

Fixatives such as Bouin's fluid are known to remove inorganic iodine from thyroid tissue whilst leaving organic iodine in situ. As stated above, the whole glands were fixed in Bouin before being counted, so that the whole gland counts can be taken as measuring the amount of iodine bound to thyroid proteins during the twenty four hours prior to dissection. The same applies, of course, to the section counts. The binding of iodine is only one aspect of thyroid function, but it is reasonable to suggest that it be taken as a general index of thyroid activity.

(A) THE THYROID GLAND.

Review of the literature.

Early development of the elasmobranch thyroid gland was briefly described by Balfour (1885) in embryos of Torpedo and Scyliorhinus (Scyllium). The thyroid appears as a ventral diverticulum from the pharyngeal wall. The diverticulum becomes solid and elongates to form a rod-like structure which remains attached anteriorly to the pharyngeal wall. Subsequently the anlage is divided into lobules by ingrowth of connective tissue; in this way the future follicles are marked out. The gland retains its connection with the pharyngeal wall throughout the period covered by Balfour's description (i.e. up to stage "P").

Ferguson (1911) described the anatomy, blood supply and histology of the gland in several species of elasmobranch. The thyroid rests upon the basi-hyal cartilage, to which its anterior margin is firmly attached by connective tissue. The blood supply is provided by the thyroid artery which may arise as a branch of the mandibular artery or as an independent vessel from the efferent loop of the first branchial cleft. Ferguson distinguished two types of cell in the follicular epithelium: the "chief" cell and the "colloid" cell. The chief cells have a granular, eosinophil cytoplasm; the nuclei are spherical, vesi: :cular, and lie near the base of the cells. The colloid cells have a "glistening, highly refractive colloid appearance", and the cytoplasm

is distinctly acidophil; the nuclei are small and deep staining, and are usually located near the apex of the cells.

The histology of the gland has been dealt with by a number of workers. Ranzi (1936b) studied thyroids of the females of seven ovoviviparous species and one viviparous species, and reported histol: :ogical changes which he related to sexual activity. During growth the thyroid has an active appearance, with small follicles, high epith: :elium and scarce colloid. At onset of sexual maturity the histologic: :al appearance varies from moderately active to quite inactive; in the latter the follicles are large, with low epithelium and abundant colloid which shows little sign of vacuolation. During gestation the glands vary from moderately active to intensely active, with the latter showing signs of hyperaemia. After parturition the thyroid returns to a state of lowered activity.

Zezza (1937) arrived at similar conclusions after studying the thyroid of Torpedo ocellata. The gestation period in this species lasts from May to September, and during much of that period the appear: :ance of the thyroid suggests hyperfunction.

Guariglia (1937) has reported that in the oviparous species S. canicula and Scyllium stellare sexual maturity is characterised by alternate storage and discharge of colloid.

Oliveresu confirmed that in Torpedo the thyroid of the female is hyperactive during gestation (1949a). In another paper (1949b) she

described the thyroid of S. canicula at various stages of the life cycle; her findings are in general agreement with those of previous workers.

Oliverecau reported that in Scyliorhinus the thyroid of the male appears to be less active (judged by histological criteria) than that of the female at all stages of development. In juvenile fish both sexes show moderately active glands. At the onset of sexual maturity the thyroids show increased activity; according to Oliverecau the male reaches this stage at a length of 30-35 cm (weight about 100 g.); a comparable stage is reached by the female at about 30 cm length.

Subsequently both sexes show reduced thyroid activity, but in the female there is a cyclical intensification of thyroid function which is correlated with reproduction. Hyperactivity is maximal when the eggs are in the oviducal glands, and abates as the eggs travel down the oviducts.

Thyroid function in elasmobranchs has received limited attention. The first recorded study with radio-active iodine was that of Teh Ping Lin and Goldberg (1951) on three North American species. They reported that forty six hours after injection of 400 μc ^{131}I autoradiographs showed the ^{131}I to be concentrated in the colloid; in general the smaller follicles contained less colloid but more iodine than the larger follicles.

Gorbman, Kisaitsky, Michel and Roche (1952) carried out autoradio-

:graphic and chromatographic studies on the thyroid of S. canicula. They were able to identify monoiodotyrosine, diiodotyrosine and thyroxine in extracts from the glands, and the evidence indicates that these substances appear in the order given. Small but significant amounts of labelled thyroxine were found seventeen hours after injection of ^{131}I. The authors concluded that synthesis of thyroxine follows the same pattern as in mammals.

Oliverean (1950) studied the thyroids of Scyllium stellare and S. canicula after treatment with phenylthiourea, aminotriazol and thiourea. She was unable to detect any significant histological changes in the thyroids of the treated fish. In a later paper Oliverean (1954) reported that autoradiographic studies revealed the absence of protein-bound iodine (^{131}I) from thyroids of the same species after treatment with thiourea and thiouracil. She concluded that, although thyroid histology remains unaffected, organic linkage of iodine is totally and rapidly blocked by such compounds.

Matty (1954) reported that thyroidectomy has no effect on oxygen consumption in S. canicula up to six weeks after operation. However, when assayed on rats extracts of dogfish thyroid caused an increase in oxygen consumption of the same order as that produced by mammalian thyroid preparations.

Oliverean (1955) subjected S. canicula to variations in water temperature (14°-20° C.) for periods of five to thirty days; she was unable

to detect any effect on thyroid histology after such treatment.

Morphology and Histology.

The thyroid of Scyliorhinus is a discrete gland, delimited by a thin capsule of connective tissue. It is located in the lower jaw, and extends forward from the anterior bifurcation of the ventral aorta to the basi-hyal cartilage. It is pear-shaped, with the bulbous por: :tion lying posteriorly and attached to the ventral aorta by strands of tough connective tissue. The anterior portion constitutes the "thyro: :glossal cord" (Watty, 1954); it stretches forward as a delicate strand of tissue which is attached anteriorly to the basi-hyal cartilage.

Histologically, the gland consists of closely-packed follicles separated by scanty connective tissue and blood spaces. The follicles vary in size; the smaller follicles tend to be grouped towards the centre of the bulbous posterior portion of the gland, whereas the larg: :er tend to lie peripherally; the thyroglossal cord is occupied by the larger type of follicle.

The follicular epithelium consists of very high columnar cells with basal nuclei. These cells are higher than comparable cells found in any other vertebrate group, averaging about 15 μ. The cyto: :plasm contains variable numbers of granular basophil inclusions which stain heavily with Heidenhain's haematoxylin (Plate 1, fig. 1) but not with Ehrlich's haematoxylin or Meyer's haemalum. These basophil gran: :ules show a great range in size and are often so numerous that cellular

detail (cell boundaries, nuclei) are obscured. The smaller granules tend to lie towards the apex of the cells, i.e. towards the lumen of the follicle; the larger tend to lie towards the base of the cells.

Occasionally very large basophil inclusions can be found in the epithelial cells (pl. 1, figs. 1 and 5). These appear to be aggre:gations of the basophil granules described above. They occupy large vacuoles with diameters many times the width of the epithelial cells. They are conspicuous objects, and will be referred to as basophil cysts.

The cytoplasm of the epithelial cells sometimes contains eosinophil inclusions; these also may range in size from very small granules to large bodies the size of the basophil cysts. However, they are neither so conspicuous nor so prevalent as the basophil inclusions, and in some glands they are absent.

The lumina of the follicles are more or less filled with eosinophil colloid; occasionally the colloid stains parly basophil, partly eosino:phil, and very infrequently a follicle may contain only basophil colloid. Occasionally free cells can be seen in the colloid: these are rounded cells with excentric nuclei, and are presumably epithelial cells which have migrated into the lumen of the follicle.

The smaller follicles usually have a high epithelium and dilute colloid* with resorption vacuoles, whereas the larger follicles tend to have a somewhat lower epithelium and dense colloid with no vacuoles; basophil colloid is perhaps more often found in small follicles than in

* Consistency of the colloid can be judged by the way in which it is cut by the microtome knife: dense or viscous colloid shows "ripple marks", whereas the dilute type cuts smoothly.

large. However, these correlations are not clear-cut, and it is extremely difficult (and perhaps inadvisable) to assess thyroid activity in Scyliorhinus on the basis of such histological criteria.

Variability. A number of workers (Guariglia, 1937; Olivereau, 1949a, 1949b; Zezza, 1937) have described histological changes in the thyroids of elasmobranchs corresponding to different stages of the life cycle. Both Guariglia and Olivereau described changes in the thyroid of S. canicula, and related those changes to the onset of sexual matur: :ity. Olivereau was the only worker to find regular changes of a cycli: :cal nature in the thyroid of the mature fish: she described the female gland as hyperactive (judged by histological criteria) after ovulation, during the period when the ova are in the oviducal glands.

Thyroids from fifty mature fish (37♂, 13♀) were examined; of these twenty three were control operations (18♂, 5♀). Besides these, the thyroids of six freshly-caught fish (4♂, 2♀) were used as additional controls; the latter confirmed the general picture seen in the thyroids of control operations.

Sections of the control glands showed a wide range of histological variation in both sexes (see below). Such variability is particularly noticeable among the males. At dissection no egg purses were found in the oviducts of any of the females, so that the variability among the female glands cannot be accounted for on the basis of the correlation noted by Olivereau.

Pl. 1, figs. 2, 3, serve to illustrate the histological variation among the controls. On the one hand (fig. 2, D44) the follicles are small and closely packed. The follicular epithelium is high; the colloid is dilute, is sometimes basophil, and shows resorption vacuoles. On the other hand (fig. 3, D72) the follicles are large and are separated by plentiful connective tissue; the follicular epithelium is relatively low; the colloid is dense, eosinophil, and shows no sign of resorption vacuoles.

The two glands also differ with respect to the basophil inclusion found in the epithelium. D 44 contains very small cytoplasmic granules, whereas D 72 contains relatively large granules and a few basophil cysts. A similar relationship can sometimes be seen within individual glands, where small, active-looking follicles may lie alongside large, inactive-looking follicles (Pl. 1, fig. 4). The former contain a high epithelium with fine basophil granules, whereas the latter contain a lower epithel: :ium with relatively large granules and occasional cysts. In fig. 4 the epithelium of the large follicles on the left of the photograph is so densely packed with large basophil granules that cellular detail is obscured.

There appears to be no significant variation between glands in the degree of vascularization. Olivereau (1949b) claims to have detected increased vascularization in the thyroids of female Scyliorhinus, occurr: :ing at the time when the ova are in process of vigorous growth. How:

:ever, in the present work no significant differences in vascularization have been found, either among control glands or between those of controls on the one hand and hypophysectomised fish on the other.

Experimental Studies.

(1) Histology.

(a) Less than 20 weeks after operation.

Sections of the thyroid glands from a number of early operat:ions had indicated that hypophysectomy does not affect thyroid histology within this period. The pituitaries of these fish were not sectioned at autopsy, and the detailed results have therefore not been included in this report.

From a later series of operations detailed results are avail:able for twelve fish. One of these (P48, table II) was a partial hypop:hysectomy. The thyroid glands showed no unusual histological features.

(b) 20-30 weeks after operation.

A total of twenty-six fish are included in this group, of which eight are controls. The thyroids of the controls show the usual range of histological variation; there were no histological features which could be attributed to experimental treatment or to captivity. This statement applies to the thyroids of all controls, regardless of length of time after operation at which dissection was carried out. It

follows that no further description of the control thyroids is necess: :ary.

Of the hypophysectomised fish in this group only one appears to merit special mention, namely D46 (rostral and neuro-intermediate lobectomy); the thyroid epithelium of D46 was found to contain a partic: :ularly large number of basophil cysts (pl. 1, fig. 5). However, there are several other fish in this group from which the same pituitary lobes had been removed, and their thyroids appear to be normal (see table II, p. 96b). One such fish (D60) has a very "active" gland, with small foll: :icles and a high epithelium; in many follicles the lumina are occluded and some of the remainder show a basophil colloid. This thyroid (D60) is not considered to be abnormal, since it lies within the range of vari: :ation shown by the controls.

(c) 31-40 weeks after operation.

There are twelve fish in this group, of which eight are con: :trols. The four hypophysectomised fish will be treated individually; in each case the condition of the pituitary at dissection is given in brackets. The results described below are summarised in table I, p. 96a.

D 47. (Rostral lobe absent; neuro-intermediate lobe represented by a remnant; ventral lobe present).

The thyroid of this fish appears to be normal.

D 51. (Rostral lobe represented by a remnant; remainder of pituitary present).

The thyroid shows signs of disorganisation. Over large areas the lumina of the follicles are occluded; in some places the follicles have lost their organization, and the epithelial cells lie scattered among strands of connective tissue (pl. 2, fig. 6). Large basophil inclusions are common among the epithelial cells; these are not so large as the basophil cysts described above, but are doubtless related to them. However, this gland is not markedly abnormal; the general picture is reminiscent of control D 60, described above.

D 19. (Rostral and neuro-intermediate lobes absent; ventral lobe present).

Thyroid follicles irregular in size and shape, with free cells in the lumina of many. The epithelium lacks basophil granula: :tion. In some areas the follicles are breaking down; this seems to be brought about by dispersal of the epithelial cells. After dispersal of the cells the colloid left in situ takes on a very dense appearance and stains intensely with haematoxylin. Strands of connective tissue appear to be invading the gland (pl. 2, fig. 7); in the path of the invading tissue the follicles show signs of disruption.

D.34. (Rostral lobe present but damaged; neuro-intermediate lobe absent; ventral lobe present).

Thyroid follicles irregular in shape and size, separated by a good

deal of connective tissue. The epithelium if high, with heavy basophil granulation obscuring cellular detail. A number of follicles show free cells within the colloid. In some follicles the colloid is broken up into rounded or angular lumps, and some of these colloidal lumps stain intensely with haematoxylin (pl. 2, fig. 8.)

These basophil colloidal bodies are similar to the isolated clumps of basophil colloid described under D 19. The large clumps seen in fig. 7 (D 19) have been left behind after dispersal of the follicular cells. In fig. 8 (D 34) the follicle is still intact, but the colloid is breaking up into separate pieces, some of which show an intense basophil reaction.

Of these four glands, D 19 and D 34 are taken to be abnormal, showing early degenerative changes. The changes common to both glands are penetration by invading connective tissue, disintegration of individual follicles, and a tendency for the colloid to form dense, basophil bodies. Both glands show an unusual number of follicles with free cells in their lumina.

(d) 41-50 weeks after operation.

This group includes five fish, of which two are controls. Two of the hypophysectomised fish (D 12 and D 13) possessed similar thyroids, and these will be described together; the third will be treated separate:ly. The condition of the pituitaries at dissection is

given in brackets.

D 12 and D 13. (D 12: rostral lobe absent; neuro-intermediate lobe represented by a remnant; ventral lobe present.
D 13: rostral and neuro-intermediate lobes absent; ventral lobe present).

Thyroid follicles irregular in size and shape, separated by a good deal of connective tissue. Among the intact follicles the epithelium of both glands is packed with basophil granules to such an extent that cellular detail is obscured. The follicular epithelium also contains a large number of basophil cysts, and in D 12 it contains some eosinophil cysts as well. In many follicles the colloid has a porous or "honeycomb" appearance. The tendency for free cells to invade the lumina of the follicles was again noticed.

Localised disintegration of follicles occurs in both glands, and is particularly well seen in D 13 (pl. 2, fig. 9). In the region photographed the follicles have lost their organization and the epithelial cells are dispersed throughout a matrix of connective tissue; fragments of colloid and basophil bodies (probably epithelial cysts) are scattered throughout the area.

D 12 contains a number of follicles similar to those shown in pl. 2 fig. 8, with the colloid breaking up into

basophil lumps. Pl. 2, fig. 10 (D 12) shows a group of basophil bodies lying within a connective tissue matrix; they appear to be colloid remnants from a follicle which has been invaded by connective tissue. The invasion of individual follicles by connective tissue will be described and figured in more detail below (see thyroid D 1).

It appears that disintegration of the follicles can occur in two ways: (a) Dispersal of the epithelial cells and simultaneous invasion by connective tissue results in the condition found in D 13 (pl. 2, fig. 9). This type of degeneration tends to occur in smaller foll: :icles where the colloid is dilute. (b) Dispersal of the epithelial cells may be preceeded by break-up of the colloid and its conversion to basophil masses as seen in fig. 8. The latter remain in situ after dispersal of the epithelial cells, and become embedded in a matrix of invading connective tissue; the result is seen in pl. 2, fig. 10. This type of degeneration tends to occur in large follicles where the colloid is viscous. Presumably such colloid cannot be easily resorbed and special measures are required to deal with it.

D 31. (Rostral lobe represented by a remnant; remainder of pituitary present).

Although the appearance of this thyroid is abnormal, it has clearly not suffered degeneration to the same extent as D 12 and D 13. The follicles are fairly regular in size, but irreg: :ular in shape (pl. 3, fig. 11). They are separated by a

large amount of connective tissue, but it is difficult to decide whether this represents an unusual condition or whether the gland is actually being invaded by connective tissue; there are indications that such invasion has commenced.

The follicular epithelium is high and contains large numbers of basophil granules which obscure cellular detail. Most follicles contain dilute colloid, and resorption vacuoles are common. A few show invasion of the lumen by free cells.

The general picture presented by this thyroid suggests dysfunction rather than degeneration; if degeneration has set in, it appears to be in its early stages.

Of the three thyroids described in this group, D 12 and D 13 show degenerative changes. These changes are well established in both glands, and can be interpreted as a later phase of the early degenerative changes described in D 19 and D 34.

(e) More than 50 weeks after operation.

There is one fish (D 1) in this class. It was dissected fifty-five weeks after operation. At dissection the rostral and neuro-intermediate lobes of the pituitary were found to be absent; the ventral lobe was present.

D 1. The thyroid shows advanced degeneration. The intact follicles are very irregular in shape and size (pl. 3, fig. 13). In the largest follicles the epithelial is low and cytoplasmic granules

are relatively scanty, but in most of the follicles the cells are high and heavily studded with basophil granules. Baso: :phil epithelial cysts are common, and in the colloid resorpt: :ion vacuoles are rare.

Much of the gland is occupied by connective tissue (com: :pare figs. 12 and 13). Pl. 4, fig. 14, shows a follicle in process of being replaced by connective tissue. The connect: :ive tissue has encroached upon and obliterated much of the original lumen; the remainder of the lumen contains some free cells and a group of basophil colloidal bodies. A few similar bodies are embedded around the periphery of the connective tissue mass. This process of replacement eventually results in whorls of connective tissue which mayor may not enclose basophil colloidal bodies. Such whorls of connective tissue are common in this gland (pl. 4, fig. 15).

In D 1 the basophil colloidal bodies are seen to have a peculiar structure of concentric rings, centred about one or more points (pl. 4, fig. 16). This structure suggests that the colloid condenses around certain focal points. As it condenses it stains intensely basophil.

There is evidence that free cells lying in the colloid constitute the focal points around which condensation takes place (pl. 4, fig. 17). The nature and origin of these cells

is not apparent. They do not resemble the free cells commonly found in the lumen of normal follicles. The cytoplasm is clear and the nuclei stain intensely; when found without a sur: :rounding envelope of dense colloid they show a thick and some: :what irregular cell membrane (pl. 4, fig. 17).

The colloid does not always condense in the manner described above to give concentric basophil bodies. In some cases conden: :sation results in large basophil masses which show no sign of concentric structure; a number of such basophil masses are seen in pl. 4, fig. 15.

Comment. The degenerative changes described present some unique features. The thyroid epithelium shows no significant reduction in height; this point will be dealt with in a later section. The operation has no detectable effect on vascularity of the gland; staining reaction of the colloid is unaffected until the degenerative changes (see below) appear; and finally resorption vacuoles are not uncommon in those glands in which degenerative changes have been described.

In Scyliorhinus the degenerative changes seem to follow the pattern outlined below:

Invasion of the gland by connective tissue is noticeable at an early stage. The invading connective tissue replaces follicles in localised areas. The follicles in the path of the invading tissue dis: :integrate. In small follicles the epithelium disperses; the small

amount of dilute colloid is broken up by strands of connective tissue and is presumably resorbed. In large follicles with relatively dense colloid the epithelial cells disperse and the invading connective tissue gradually encroaches upon the lumen; simultaneously the colloid condenses and becomes markedly basophil. In most cases condensation is centred on a few focal points consisting of characteristic cells; the colloid condenses about these cells with a pattern of concentric rings. In other cases the process of condensation results in a rela: :tively large colloidal mass which shows no sign of concentric structure. The invading connective tissue continues to encroach, and finally surr: :ounds the basophil colloidal bodies. There is no evidence that the latter are removed or destroyed. They lie embedded among the connect: :ive tissue in all the glands showing degeneration.

The above results are summarised in Tables I and II. Inspection of the tables brings to light the following points:-

(a) Thyroid degeneration is a function of time; degenerative his: :tological changes can be detected some thirty-seven to thirty-eight weeks after operation. In the female they are clearly noticeable at that time; in the male they are not yet so well-established, and the evidence presented here indicates that degenerative changes might appear earlier in the female than in the male. However, the limited number of fish makes it unjustifiable to press this point.

TABLE I.

Histology of the thyroid after hypophysectomy.

Fish	Condition of pituitary at dissection			Time after operation (weeks)	Histological appearance of thyroid
	Rostral lobe	N-int. lobe	Ventral lobe		
D 46 ♂	-	-	Present	27	Abnormal?
D 47 ♂	-	*	Present	35	Normal.
D 51 ♂	*	Present	Present	37	Signs of disorganisation.
D 34 ♀	Present(x)	-	Present	37	Early degenerative changes.
D 19 ♀	-	-	Present	38	
D 12 ♀	-	*	Present	42	Degeneration.
D 13 ♀	-	-	Present	42	
D 31 ♀	*	Present	Present	45	Early degeneration?
D 1 ♂	-	-	Present	55	Advanced degeneration.

* Remnant of lobe remains in situ.

- Lobe absent.

(x)This lobe present but damaged.

TABLE II.

Histology of the thyroid after control operations and hypophysectomy.

Fish	Condition of pituitary at dissection			Time after operation (weeks)	Histological appearance of thyroid.
	Rostral lobe	N-int. lobe	Ventral lobe		
♂ (22)	Controls)	pituitaries intact		0-50	)Wide histolog:
♀ (7)	Controls)			0-45	) :ical variation)
♂ (7)	*	Present	Present	24-30	No significant histological diff. between these and controls.
♀ (2)	*	Present	Present	24	
P 77 ♂	-	Present	Present	26	
D 3 ♂	-	-	*	29	"
P 64 ♂	-	-	Present	27	"
D 40 ♀	-	-	Present	23	"
D 60 ♂	-	*	Present	29	"
P 63 ♂	*	*	Present	25	"
P 71 ♂	-	Present	-	23	"
P 48 ♂	Present	-	-	13	"
P 42 ♂	Present	Present	-	24	"

* Remnant of lobe remains in situ

- Lobe absent.

(b) Degeneration is progressive, and about a year after oper: :ation the thyroid presents an appearance that can be described as atrophic, with many follicles replaced by connective tissue.

(c) Thyroid degeneration is not dependent on absence of the ventral lobe of the pituitary. In all cases the ventral lobe was present (table I). Moreover, severe damage to the ventral lobe has no effect on thyroid histology up to twenty nine weeks after opera: :tion (see D 3, table II).

(d) The balance of evidence indicates that the rostral lobe is responsible for thyroid control. An obstacle to that view is the fact that the rostral lobe of D34 (table I) was found to be present at dissection. However, this lobe was smaller than normal, and show: :ed clear signs of damage (invasion by connective tissue and localised disintegration of the epithelium); it is possible that its function had been impaired to a greater extent than the histological picture suggested.

(e) Alternatively, table I may be taken to suggest that removal of either the rostral lobe or the neuro-intermediate lobe causes thyroid degeneration. Interpreted in terms of a thyrotrophic hormone, this implies that both lobes contribute to synthesis of thyrotrophin.

There is no record in other vertebrates of cooperation be: :tween different parts of the pituitary to produce thyrotrophin; more: :over, it is well established that in other vertebrates the partes

intermedia and nervosa are not concerned with thyroid control. In any case the results presented are adequately accounted for by a more acceptable alternative (see 'd' above).

(2) Thyroid Cell Heights.

Thyroid cell heights are given in the Appendix for those fish dissected more than twenty weeks after operation. The figures allow a comparison between three groups (i.e. operational categories) of fish; all the fish considered are males:-

	Operation	No. of fish in group.	Mean cell height for group.	S.D. for group.
(a)	Controls	14	82.9	7.5
(b)	Rostral and M.-int. lobes removed	6	82.3	26.5
(c)	Rostral lobe removed	8	78.5	7.0

The 'mean cell height for group' is derived by taking the sum of the mean cell heights (Appendix) and dividing by the number of fish in the group. The units used are divisions on the micrometer eyepiece. The 'standard deviation for group' measures the dispersion of the means about the mean for the group.

The "t" test (formula given on p.103) shows no significant difference at the 5% level between the cell heights of the three groups. These figures therefore support the conclusion reached by histological study, namely, that hypophysectomy does not result in a decrease in thyroid cell height.

(3) Thyroid Weights.

Tables III and IV show the relationship between thyroid weight and body weight in a number of males, including controls and hypophysectomies. The number of observations is limited, but there are indications that removal of the rostral lobe alone, or removal of the rostral and neuro-intermediate lobes together, has no effect on thyroid weight. Among controls the ratio thyroid wt./body weight (x 1000) has a mean value of 40.2 and a standard deviation of 11.6 (table III). Both operated groups (table IV) have mean values for this ratio which are not significantly different from the mean of the controls.

(4) Studies Using Radioactive Iodine (^{131}I).

Radiation counts. Sixteen males were injected with radio-iodine twenty four hours before dissection. Radiation counts from the thyroids of these sixteen fish are given in tables III and IV. The glands were counted after removal of inorganic iodine (see p.78). Whole gland counts were related to weight of the gland and the result expressed as counts per minute per mg. thyroid tissue (column B).

Representative sections (10μ) from about the middle of each gland were also counted. The counts were related to the areas of the sections, and the result expressed as counts per minute per unit area of section (column C).

TABLE III.

(All fish listed are males).

Column A : Ratio of thyroid weight (mg.) to bodyweight (g.) x 1000.

Column B : Ratio of whole gland counts, recorded by scintillation counter after 24 hours in fixative, to weight of thyroid (i.e. corrected counts per minute per mg.).

Column C : Ratio of section counts to section areas. Sections of 10μ counted in end-window counter; areas of sections determined by projecting adjacent sections at constant magnification onto squared paper (i.e. corrected counts per minute per unit area).

Group (a) Controls: pituitaries left intact.

Fish	Time after operation (weeks)	A	B	C	Autoradiograph
D 2	50	32	-	-	
D 44	35	30	-	-	
D 45	37	34	57	38	trace
D 48	38	33	-	-	
D 56	34	35	-	-	
D 57	37	45	191	240	+
D 78	31	35	224	262	++
P 9	25	40	-	-	
P 26	25	36	382	148	++
P 40	24	61	-	-	
P 49	23	47	-	-	
P 55	23	65	-	-	
P 58	19	24	-	-	
P 70	25	46	331	498	++
Mean		40.2	237	237.2	
S.D.		11.6	127	170.5	

TABLE IV.

(All fish listed are males).

Columns A, B and C as for Table III.

Fish	Time after operation (weeks)	A	B	C	Autoradiograph
Group (b) Rostral and neuro-intermediate lobes removed.					
D 1	55	47	-	-	
D 47	35	45	-	-	
P 63	25	33	311	318	++
P 64	27	35	86	85	+
	Mean	40	198.5	201.5	
	S.D.	7.1	-	-	
Group (c) Rostral lobe removed.					
D 51	37	24	309	201	+
D 82	27	36	206	92	trace
D 84	30	31	449	435	++
P 52	26	30	77	74	+
P 54	24	36	303	53	++
P 60 *	24	56	242	107	++
P 61	27	51	30	32	+
P 62	24	32	113	51	+
P 77	26	27	36	66	+
	Mean	35.9	196.1	123.4	
	S.D.	10.8	143.5	126.8	
Mean of (b) + (c)		37.1	196.5	137.6	
S.D. of (b) + (c)		9.7	137.8	128.8	

For further information on the pituitaries refer to Appendix.

* P60: ventral lobe of pituitary represented by a remnant.

The figures in columns B and C are a measure of the amount of iodine bound to thyroid proteins during a twenty-four hour period. Tables III and IV show that the ratio in column B generally parallels the ratio in column C; however, the factor B/C is not constant.

Tables III and IV allow a comparison between three groups of fish: (a) controls, (b) rostral and neuro-intermediate lobectomies, and (c) rostral lobectomies. Comparing the mean values shown in col: :umns B and C:- the means for group (c) are smaller than those of group (a), but the standard deviations of the two groups are so large that the differences are clearly not significant. There are only two obser: :vations in group (b), so that calculation of a standard deviation is redundant; the mean values for this group are again lower than those of the control group (a), but again the differences are not statistically significant.

Table IV shows the means and standard deviations for groups (b) and (c) taken together. The means are again lower than those of the control group (a), and the standard deviations are again large; the differences between the means are not statistically significant (see 'Statistical Analysis').

It is clear from these figures that the amount of iodine bound to thyroid proteins varies considerably, both among the controls and among the hypophysectomised fish. The mean values suggest that rostral lobectomy is followed by a decline in I-binding capacity of the thyroid,

but the figures given cannot establish that such a decline does in fact occur.

One further point may be mentioned: Radiation counts are available for eleven of the thirteen fish listed in groups (b) and (c) of table IV. Those eleven fish were dissected thirty-seven weeks or less after operation. It follows that any trend revealed by the ^{131}I counts applies to the first thirty-seven weeks after operation. The figures for those counts suggest a decline in functional activity of the thyroid after rostral lobectomy (see above). If such a decline does occur it can be taken as occurring within thirty-seven weeks of the operation, i.e. prior to the onset of histological degeneration.

Autoradiography. Pl. 5, fig. 18 shows autoradiographs of thyroids P70 and P63. The sections were mounted side-by-side on the same slide and given the same treatment throughout. The radio-activity is concentrated in the colloid. Comparison with the control (P70) shows that loss of the rostral and neuro-intermediate lobes has not appreciably affected I-uptake in P63.

Pl. 5, fig. 19 shows a similar preparation, with thyroid sections of D78 (control) and P77 (rostral lobectomy) mounted side-by-side. In this case there is an appreciable difference between the two glands: that of P77 shows markedly less radio-activity than that of the control.

The last columns of tables III and IV indicate the degree of blackening shown by the autoradiographic films. In assessing the

degree of blackening two factors are involved: extent and intensity of blackening. However, these two factors usually go together; a section which shows extensive blackening usually shows intensive blackening as well, so that in most cases the assessment presented no problem. In doubtful cases the final assessment was based on extent rather than intensity of blackening.

Inspection of tables III and IV reveals that the trend seen in fig. 19 is not constant. One of the controls (D45), for instance, shows only a trace of blackening, whereas blackening is maximal in three rostral lobectomies. However, the proportion of glands showing maximal I-uptake is higher among the controls than among the hypophy: :sectomies (groups 'b' and 'c').

Although there are exceptions, the degree of blackening tends to parallel the count ratios shown in columns B and C. The autoradio: :graphs therefore tend to support the conclusions reached from a study of the counts: they suggest that rostral lobectomy is followed by a decline in I-binding capacity of the thyroid.

Statistical Analysis.

The 't' test was used to assess the significance of the numer: :ical results given in tables III and IV. A close approximation to 't' is given by the formula:-

$$t = \frac{m_1 - m_2}{\sqrt{\frac{s.d._1^2}{n_1} + \frac{s.d._2^2}{n_2}}}$$

where m_1 and m_2 are the arithmetic means of the two samples to be compared, $s.d._1$ and $s.d._2$ are the respective standard deviations, and the samples consist of n_1 and n_2 observations respectively.

Values for s.d. are provided by the formula:-

$$s.d. = \sqrt{\frac{\sum(n-x)^2}{n-1}}$$

where x represents the individual observations comprising the sample.

In all cases the 5% level of significance was adopted. The test was applied to the results listed in columns A, B, and C of tables III and IV. There was no significant difference between the controls (group 'a') and the hypophysectomised fish (groups 'b' and 'c' taken together) with respect to the ratios given in the tables.

Comment. From the results presented above the following points emerge:-

(a) There appears to be no significant reduction in thyroid cell height after removal of the rostral and neuro-intermediate lobes of the pituit: :ary.

(b) There appears to be no significant reduction in thyroid weight after

removal of the same pituitary lobes. This appears to be true even for those thyroids in which degenerative histological changes were found (e.g. D1, table IV); presumably the weight is maintained in such cases by the connective tissue which replaces the glandular tissue.

(c) Radiation counts do not show any significant difference between the thyroids of controls and those of hypophysectomised fish. However, the means suggest a tendency towards lower counts in groups (b) and (c) of table IV; in other words they suggest that removal of the rostral lobe of the pituitary may adversely affect thyroid function. This conclusion is supported by the autoradiographic studies.

(d) The suggested decline in thyroid function precedes the histological changes already described.

Discussion.

The pioneer work of Smith (1916) on tadpoles of Rana boylei showed that the hypophyseal region of the pituitary exercises a trophic effect on structure and function of the thyroid gland. This was confirmed by Allen, (1917). In a later paper Smith and Smith (1922) reported successful replacement therapy using anterior pituitary extract of mammals, and demonstrated that thyrotrophin production is localised in the anterior pituitary.

Subsequent work has shown that hypophysectomy causes some degree of involution of the thyroid in all vertebrate classes. This effect

has been described in mammals by Smith (1927, 1930), Rowlands (1935) and others; in birds by Rowlands (1935); in reptiles by Schaefer (1933) and Hellbaum (1936); in amphibians by Tuchmann-Duplessis (1945); and in teleost fish by Vivien (1941), Buser-Lahaye (1953), Pickford (1953) and Chavin (1956).

The main features of thyroid involution are decrease in height of the epithelial cells and distention of the follicles with colloid. Rowlands (1935) found a decrease in thyroid weight in the ferret and hedgehog, but not in the guinea-pig. Smith (1930) reported a weight decrease in the rat thyroid and reduction in size of the thyroid in tadpoles of Rana boylei (1916). Tuchmann-Duplessis (1945) reported decreased vascularity in the thyroid of Triturus (Triton). Changes in staining reaction of the colloid were reported by Pickford (1953) who noted in Fundulus a tendency towards acidophil colloid after oper: :ation, and by Tuchmann-Duplessis (1945), who reported in Triturus a change from chromophobe to chromophil colloid. Rowlands (1935) men: :tioned the absence of resorption vacuoles from the colloid of mammals and birds after hypophysectomy.

The histological changes mentioned above can be detected short: :ly after hypophysectomy. Smith (1930) found significant changes in the rat thyroid after ten days, and Hellbaum (1936) reported the same in the garter snake; Tuchmann-Duplessis (1945) found that in Triturus they become noticeable about the third week after operation, and

Vivien (1941) noted their appearance some fifteen days after operation in Gobius. It seems that by about the third week after hypophysect: :omy most vertebrates begin to register histological changes in the thyroid.

The histological changes described by previous workers (review above) are quantitative rather than qualitative. They indicate a hypothyroid condition, but the gland remains intact and continues to function at a reduced level of activity. Salter (1950) has concluded, from work done on mammals, that hypophysectomy is followed by a hypo: :thyroid condition in which the basal metabolism of the animal falls to about 80% of normal, but the symptoms of thyroid deficiency stop short of myxedema. "Apparently there is some natural activity on the part of the thyroid which does not require the so-called tonic stimul: :ation of the pituitary hormone, at least for a time". After a pro: :longed period in this condition further atrophy of the thyroid may occur.

The present work has shown that the histological changes in the dogfish thyroid are qualitatively different from those described above. In Scyliorhinus the significant features of thyroid involution are dis: :integration of the follicles and their replacement by connective tissue. These changes do not appear till some considerable time (about thirty-seven weeks) after hypophysectomy; the evidence indicates that they are progressive and would eventually result in complete replacement

of the gland by connective tissue. It is safe to conclude that these changes would cause a progressive decline in thyroid function, culminating eventually in an athyroid condition.

It is possible that the changes described in Scyliorhinus correspond to the second phase of involution described by Salter. In that case two possibilities must be considered: either the earlier phase of involution (in which thyroid function declines to a "resting level") is not represented in elasmobranchs, or if it does occur it is not accompanied by any significant histological change.

There is some evidence in favour of the latter alternative. Although no significant histological changes could be detected during the thirty-seven weeks following hypophysectomy, there is reason to suspect that a decline in thyroid function might have occurred during that period. Evidence to that effect is provided by the radio-activity counts, which suggest a fall in I-uptake during the period in question. A fall in I-uptake by the thyroid is known to occur in mammals after hypophysectomy (Leblond, She and Chamorro, 1940; Randall and Albert, 1951). The ^{131}I studies reported here suggest that a similar fall might occur in elasmobranchs. However, the evidence on this point is not conclusive, and if a fall in I-uptake does occur it is probably slight.

The correlation between thyroid atrophy and hypophysectomy clearly indicates the presence of a thyrotrophic hormone in elasmo-

:branchs. Questions concerning the nature of that hormone and its effect on thyroid function must await future work. The only state: :ment that can safely be made at present is that its absence event: :ually results in atrophy of the thyroid.

Regarding the site of production of the thyrotrophic hormone: The histological evidence clearly excludes the ventral lobe of the pituitary. The histological evidence also indicates that the neuro-intermediate lobe alone is not responsible for thyroid control (table I). There is some suggestion that both the rostral and neuro-inter: :mediate lobes together might be responsible, but this possibility has already been discussed and rejected (p. 97).

The histological evidence favours the view that the rostral lobe is the site of thyrotrophin production. This evidence is supp: :orted by the ^{131}I studies which suggest a decline in thyroid function after rostral lobectomy. However, further information concerning the site of thyrotrophin production is clearly desirable. A number of fish have been retained for dissection at a later date, and it is hoped that they will provide clear evidence on this point.

In higher vertebrates the thyrotrophic hormone is produced by the pars anterior of the pituitary. It is now generally accepted that the rostral lobe is the homologue of the pars anterior (see re: :view of literature on the pituitary), the results presented here therefore indicate that the rostral lobe is also the analogue of the pars anterior - at least in so far as the thyrotrophic hormone is concerned.

Summary.

Hypophysectomy results in degenerative histological changes in the thyroid of Scyliorhinus. These changes can be detected about thirty seven weeks after operation and become progressively more pro: :nounced. The histological evidence indicates that thyroid control is mediated by the rostral lobe of the pituitary, and shows, beyond reasonable doubt, that the ventral lobe is not involved.

There appears to be no significant reduction in thyroid cell height after removal of the rostral and neuro-intermediate lobes of the pituitary. There appears to be no significant reduction in thy: :roid weight after removal of the same pituitary lobes; presumably the weight is maintained, after onset of histological degeneration, by the connective tissue which invades the gland.

Studies with radioactive iodine do not show any significant difference (up to thirty seven weeks after operation) between the thy: :roids of controls and those of hypophysectomised fish, but such stud: :ies suggest a decline in thyroid function after rostral lobectomy.

The significance of these results is discussed. There is clear evidence for the presence of a thyrotrophic hormone, and it is tentatively concluded that the rostral lobe of the pituitary is res: :ponsible for production of thyrotrophin.

(B) THE TESTES.

Review of the Literature.

Chieffi (1949, 1950) has described the early development of the gonad in Torpedo and Scyliorhinus. He distinguished a medulla and a cortex in the gonad of both sexes. In the Torpedo embryo the germinal cells, when first recognizable, are found in the splanchno: :pleur, the mesentery, and the fold formed by the latter and the somat: :opleur. Later they migrate into the genital ridge and take up posit: :ion in the cortex of the gonad. In the female the germ cells remain in the cortex, but in the male they migrate from the cortex to the medulla.

According to Chieffi, in older embryos the medullary tissue is replaced by leucocytes. Presumably those leucocytes form the epigonal tissue.

Policard (1902), working on young Rajidae, described the "stroma" of the testis as composed of lymphocytes and mononuclear leucocytes. The tissue referred to is the epigonal organ of later workers. Poli: :card assigned to the stroma a twofold function: formation of leucocytes and nutrition of the spermatic ampoules.

Moore (1894) described the testis tubules of Scyliorhinus (Scyllium) as composed of "foot and semen cells". In young tubules the foot cells are aggregated around the lumina and the semen cells are located outside the foot cells. The semen cells divide and

arrange themselves in layers. When the semen cells are three or four layers deep the foot cells migrate through them "with a singular amoeboid movement" and take up position against the bounding membrane of the tubule. In the following year Moore (1895) described at some length the maturation divisions in the testes of several elasmo: :branch species.

Stephan (1902a) held that the foot cells give rise to germinal cells, and questioned whether the migration described by Moore does in fact occur. He expressed the view that the definitive Sertoli cells are located around the periphery of the tubule from the time the latter is formed.

The same worker (Stephan, 1902b) studied the fate of the Sertoli cells after discharge of the spermatozoa. The Sertoli nuclei divide amitotically and the cells proliferate to refill the evacuated tubules. Somewhat later the tubules break down and the liberated cells contri: :bute to the adjacent tissue. From the description given this adjac: :ent tissue is clearly the epigonal tissue. Stephan's description was interpreted by Mathews (1950) as "implying that part at least of the epigonal organ" is derived from Sertoli cells, an implication with which Mathews strongly disagreed.

Maximow (1923) reported that the epigonal tissue of S. canicula produces leucocytes.

Battaglia (1925) studied the interstitial cells of the testis

in four species of elasmobranch, including S. canicula. From their first appearance, in the zone of newly formed tubules on one side of the testis, they have the appearance of connective tissue cells, except for the fact that this cytoplasm contains some lipoid granules. Passing across the testis the tubules become progressively more mature; the interstitial cells become larger, their lipoid content increases, and they acquire a rounded, epithelial aspect. Battaglia suggested that the interstitial cells of elasmobranchs are analogous to those of mammals.

Matthews (1950), in a treatise on reproduction in the basking shark, described the morphology and histology of the testis. Each testis is surrounded by "a mass of lymphoid tissue and erythrocytes" which constitutes the epigonal organ. He reviewed the literature on the epigonal organ. Mathews found that histologically the epigonal tissue of Raia is similar to that of the basking shark, and in both species "there can be no doubt" that it produces erythrocytes as well as leucocytes.

Matthews also described and figured migration of the Sertoli cells. When first distinguished they form an unmistakable layer of cells surrounding the lumen of the young tubule. He referred to them at this stage as spermatogonia on the grounds that they appear to contribute, by division, to the number of germinal cells. However, Matthews seems to be incorrect in this; Fratini (1953) has described the cells in question as derived from connective tissue elements, and it has not

been confirmed that they contribute to the germinal elements. After the germinal tissue has increased to four or five layers of cells migration of the "inner cells" takes place; the latter pass outward between the germinal cells and take up position around the periphery of the tubule to become the definitive Sertoli cells.

Mathews found that shortly after the spermatozoa are discharged from the ripe tubules the latter shrink in size and the Sertoli cells begin to degenerate.

Fratini (1953) described spermatogenesis in the testis of S. canicula. Germ cells and connective tissue cells are located against the ventral border of the testis. They migrate toward the dorsal side of the testis and multiply to form seminiferous tubules. Maturation of the germ cells takes place while the tubules are being shifted dorsally, and the divisions are synchronous for all the germinal cells in a given tubule. Finally, the mature spermatozoa are liberated into collecting tubules on the dorsal side of the testis.

Fratini confirmed that migration of the Sertoli cells takes place. Before the tubules are differentiated the germinal cells are irregularly mixed with Sertoli cells, the latter being derived from connective tissue elements. At a later stage the Sertoli cells take up a central position in the tubule, bordering on the lumen. Finally they migrate towards the periphery of the tubule; migration occurs when the germinal cells enter upon the leptotene stage of the first meiotic prophase.

Ford (1920) contributed some information on sexual development

and breeding habits in five species of dogfish. He reported that sexual maturity is usually attained by S. canicula at a length of 57 - 60 cm. It is not clear what criterion of sexual maturity Ford used in the case of the male.

The effect of hypophysectomy on the elasmobranch testis has been investigated by Vivien. In the first of two papers (Vivien, 1940) he reported that hypophysectomy in S. canicula is followed by a progressive decline in the testis weight/body weight ratio.

The following year (Vivien, 1941) he published a discursive paper on the effects of hypophysectomy in Scyliorhinus and Gobius (Teleostei). This paper has already been reviewed and only those findings related to the testis will be mentioned here.

Vivien reported a sexual cycle in Scyliorhinus, with a reproductive phase from February to July. During the resting phase of the cycle he was unable to detect a reduction in testis volume, but reported a change in macroscopic appearance.

Prior to the onset of puberty hypophysectomy appears to have no effect on the testis. If carried out at the beginning of puberty the testis ceases development. If performed on the adult the result is atrophy of the testis, with cessation of spermatogenesis. These findings are stated in the form of conclusions; Vivien gave no detailed results. It is difficult to see how he could have arrived at such conclusions since, on his own admission, both his records and his histological material had been destroyed (see p. 36).

Morphology and Histology.

The testes of Scyliorhinus are paired structures; they lie in the abdominal cavity against the dorsal body wall, to which they are attached by the mesorchia. They are oval in cross-section and elongated, stretching from a point near the oesophagus to the rectal gland near the cloaca. However, not all of this structure consists of germinal tissue. The germinal tissue, or testis proper, occupies approximately the anterior two thirds of this elongated structure; the posterior one third consists of lymphomyeloid tissue which constitutes the "epigonal organ".

The epigonal tissue is not confined to the posterior third of the testis (for convenience the whole structure will be referred to as "testis"); it continues anteriorly as a thin layer lying along the dorso-lateral border of the germinal tissue. This layer of epigonal tissue becomes progressively thinner towards the anterior end of the testis.

The close association of germinal tissue and epigonal tissue in elasmobranchs has been noted by many workers, and the epigonal organ has been discussed at some length by Matthews (1950). It is now generally held to be a blood-forming organ, not directly concerned with the germinal portion of the testis. However, it is quite impossible to separate the two tissues, and all testis weights in this thesis include the weight of the epigonal organ.

Histologically the testis shows a convenient separation of the various stages of spermatogenesis. A cross-section at any point along the anterior two thirds shows the same picture; the following is a description of a typical section:-

Along the mesial-ventral border runs the "germinal line", consisting of primordial germ cells (pl. 5, fig. 20). The germinal line has also been referred to as the tubulogenic zone (Fratini, 1953). The cells of the germinal line multiply and give rise to the semini: :ferous tubules and their contained germ cells; as they multiply they migrate dorso-laterally from the germinal line. The result is a zone of young tubules, arranged in a semi-circle outside the cells of the germinal line. These young tubules contain spermatogonia (pl. 5, fig. 21).

Shortly after the tubules are formed they acquire a lumen. The lumen is surrounded by a single layer of cells of characteristic appearance: their nuclei are elongated in a direction radial to the centre of the tubule; cytoplasm is scanty and the cells are closely packed. Outside this layer of cells is located the germinal tissue.

In contrast to the cells just described, the germinal cells have rounded nuclei and plentiful cytoplasm. In newly-formed tubules there is a single layer of germinal cells, but they soon increase in number to form a layer several cells thick. External to the germinal tissue there is the bounding membrane of the tubule.

It is now agreed that the radial cells surrounding the tubular

lumen eventually migrate to the periphery of the tubule to become definitive Sertoli cells; however, that interpretation was at one time a matter of dispute. Migration of the "foot cells" was first described by Moore (1894), but Stephan (1902a) questioned whether it did in fact occur; Stephan held the view that the cells surrounding the tubular lumen gave rise to germinal cells, and had no connection with the Sertoli cells. Subsequent work has confirmed Moore's interpretation (Matthews, 1950; Fratini, 1953); Matthews has published photomicrographs which show, beyond reasonable doubt, that migration does take place.

From the point of view of this work, the main interest of these primordial Sertoli cells lies in the fact that they are diagnostic of the spermatogonia region. By the time the germinal layer is four to five cells deep they can no longer be seen around the tubular lumen. According to Fratini (1953) they migrate to the periphery of the tubules at the beginning of leptotene.

The region of spermatogonia passes distally* into the region of primary spermatocytes. The latter is characterised by germinal cells with large nuclei and abundant cytoplasm (pl. 5, fig. 22). The Sertoli cells are now arranged around the periphery of the tubules; they can be readily distinguished since their nuclei are light-staining

* The proximal tubules are those tubules lying nearer the germinal line; the distal tubules are those lying nearer the dorso-lateral border of the testis (see pl. 5 fig. 20). The terms "proximally" and "distally" will always be used in that sense.

and are somewhat flattened parallel to the tubular membrane; in contrast the spermatocytes have regularly-rounded nuclei which stain heavily with haematoxylin. Many of the tubules in this region have no lumen; the lumina have doubtless been occluded by proliferation of the germinal cells.

Distal to the primary spermatocytes lies the region of secondary spermatocytes. The latter is characterised by germinal cells with relatively small nuclei, the nuclear diameter being about half that of the primary spermatocytes (fig. 22). Although reduction in size of the nucleus is the most prominent feature distinguishing this region from the last, it should be pointed out that the cell size is correspondingly reduced. However, the cell boundaries are not easily seen, and nuclear size affords the quickest and most reliable method of identification.

The region of secondary spermatocytes passes distally into the spermatid region (pl. 6, fig. 23). The nuclei here are slightly smaller and stain more intensely than those of the secondary spermatocytes. In some tubules they are seen to have a radial arrangement with respect to the centre of the tubule, suggesting that the spermatids have taken up definite positions relative to the Sertoli cells. Apart from their nuclei the Sertoli cells cannot be distinguished.

The spermatid region passes into the region of spermatozoa. The transition zone between the two regions contains a number of

tubules in which the spermatid nuclei are undergoing transformation into sperm heads (pl. 6, fig. 24). The spermatozoa are oriented with their heads toward the periphery of the tubules; the tails occupy the lumina of the tubules. They are grouped in clusters, and each cluster is associated with a Sertoli cell (pl. 6, fig. 25). In the proximal tubules each cluster is composed of a loose aggregation of spermatozoa, but in the distal tubules the clusters consist of closely-compacted spermatozoa, and individual spermatozoa can no longer be distinguished.

In this region the tubules are seen to contain a number of hyaline bodies lying within the Sertoli cells on a level with the middle pieces of the spermatozoa. These bodies stain with eosin and are slightly smaller than the Sertoli cell nuclei. They have been described by a number of workers (Matthews, 1950), but their nature and purpose are not known.

The outermost tubules of this region have discharged their spermatozoa and are in process of involution. According to Stephan (1902b) the Sertoli cells proliferate at this stage, with amitotic division of their nuclei. They fill the evacuated tubules and proceed to ingest the debris left in the tubules. The tubules then break down and liberate the Sertoli cells which mix with the adjacent cells of the epigonal tissue.

Stephan did not regard the epigonal organ as a separate entity; he considered it to be part of the testis. His description implies that the epigonal tissue is built up, in part at least, of Sertoli

cells released by the disrupted tubules.

Stephan's work was questioned by Matthews (1950). Referring to the "opinions" expressed by Stephan, he clearly disagreed with the course of events described by the latter. Matthews found that after discharge of the spermatozoa the Sertoli cells degenerate, and implied that they do not contribute to the epigonal tissue. This view was shared by Fratini (1953).

Throughout the germinal portion of the testis a number of collector tubules can be found. Their diameter is small, and they lie coiled between the spermatogenic tubules. They were identified by Matthews as part of the rete testis.

Interstitial cells. The inter-tubular tissue in Scyliorhinus consists of scanty connective-tissue fibres among which can be found a few small, fusiform cells. The cells have the appearance of connect: :ive-tissue elements.

The question arises whether these cells can be regarded as ana: :logous with the interstitial cells of higher vertebrates. The litera: :ture on the point is conflicting: according to Stephan (1902b) they do not have the appearance ("la constitution") of the interstitial cells found in higher vertebrates. He suggested that the endocrine role of the interstitial cells in higher vertebrates is performed in selachians by the Sertoli cells after the spermatozoa have been discharged from the tubules.

Battaglia (1925) studied the interstitial cells in four elasmo: :branch species. Tracing their development from the germinal line to the spermatozoa region he found them increasing in size and lipoid content. He considered them to be derived from connective tissue, and attributed to them an endocrine function similar to that found in mammals.

Matthews (1950) mentioned the interstitial cells of Heterhinus, and noted that they tended to aggregate in groups, but made no suggestion as to their possible function.

It will be apparent from this review that there is no real evidence that the interstitial cells of elasmobranchs have an endocrine function. The work of Battaglia is suggestive, but the presence of lipoid does not demonstrate an endocrine function. From the sections studied during the course of this work, there is no reason to think that the interstitial cells of Scyliorhinus are anything more than connective tissue elements. They do not appear to be affected by hypophysectomy, but a lipoid study would be necessary before that state: :ment could be made with any confidence. Since they showed no histo: :logical changes in any of the operated fish they will receive no further attention here.

Variability. Vivien (1941) was the only worker to mention a seasonal change in the testis of Scyliorhinus. He recorded a change, in macroscopic appearance, from a uniform, creamy yellow during the

resting period (August - January) to milky white with an acinar structure ("structure acineuse") when filled with spermatozoa.

The testes studied during the course of this work showed no histo: :logical changes which could be interpreted as seasonal. The only changes noted are correlated with size (and presumably with age) of the fish: the larger the animal, the larger the cross-sectional area of the testis and the greater the amount of spermatogenic tissue. However, the relative number of tubules in each region remains fairly constant, regardless of testis size.

Sexual maturity. All the fish used in this work, with one exception, were sexually mature. The criterion adopted for sexual maturity was presence of spermatozoa in the outermost region of the testis. The single exception was P 70 (control, length 54 cm.) the testis of which did not contain spermatids or spermatozoa. According to Ford (1920) sexual maturity is attained by both sexes of Scyliorhinus at a length of 57 - 60 cm. P 70 was below this critical size, and the absence of spermatids and spermatozoa was attributed to sexual immaturity.

Two other fish were below the critical size given by Ford; these were D 60 (54 cm.) and D 80 (52 cm.). However, the testes of both contained spermatozoa and they were classed as mature fish.

Since the fish used in this work (with the single exception of P 70) were sexually mature, it follows that the effects to be described apply only to mature fish, i.e. to fish whose testes contain all stages

of spermatogenesis. The present work allows no statement as to the effect of hypophysectomy on the immature testis.

Experimental Studies.

Number of Fish Involved.

The testes of fifty eight fish were sectioned and examined; of these twenty eight were controls. One of the controls (P 70) was found to be immature, and will not be considered further. The remaining thirty fish were total and partial hypophysectomies.

Possible Effect of Inanition.

Nine fish showed varying degrees of resorption of the spermatids and/or spermatozoa. These fish are listed in Table V (p. 123a) which shows the condition of their pituitaries at dissection. Four were controls, with intact pituitaries; three of the remaining five showed all lobes of the pituitary represented (though some lobes were present as remnants).

The testis of D 48 will be described first, since it shows resorption affecting both of the regions in question. Spermatogenesis appears to be normal up to the secondary spermatocyte stage. Some of the proximal tubules of the spermatid region appear normal, but the distal tubules show degenerative phenomena. In the latter the spermatids tend to be

TABLE V.

Regression of Testes: Resorption of Spermatids - Spermatozoa.

Fish	Wt. gain (+) or loss (-) in g.	Pituitary at dissection			Time after operation (weeks)
		Rostral lobe	N.-int. lobe	Ventral lobe	
D 3	- 15	-	-	*	29
D 44(C)	+ 42	present	present	present	35
D 45(C)	- 20	present	present	present	37
D 46	- 210	-	-	present	27
D 48(C)	- 50	present	present	present	38
D 50	- 20	*	*	present	20
D 51	+ 133	*	present	present	37
D 78(C)	- 45	present	present	present	31
P 61	- 148	*	present	present	27

(C) Controls.

* Remnant of lobe remains in situ.

\- Lobe absent.

clumped together in scattered groups. The nuclei are irregular in size; some are very large (about the size of primary spermatocyte nuclei).

Moving towards the spermatozoa, signs of regression become steadily more pronounced (pl. 6, fig. 26). In the transition zone between the two regions, where the spermatids are in process of changing to spermatozoa, many of the spermatids have been resorbed; this is clear from the small numbers seen in some of the tubules. High magnification shows nuclear material as threads and blobs of irregular size and shape.

The next region contains no spermatozoa. All the tubules show nuclear debris; most contain large hyaline, eosinophil inclusions which appear to be related to the hyaline inclusions found in the Sertoli cells of normal tubules. The background material in these tubules is provided by the Sertoli cells, the cytoplasm of which appears to form a syncitium. Resorption of the germinal elements has apparently been carried out by the Sertoli cells.

In most of the testes comprising this group (Table V) resorption has not progressed as far as the spermatids; it is more or less confined to the spermatozoa. D 73 is typical: many of the distal spermatozoa tubules of D 73 appear normal, but the proximal tubules show resorption phenomena similar to those described above.

In the affected tubules of D 73 the spermatozoa show a tangled

arrangement which is not characteristic of normal spermatozoa; scattered here and there are threadlike fragments of nuclear material which suggest break-up of the spermatozoa. Many of the tubules con: :tain clumps of spermatid nuclei which have not effected the change to spermatozoa. These nuclei lack the regularly-rounded shape of normal spermatids. They lie within a vacuolated cytoplasm which has the appearance of a syncitium; the latter might well have been formed from the cytoplasm of the Sertoli cells, but it is impossible to be certain.

There are two testes in this group which merit special attention, namely D 3 and D 45. In both of these resorption has affected the spermatids, and particularly those tubules showing transition stages between spermatids and spermatozoa.

In the case of D 3 all stages up to secondary spermatocytes are present, and so also are spermatozoa, but spermatids are absent. Pl. 7, fig. 27 shows a cross-section of the testis coloured with Sudan Black. The region of degeneration is seen to be strongly sudanophil, indicating the presence of lipoids. It stretches in a clearly-defined band across the testis. Pl. 7, fig. 29 shows part of the affected region at higher magnification; for comparison pl. 7, fig. 28 shows a similar region from a control testis (D 4) in which these resorption phenomena do not appear.

The tubules in the affected region of D 3 are shrunken and

filled with a syncitial, vacuolated cytoplasm. They show clumps of nuclear material, among which it is possible to recognise occasional spermatids and transition stages between spermatids and spermatozoa (pl. 7, fig. 30).

Testis D 45 (control, pl. 8, fig. 31) is similar to D 3, but resorption has affected the transition stages between spermatids and spermatozoa rather than the spermatids sensu stricto. A few proximal spermatozoa tubules also show signs of resorption.

Comment. Testis D 3 will be commented on at some length in a later section. The lipoidal degeneration seen in D 3 has affected a well defined portion of the germinal epithelium. This type of effect is characteristic of hormonal deficiency, and there is some suggestion that the condition in D 3 might be a long-term effect of ventral lobectomy; however, the effect in question cannot be attributed to hormone deficiency for the following reasons:-

(a) A similar, though less clearly defined, effect has appeared in a control, D 45.

(b) A second control, D 48, shows resorption of both spermatids and spermatozoa. In the remaining fish of the group resorption phe: :nomena are more or less confined to the spermatozoa.

Clearly these testes can be interpreted as a series in which resorption may affect either the spermatids or the spermatozoa or both.

There is no case for attributing resorption to pituitary deficiency, since the effects in question appear in controls as well as in hypophysectomised fish.

Attempts to explain these resorption phenomena have not been fully convincing. The factors likely to be responsible will be dis: :cussed under the following heads:-

(1) Seasonal change. The literature bearing on this point has already been mentioned. Vivien (1941) reported changes in the macroscopic appearance of the testes of Scyliorhinus which he corre: :lated with reproduction. Vivien's report has not been confirmed at this laboratory; the testes of Scyliorhinus have a fairly uniform appearance at all seasons of the year.

According to Vivien the reproductive cycle of Scyliorhinus shows a resting period from August to January. This is, presumably, the period during which such effects as those described above could be expected to appear. However, most of the fish listed in Table V were dissected in February and March; the remaining fish of the group (Table V) were dissected in July, October and December. Moreover, if the phenomena described are seasonal effects they could be expected to appear in the testes of a number of other fish dissected at the same time as those in Table V. That they do not appear in the other fish can be taken as further evidence against this hypothesis.

(2) Inanition. This seems the most likely explanation of the observed effects. Table V shows the weight gain (+) or loss (-) of each of the fish listed. Most show a loss of weight over the period of the experiment.

However, many of the other fish used in this work also suffered a weight loss, and showed no signs of the resorption phenomena described. It may also be noted that two of the fish in Table V showed a gain in weight.

According to Selye (1949) inadequate diet may cause involution of the testis in higher vertebrates. A number of workers have reported on the effect of inanition in Amphibia, and the literature on this work has been summarised by Van Oordt (1956); starvation has been found to inhibit spermatogenesis in some species but not in others.

Inanition seems to provide the most plausible explanation of the effects described above, since there is some question as to whether the diet used was adequate. It is not clear why inanition effects should appear in these few fish and not in others; all the fish used in this work were given the same diet and the same amount of food (36 g. herring per week). Nor is it clear why in some cases there should be resorption of spermatozoa and in other cases resorption of spermatids. However, no other hypothesis seems to offer a more adequate explanation of the observed effects.

(3) Other factors. The literature on the various "stimuli" capable of causing involution of the testis has been discussed by

Selye (1949). His conclusions apply to higher vertebrate, but the stimuli in question may be mentioned here. They include disease, age, temperature, light, stress and others. None of these can be demonstrated to apply in the present case. The fish used in this work were kept under identical environmental conditions, and most appeared normal and in good health at dissection; only two were dissected post-mortem (D 46 and D 48) and their testes showed no signs of histolysis. Age must be regarded as an uncontrollable variable; there is no reason to think that the fish in Table V were older than most of the others used in this work.

It may be concluded that the cause of the degenerative changes described above is not clear. In the absence of a more convincing explanation the changes in question may be tentatively ascribed to inanition.

Testis D 3. As mentioned above, the degeneration found in the spermatid region of D 3 is limited to a particularly well defined portion of the germinal epithelium. There are certain points relating to this fish which deserve attention, and further comment seems advisable.

In D 3 the ventral lobe of the pituitary was found to have been severely damaged (see p. 137) whereas the ventral lobes of the other fish in the group were intact at dissection (Table V). It will be shown later that certain degenerative changes affecting the early stages of spermatogenesis are correlated with ventral lobectomy (D 3 is one of the fish which showed such changes) and it seems possible

that the spermatid resorption found in D 3 was also an effect of ventral lobectomy. Furthermore, D 3 was dissected twenty nine weeks after operation; this represents a longer "survival period" than that recorded for any other fish after ventral lobectomy. It follows that the spermatid resorption described above could well be a long-term effect of ventral lobectomy.

However, on the evidence available there appears to be no alter: :native but to regard D 3 as one of the series treated above. While doing so it might be advisable to make some reservation in case future work should show the spermatid resorption found in this testis to be a real effect of hypophysectomy.

Effect of Hypophysectomy.

Nine of the thirty hypophysectomised fish showed degenerative changes which were confined to the early stages of spermatogenesis (spermatogonia and primary spermatocytes). For reasons which will appear later, this is considered to be a real effect of hypophysectomy.

The fish in question are listed in Table VI (p. 135a), the first column of which is an attempt to assess the extent of degenera: :tion in each testis.

In every case there is a more or less well defined band of degenerating tubules stretching across the testis (pl. 8, fig. 32). This band usually lies between the spermatogonia and primary spermato:

:cytes (pl. 8, fig. 33), but in one case (P 60, see below) the zone of degeneration intrudes among the primary spermatocytes.

In the testes in question the proximal tubules of the sperma: :togonia region appear normal. Passing distally, signs of degenera: :tion usually appear in those tubules with four or more layers of spermatogonia; sometimes they can be detected in tubules with two to three layers of spermatogonia. In these tubules the spermatogonial nuclei stain intensely; they lose their regularly-rounded shape and show signs of disintegration. Adjacent nuclei may be clumped together to form isolated groups of four or more (pl. 9, fig. 34).

Within a very short distance of the point at which these changes appear the tubules show a marked reduction in the number of germinal nuclei. Pl. 9, fig. 34 shows a tubule bordering the zone of degenera: :tion in which the number of spermatogonial nuclei is estimated at less than half the normal complement.

In contrast to the germinal nuclei, the Sertoli nuclei do not appear to be affected. Soon after the above changes appear the pri: :mordial Sertoli cells disperse from their positions around the lumen of the tubule (pl. 9, fig. 34). However, they do not take up position around the periphery of the tubule, as is normally the case; instead they lie scattered among the degenerating spermatogonia.

From this description it would appear that degeneration of the spermatogonia is synchronous with migration of the primordial Sertoli

cells. However, there is some reason for thinking that the dispersal of the primordial Sertoli cells described above does not correspond to the migration seen in normal tubules. That view is suggested by two observations: first, the Sertoli cells apparently do not reach their definitive positions against the tubular membrane; secondly, their dispersal occurs at an earlier stage than migration. Pl. 9, fig. 34 shows that dispersal has occurred in a tubule containing two to three layers of spermatogonia; migration occurs at a much later stage, when the tubules contain four or five layers of spermatogonia.

Later stages of the degenerative process are seen in pl. 9, fig. 35. There is progressive reduction in the number of germinal nuclei, usually accompanied by shrinkage of the tubules. Fragments of dark-staining nuclear material are scattered throughout a cyto: :plasmic matrix. The latter has the general appearance of a syncitium, but a few cell boundaries can be detected. The Sertoli nuclei remain normal in appearance, but their numbers become reduced as the degenera: :tive process nears completion.

Distally the zone of degeneration gives way to intact tubules containing primary spermatocytes. The transition from degenerating to intact tubules is often sudden (pl. 9, fig. 35) but in some areas it is gradual, i.e. the zone of degeneration has begun to intrude on the primary spermatocytes. In such areas the proximal spermatocyte tubules show early degenerative changes similar to those described

above (pl. 9, fig. 36). Clumping of the spermatocyte nuclei, in groups of four or more, is a particularly noticeable feature.

In one testis (P 60) there is evidence that the zone of degeneration may not always be confined to the transition region between spermatogonia and primary spermatocytes. In the area shown in pl. 10, fig. 37 the zone of degeneration lies between primary and secondary spermatocytes.

In P 60 the affected tubules are shrunken and most of their germinal elements have been resorbed. They contain a few scattered nuclei which are easily recognizable as those of primary spermatocytes. These lie within a cytoplasmic matrix which fills each tubule. A few Sertoli nuclei can be seen about the periphery of the tubules. There is a sharp transition between the zone of degeneration and the secondary spermatocytes; the latter show no sign of degenerative changes.

The affected tubules in P 60 differ, in some respects, from those described above (compare figs. 34, 35 and 37). Clumping of the nuclei is rare in P 60, and nuclear debris is seldom seen. However, other testes show that one type of picture can merge into the other, and there is no question of two distinct resorptive processes being involved.

The testes of two fish (P 42 and P 71) contained no primary spermatocytes. The zone of degeneration is bounded proximally by

spermatogonia, in which degenerative changes can be detected, and is succeeded distally by secondary spermatocytes (pl. 10, fig. 38). There is no sign, in the testes of either fish, of degenerative changes among the secondary spermatocytes.

It is not difficult to suggest an explanation for the absence of primary spermatocytes from the testes of these two fish. Gradual encroachment of the zone of degeneration could have destroyed most (perhaps all) of the primary spermatocytes; presumably the contents of any tubules not affected by degeneration would eventually have made the change to secondary spermatocytes, and the condition existing in these testes would have resulted.

To pursue the point: had these fish been allowed to live the secondary spermatocytes would presumably have given rise to spermatids. In due course the spermatids would have given rise to spermatozoa, which would eventually have been discharged. The end point would have been a testis containing no germinal tissue except possibly early spermatogonia (there is no evidence that early spermatogonia are affected by the degenerative changes described). It follows that in theory the degenerative changes described, though limited in extent, could ultimately have resulted in complete aspermia.

Traces of Degeneration.

Three fish showed traces of the condition described above.

They are listed in Table VII A (p. 135b). One of these (P 66) was a control. The testes of all three showed a few tubules, in the spermatogonia-primary spermatocyte region, in which signs of degenera: :tion could be detected. Pl. 10, fig. 39 shows a group of such tubules from P 66; clumping of the nuclei is apparent, and fragments of dark-staining nuclear material can be detected in places.

Signs of degeneration were not considered to be sufficiently marked in these three fish to warrant their inclusion in Table VI. The fact that one was a control is significant: it suggests that the transition from spermatogonia to primary spermatocytes should be regarded as otirical to the process of spermatogenesis.

Comment. The degenerative changes described are confined to the spermatogonial and primary spermatocyte regions of the testis. There is no evidence that other regions of the testis are affected. There is no reason to think that degeneration was a transient phenomenon; the absence of primary spermatocytes from P 42 and P 71 indicates that degeneration had been in progress for some time in those testes.

Tables VI and VII show that the changes described are correlated with hypophysectomy. They occur in hypophysectomised fish (Table VI), but, with one exception (P66, Table VII A), they are absent from controls (Table VII B).

Inspection of Table VI allows a more definite statement on the

TABLE VI.

Degenerative changes affecting spermatogonia - primary spermatocytes.

Fish	Extent of degeneration	Time after operation (weeks)	Pituitary at dissection		
			Rostral l.	N-int.l.	Ventral l.
D 3	x	29	-	-	*
P 7	x	14	-	-	-
P 48	x	13	present	-	-
P 60	x	24	*	present	*
P 37	xx	15	-	*	-
P 36	xxx	8	present	present	-
P 80	xxx	5	-	-	*
P 42	xxxx	24	present	present	-
P 71	xxxx	23	-	present	-

* Remnant of lobe remains in situ.

\- Lobe absent.

TABLE VII.

A. Traces of degeneration in spermatogonia - primary spermatocyte zone.

Fish	Time after operation (weeks)	Pituitary at dissection. Rostral lobe	N.-int. lobe	Ventral lobe.
D 55	6	-	ŧ	present
P 56	9	ŧ	-	present
P 66(C)	6	present	present	present

B. Spermatogonia and primary spermatocyte regions of testis normal.

4	5 - 55	-	-	present
1	26	-	present	present
3	5 - 35	-	ŧ	present
1	27	ŧ	-	present
3	4 - 25	ŧ	ŧ	present
7	24 - 37	ŧ	present	present
26(C)	3 - 50	present	present	present

(C) Controls.

ŧ Remnant of lobe remains in situ.

\- Lobe absent.

point. In six of the nine fish listed the ventral lobe of the pituitary was found to be absent; in each of the other three cases the ventral lobe was represented by a remnant. It can therefore be stated that the degenerative changes described are correlated with absence of all or most of the ventral lobe.

On the other hand the degenerative changes in question cannot be related to absence of the rostral lobe or to absence of the neuro-intermediate lobe of the pituitary. Table VI shows that the rostral lobe was intact in three of the fish concerned and a remnant was present in a fourth; the neuro-intermediate lobe was intact in four fish and a remnant was present in a fifth. It follows that the de: :generative changes described in the testes cannot be attributed to absence of these lobes.

As stated above, a remnant of the ventral lobe was found in three of the fish listed in Table VI (D 3, P 60 and P 80). The term "remnant" is open to different interpretations, and, since it assumes some importance in the present context, there is a case for stating more precisely what the term implies.

When applied to the rostral or the neuro-intermediate lobe the term "remnant" can be defined with a fair measure of precision. It can be taken to mean a portion whose size does not exceed 10% that of the average lobe.

The size is estimated by examination of serial sections. Though simple in principle, this method was not always easily applied;

and, since it was clearly incapable of measuring functional capacity, no attempt was made to reduce the estimate to a precise figure for each gland. However, it could reasonably be expected that functional capacity would be roughly proportional to the amount of tissue present, and it was felt that an estimate of the kind given above was in order.

With regard to the ventral lobe the position is not so straight:forward. The problem encountered was inherent in the surgical procedure used. The ventral lobe was not dissected; instead, an attempt was made to destroy it by cautery and seeker. Obviously, this procedure could result in extensive damage while leaving much of the tissue in situ. This did in fact occur in two of the fish listed in Table VI (D 3 and D 60).

D.3 is a case in point. Pl. 11, fig. 41 shows a parasagittal section through the ventral lobe. For comparison pl. 11, fig. 40 shows a similar section from a control (D 4). The control section shows a ventral lobe with a large lumen and numerous peripheral divert:iculae. The epithelium is high columnar, and the lumen contains an eosinophil colloid. There are a number of small blood vessels between the peripheral diverticulae.

In contrast, the ventral lobe of D 3 shows only traces of a lumen (fig. 41); the epithelial cells are pyknotic, and there are few blood vessels. The general appearance suggests that this lobe had suffered considerable damage.

It is questionable whether any purpose could be served by attempting to assess the size of the remnant in D 3. As far as the lumen is concerned, destruction is almost complete. Much of the epithelium is still present, but its condition is such as to suggest that the size of the remnant would be a most inadequate index of functional capacity.

The ventral lobe of P 60 is similar to that of D 3, and the problem presented is the same. The ventral lobe of P 80 shows more extensive damage than the other two; since testis degeneration in P 80 is quite marked, it seems advisable to describe the ventral lobe.

Over much of its width the ventral lobe of P 80 has been com: :pletely destroyed (pl. 11, fig. 42). Laterally there is a remnant which appears to have escaped damage. Pl. 11, fig. 43 shows a section through the remnant. The lumen is patent and contains colloid; the epithelium is high columnar and there are a number of blood vessels in the vicinity. Although small, its size is that typical of an extreme lateral section.

It will be apparent from the foregoing that the ventral lobes were severly damaged in these three fish. In the six other fish listed in Table VI the ventral lobes were completely destroyed.

Ventral lobectomy was attempted in eighteen other fish without success. In these the ventral lobe either showed no sign of damage or showed localised traces of damage, the latter so slight that the

lobes in question were considered to be intact. These eighteen fish appear among others listed in Table VII; their ventral lobes are classified as "present".

It has been pointed out that testis degeneration is correlated with hypophysectomy. The evidence presented in Table VI has shown a decided correlation between the degenerative changes in question and absence of, or severe damage to, the ventral lobe of the pituitary. The "negative" evidence in support of that thesis is presented in Table VII. Though less striking than the positive evidence already dis: :cussed, it is no less significant.

First, there is a case for excluding from the discussion the three fish listed in Table VII A. The degenerative changes in the testes are not sufficiently marked to warrant inclusion of these fish in Table VI; on the other hand the testes could hardly be classed as "normal". Perhaps the signs of degeneration should be regarded as defining a critical stage in the process of spermatogenesis, i.e. a stage at which spermatogenesis could be expected to break down under adverse physiological conditions. Table VII A shows that the condition of these testes cannot be attributed to hypophysectomy: one fish was a control, with an intact pituitary; of the other two, one showed a remnant of the rostral lobe (P 56) and the other showed a remnant of the neuro-intermediate (D 55). The ventral lobes were present in all three.

Passing on to section B (Table VII), the evidence presented shows: (a) that presence of the ventral lobe is correlated with a histologically normal testis; (b) that ablation of the rostral lobe and/or the neuro-intermediate lobe does not affect the testis.

Fish D 1 may be cited as a case in point. It was dissected fifty five weeks after operation. Serial sections of the pituitary region confirmed the absence of the rostral and neuro-intermediate lobes, but showed the ventral lobe to be intact (pl. 12, fig. 44). It may be noted in passing that this was one of the fish in which an attempt had been made to destroy the ventral lobe by seeker. The sections showed no evidence of damage to the ventral lobe.

The testes of D 1 appeared normal, with all stages of sperm: :atogenesis represented. There was no sign of degenerative changes in the spermatogonia-primary spermatocyte regions (pl. 12, fig. 45), or in any other part of the testis.

Table VI raises one further point. It might have been expected that the extent of degeneration in each testis would depend on time after operation; i.e. that degeneration would be slight in fish dis: :sected shortly after operation, and would become more marked as "time after operation" increased. However, Table VI shows no corre: :lation between the two parameters.

The technique of ventral lobectomy provides a possible explana: :tion. There is no means of telling the extent of the damage inflicted

on the ventral lobe at the time of operation. Even when a ventral lobe is absent at dissection there is no guarantee that destruction was entire at operation. In such circumstances it is clearly im: :possible to say at what point in time the ventral lobe becomes in: :capable of supporting spermatogenesis. This statement, of course, assumes the validity of the conclusion that gonad function is under control of the ventral lobe.

Testis Weights.

Text fig. 6 (p.142) is a graphical presentation of the testis weights of those fish listed in Tables VI and VII. The weights are related to body weight and plotted against "time after operation". The graph makes no allowance for pituitary remnants found in certain fish at dissection; to allow for such remnants would have unduly complicated the picture.

The plot shows a wide scatter which makes interpretation diffi: :cult but also serves as a measure of dispersion. There is perhaps a slight decline in testis weight, in controls as well as hypophy: :sectomies, with time after operation. There is no evidence that hypophysectomy causes a decline in testis weight/body weight ratio. However, the number of fish in each operational category is small, and the possibility that a slight decline occurs is not excluded by the evidence presented here.

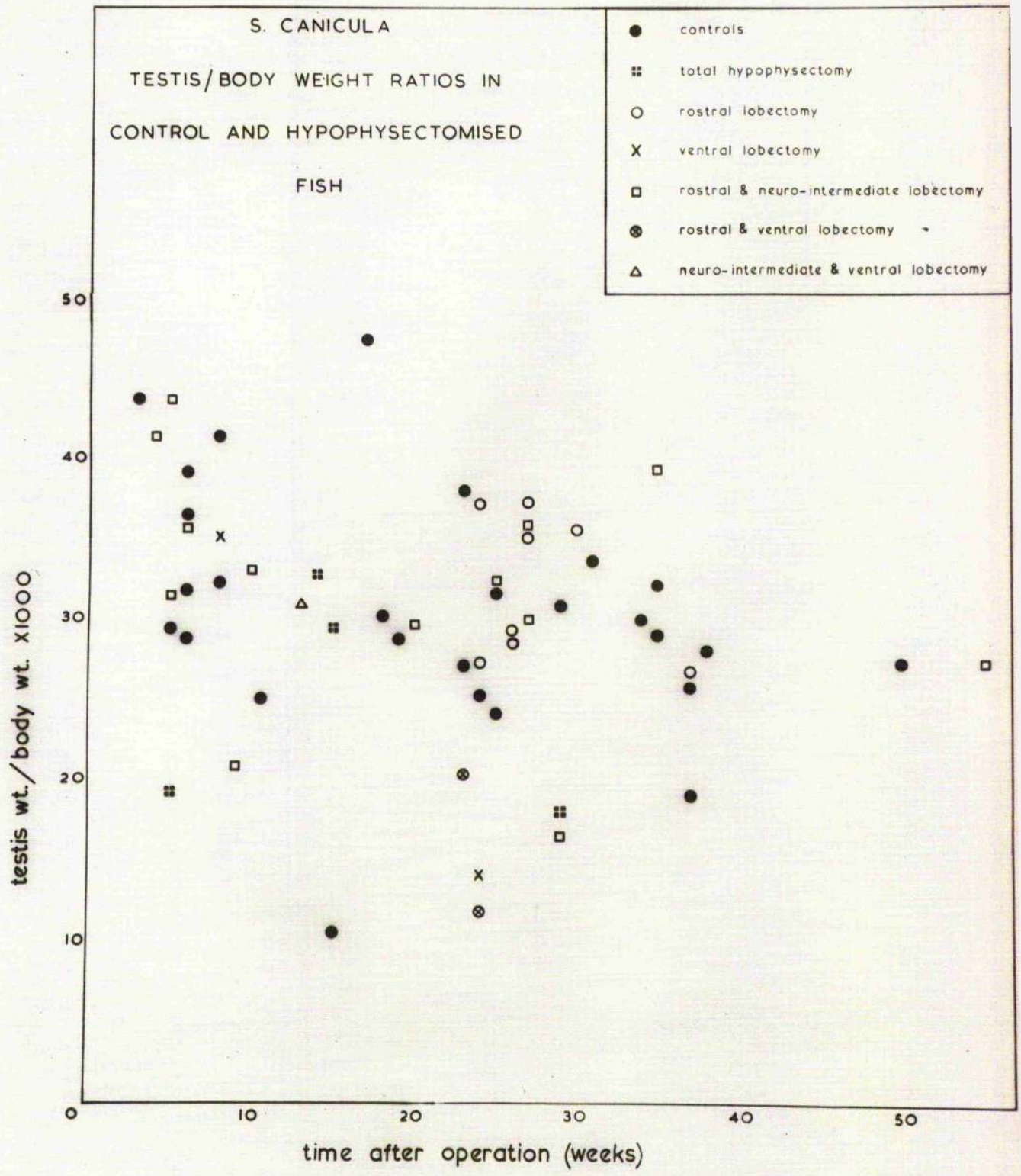

S. CANICULA
TESTIS/BODY WEIGHT RATIOS IN
CONTROL AND HYPOPHYSECTOMISED
FISH
controls
total hypophysectomy
rostral lobectomy
ventral lobectomy
rostral & neuro-intermediate lobectomy
rostral & ventral lobectomy
neuro-intermediate & ventral lobectomy
testis wt./body wt. X1000
time after operation (weeks)
0
10
20
30
40
50

Fig. 6

It can be said that the figures on which this graph is based do not corroborate the figures published by Vivien (1940). According to Vivien the testis/body weight ratio falls steadily after hypophysectory, reaching a value of .006 (i.e. 6.0 on the scale used here) some thirty six weeks after operation. When plotted Vivien's figures fall on a diagrammatic curve. However, he did mention that certain fish were left out of his statistical analysis ("ceux présentant un état physiologique déficient on été écartes des statistiques")!

Discussion.

The evidence presented here constitutes a strong case for the hypothesis that spermatogenesis in Scyliorhinus is controlled by the ventral lobe of the pituitary. Such control is presumably exercised, as in higher vertebrates, by means of one or more gonadotrophic hormones, and some consideration of the pituitary gonadotrophins is in order.

The work on higher vertebrates, particularly mammals, has established the presence of three pituitary gonadotrophins: the follicle stimulating, luteinising and luteotrophic hormones (FSH, LH and LTH). No physiological function has been ascribed to LTH in the male, although the hormone occurs in the male pituitary (Creep and Chester Jones, 1950).

Although a great deal of work has been done on FSH and LH in higher vertebrates it is still not clear what part each plays in controlling testis function (see reviews by Turner, 1948; Creep and

Chester Jones, 1950, Evans and Simpson, 1950; Nelson, 1952). Early work led to the view that FSH controls spermatogenesis, whereas LH controls the interstitial tissue. Later it was shown that low dosages of LH were capable of maintaining the spermatogenic epithe: :lium, and that under certain circumstances androgen is also capable of maintaining spermatogenesis. Such findings have led to the ex: :treme view that spermatogenesis is controlled by androgen, and the pituitary gonadotrophins merely serve to maintain an adequate supply of androgen (Burrows, 1949).

It is now generally held that FSH and LH act synergistically. Spermatogenesis is controlled primarily by FSH while LH controls the interstitial cells; the latter is also capable of affecting spermato: :genesis through the medium of androgen produced by the interstitial cells.

Little is known of the gonadotrophins of fish. The literature on the subject has been reviewed by Hoar (1955) and by Pickford and Atz (1957). There seems no reason to doubt that the gonads are under pituitary control, both in teleosts and in elasmobranchs. The pres: :ence of FSH and LH has not been established, but specific versions of both hormones may well be present: the gonadotrophins of higher verte: :brates are protein hormones and there is evidence that they are group-specific and even species-specific (Creaser and Gorbman, 1939; Witschi, 1955; Noble and Plunkett, 1955; Pickford and Atz, 1957).

Hypophysectomy has been found to cause regression of the testis in all vertebrate groups. All workers have reported adverse effects on spermatogenesis. A brief review of the literature will show the changes brought about by hypophysectomy and the time required for those changes to appear.

In the rat testis Smith (1927) found regressive changes fifteen days after operation. Smith (1930) reported decrease in size of the seminiferous tubules and noted that thirty four days after operation only spermatogonia and degenerating spermatocytes are present. Collip, Selye and Thomson (1933) carried out hypophysectomy on a large number of rats; they confirmed that testicular atrophy follows the operation and mentioned "reduction ..... of germinal epithelium" as one of the effects. Selye (1949) was somewhat more specific: he reported that twenty eight days after hypophysectomy only spermatogonia and primary spermatocytes are present in the rat testis.

Perhaps the most detailed account of the changes in the rat testis following hypophysectomy is given by Coombs and Marshall (1936). The seminiferous tubules show reduction in diameter and lipoid degeneration. These changes appear in fourteen days, at which time a reduction in the number of spermatozoa is noticeable. By the fourth week after operation the above changes are pronounced and spermatozoa are no longer present.

The literature on the effects of hypophysectomy in birds is

not extensive. Hill and Parkes (1934) reported testicular atrophy in a number of species; two weeks after operation spermatozoa are absent, and by five weeks only spermatogonia remain. Coombs and Marshall (1956) reported that within seventeen days of operation the testis tubules of cockerels show degenerative changes but they give no details of the effect on spermatogenesis.

Schaefer (1933) has given a sketchy account of testis degenera: :tion in the garter snake; his mention of "degenerating spermatocytes" should perhaps not be taken literally. Wright (1956) has described the testis of Agama at thirty four to thirty nine days after operation; spermatogonia and spermatocytes are abundant at that time, but sperma: :tids are rare and spermatozoa absent.

The effect of hypophysectomy on the amphibian testis has been described by Caullery (1940), Tuchmann-Duplessis (1945), Van Oordt and Van Oordt (1955) and by Van Oordt (1956). The pattern of degeneration is the same; the spermatozoa are first affected, and eventually the germinal tissue is reduced to spermatogonia. The earliest effect was reported by Van Oordt and Van Oordt on Rana temporaria; in this species the germinal elements are reduced to "primary spermatogonia" within four: :teen days if the operation is performed in June.

In teleosts the effects are similar. Matthews (1939) and Pickford (1953) found that in Fundulus only the spermatogonia remain unaffected. Caullery (1940) has reported that in Gobius spermatids

are absent ninety four days after operation, but some spermatozoa are still present. This is the only case in which disappearance of the spermatids is reported to precede that of the spermatozoa. The earliest effect was reported by Matthews on Fundulus; he particularly mentioned two fish, hypophysectomised in October, which showed marked reduction of the spermatids and spermatozoa after ten and thirteen days.

This review is not exhaustive, but it serves to show that hypophysectomy is followed by a progressive reduction of the germinal elements. The oldest elements are first to disappear (except possibly in Gobius); they are followed by progressively younger elements till finally the testis contains only spermatogonia.

The pattern of testicular regression which emerges from this review of the literature suggests that the gonadotrophic hormones exert a generalised effect on the spermatogenic epithelium, i.e. that they exert a trophic influence on all stages of spermatogenesis. However, there is reason to think that such is not the case. Van Oordt and Van Oordt (1955) and Van Oordt (1956) have suggested that in Rana gonadotrophin deficiency affects the spermatogonia, which apparently lose their capacity for mitotic division. Gaarenstroom and de Jongh (1946) put forward the hypothesis that in mammals FSH stimulates production of spermatogonia; Nelson (1952) re-examined that hypothesis in the light of further evidence of a clinical nature and concluded

that in man FSH stimulates production of spermatogonia and formation of primary spermatocytes.

In Scyliorhinus the histological changes attributed to hypophysectomy were confined to the spermatogonia and primary spermatocytes. In the light of Nelson's work it may reasonably be suggested that a hormone of the FSH type is present in elasmobranchs, and that the degenerative changes described in the testis of Scyliorhinus were directly due to a deficiency of that hormone.

It is relevant here to note that the elasmobranch testis has proved particularly suitable for this study in that the effects attributed to hypophysectomy could be localised with a measure of precision which appears to be unique; it has been possible to determine that the effects in question were confined to the spermatogonia and primary spermatocytes. Judging from the literature reviewed above, this advantage has not been enjoyed by other workers, and it is undoubtedly due to the well marked zonation of the dogfish testis.

In the testis of Scyliorhinus the various stages of maturation are set out in a clearly defined sequence; all the tubules composing a given region contain germinal elements at the same stage of maturation. Only in urodele amphibians does the testis show a similar arrangement. In all other vertebrates each testis tubule contains germinal cells in all stages of maturation, an arrangement that does not lend itself to the study of localised changes affecting a particular stage of spermatogenesis. This might account for the fact that other workers have

been unable to demonstrate a localised effect on the germinal epith: :elium after hypophysectomy.

One further point remains to be considered: the literature re: :viewed above has shown that histological changes can be detected in the testes of mammals, birds, amphibians and teleosts some two weeks after hypophysectomy. In Scyliorhinus the changes attributed to hypophysectomy were first seen five weeks after operation (P 80, Table VI). At that time they were already well established, and it seems not unreasonable to suggest that the time of onset in Scyliorhinus is similar to that in other vertebrates, namely about two weeks after hypophysectomy.

The results achieved in this study have led to the conclusion that the ventral lobe of the elasmobranch pituitary produces gonado: :trophin(s). However, the evidence presented is not complete. There is a clear need for further investigation by replacement therapy, using ventral lobe extracts as well as gonadotrophins from other sources. Assays of ventral lobe material for gonadotrophic activity are also indicated. Finally, it is suggested that the method of ventral lobect: :omy outlined on p. 65 should be employed in future work involving that operation, since it shows promise of ensuring complete removal of the ventral lobe; the method suggested has a further advantage in that the remainder of the pituitary is not exposed and suffers no damage from the operation.

Summary.

The testes of certain experimental fish showed resorption of the spermatids and/or spermatozoa. These resorption phenomena were found in controls and in hypophysectomised fish, and were tentatively attributed to inanition.

Hypophysectomy is followed by degenerative histological changes in the testis of Scyliorhinus. These changes affect the spermatogonia and primary spermatocytes and are correlated with absence of, or severe damge to, the ventral lobe of the pituitary. There appears to be no significant change in testis weight after hypophysectomy.

The significance of these findings is discussed. It is con: :cluded that the ventral lobe of the pituitary produces gonadotrophin(s), and the presence of a hormone of the FSH type is suggested.

Phylogenetic Implications.

De Beer (1926) has produced some evidence for the view that the ventral lobe of the elasmobranch pituitary is the homologue of the pars tuberalis. If it is accepted that the ventral lobe produces gonadotrophin(s), it follows that the elasmobranch homologue of the pars tuberalis is responsible for gonad control. This condition would be unique. It is well established that in other vertebrates the pars anterior is concerned with gonadotrophin production; the pars tuberalis is not known to produce hormones.

If we concede that the elasmobranch pituitary represents a "primitive" condition, it follows that two evolutionary changes must have occurred simultaneously: the ventral lobe migrated dorsally and became associated with the tuber cinereum to form the pars tuberalis; at the same time it lost its gonadotrophic function, which became transferred to the pars anterior.

Transfer of trophic function is not unknown; in those verte:brates which lack a discrete pars intermedia (cetaceans, manatee, armadillo, chicken) the chromatophore-expanding hormone is present in the pars anterior (Waring and Landgrebe, 1950).

If the ventral lobe is not the homologue of the pars tuberalis, the obvious interpretation is that in elasmobranchs the pars anterior is divided into two portions: the rostral and ventral lobes. One of these subdivisions (the rostral) probably produces thyrotrophin, and the other (the ventral) produces gonadotrophin. Either or both

portions of the pars anterior may of course produce other hormones besides those considered here.

No other vertebrate shows a morphological division of the pars anterior, with a corresponding division of function. In teleosts the adenohypophysis is not clearly divided into lobes, so that it is not possible to speak of a pars anterior. From amphibians to mammals the pars anterior is a discrete entity which produces both thyrotrophin and gonadotrophin.

There is, however, much work to be done before the phylogenetic significances of the elasmobranch pituitary can be assessed. The technique of ventral lobectomy used in this work was somewhat crude, and the results must be accepted with a certain reserve, but this much can be said with assurance: it is no longer possible to dismiss the ventral lobe as being without physiological significance. It is to be hoped that this work will direct attention to the ventral lobe, the physiology of which has been too long ignored.

Plate 1.

Fig. 1. Thyroid D29 (control). Epithelium high columnar, with basal nuclei and granular basophil inclusions in the cytoplasm. A number of basophil "cysts" (arrow) are seen in the epithelium. x270.

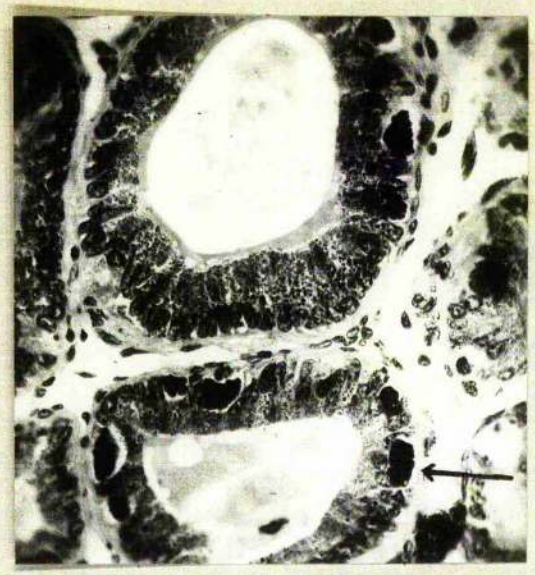
Fig. 2. Thyroid D44 (control). Follicles small, with high epithelium; some contain basophil colloid. x50.

Fig. 3. Thyroid D72 (control). Follicles markedly variable in size; colloid eosinophil. x50.

Fig. 4. Thyroid D39 (control). Portions of two large follicles on left show relatively low epithelium densely packed with basophil inclusions. Smaller follicles on right with high epithelium and scanty cytoplasmic inclusions. x240.

Fig. 5. Thyroid D46. Epithelium contains large number of basophil cysts. X252.

Plate 1.



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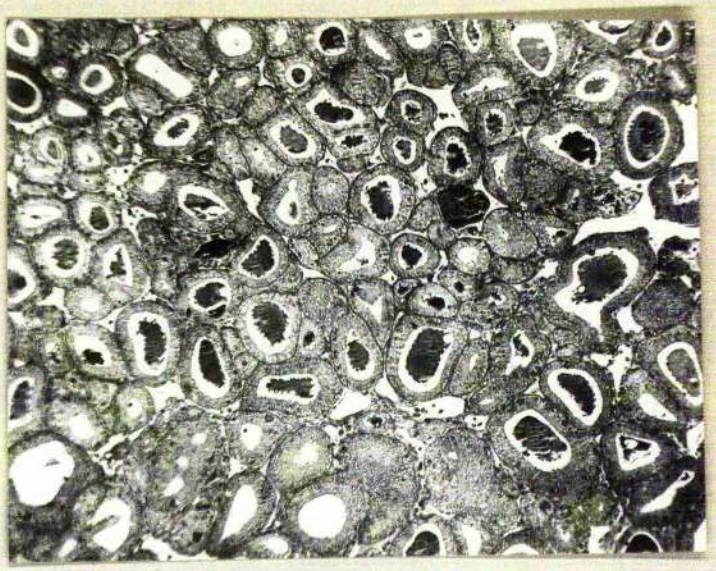

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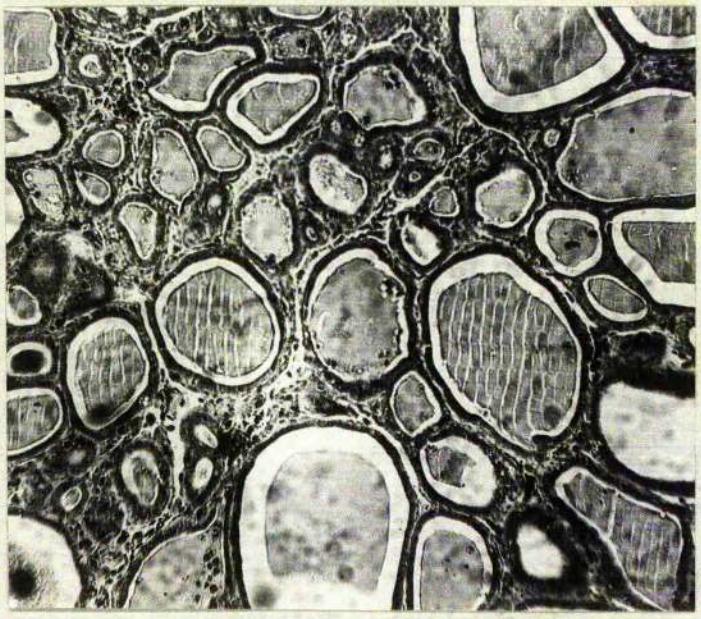

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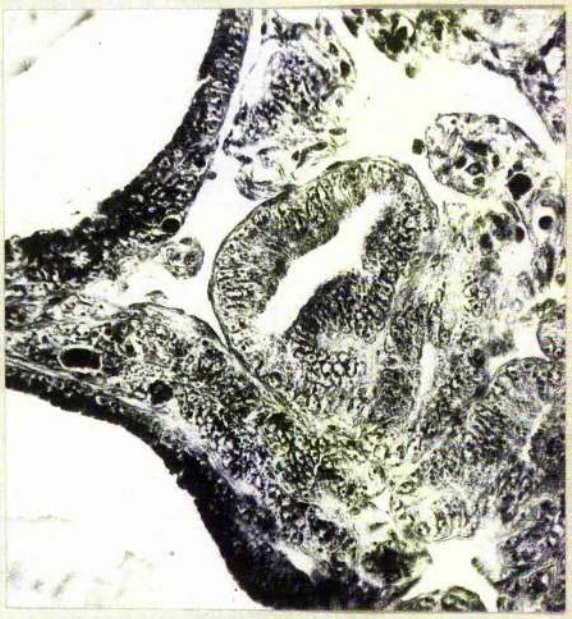

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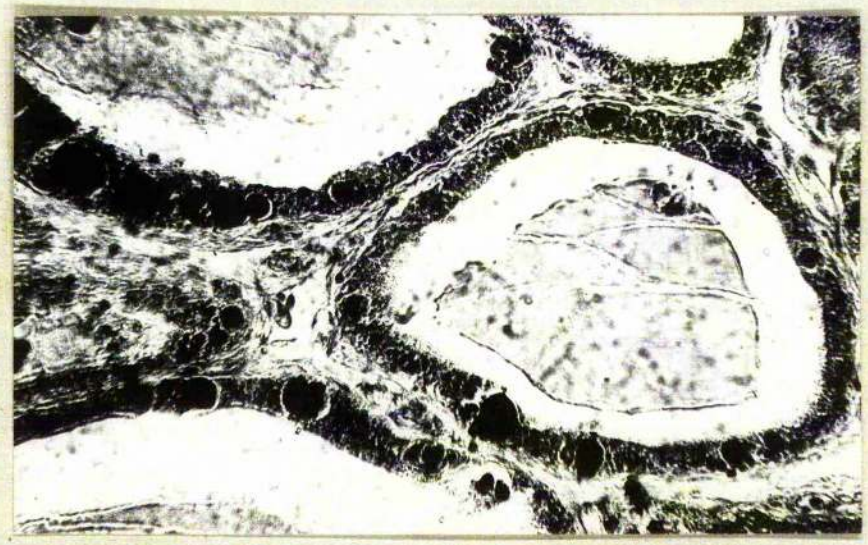

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Plate 2.

Fig. 6. Thyroid D51. Localised disintegration of follicles. Basophil bodies are seen scattered throughout the area. x150.

Fig. 7. Thyroid D19. Area being invaded by connective tissue. Note basophil colloidal masses (arrow) surrounded by connective tissue. x60.

Fig. 8. Thyroid D34. Follicle showing break-up of colloid. A number of more-or-less rounded bodies have separated from the remainder of the colloid; these bodies are intensely basophil. x250.

Fig. 9. Thyroid D13. Localised disintegration of follicles. Epithelial cells and basophil bodies (probably epithe: :lial cysts) are scattered throughout the area, and a "porous" colloid can be seen in places. x100.

Fig.10. Thyroid D12. A connective tissue mass which has probably replaced a follicle. Note basophil colloid: :al bodies scattered throughout the connective tissue. x150.

Plate 2.

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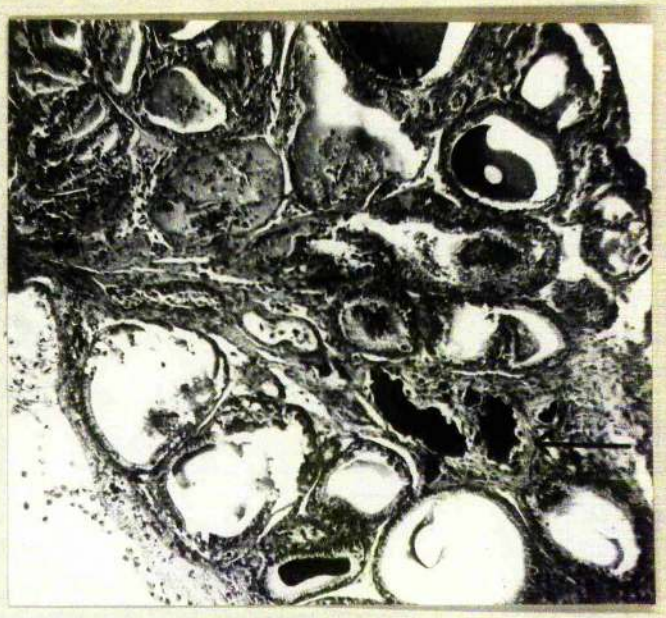

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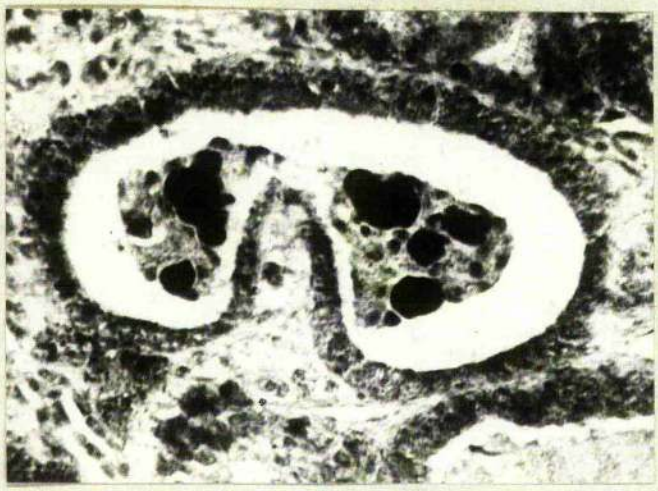

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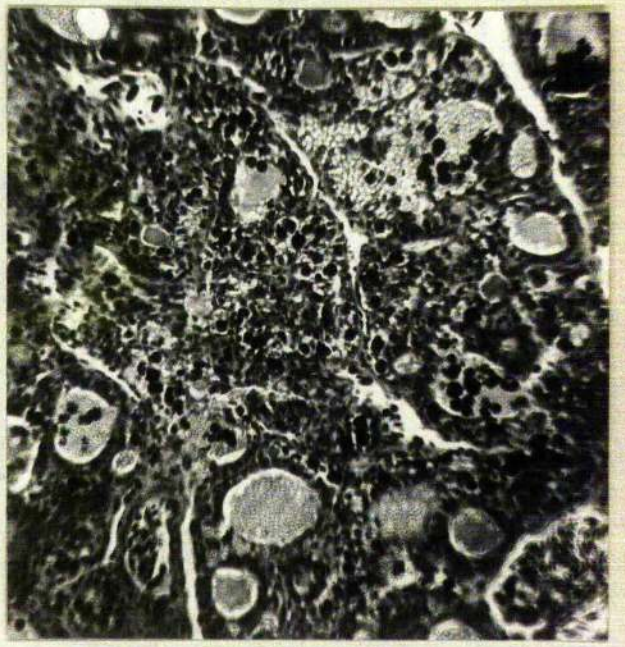

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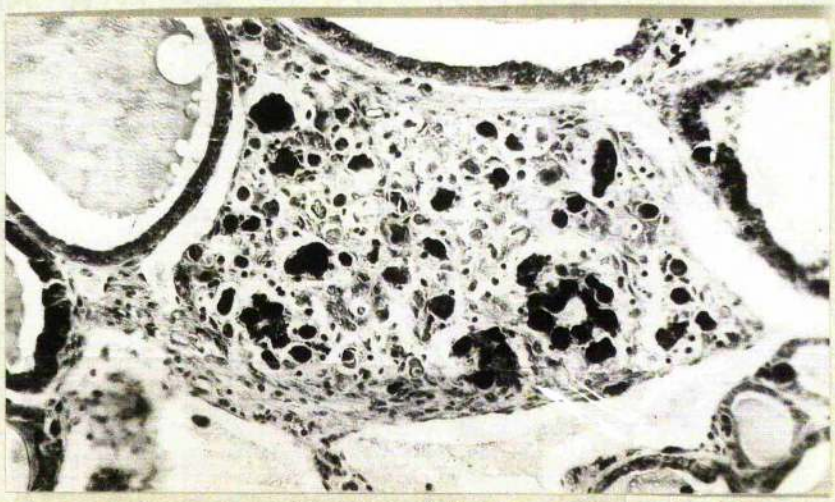

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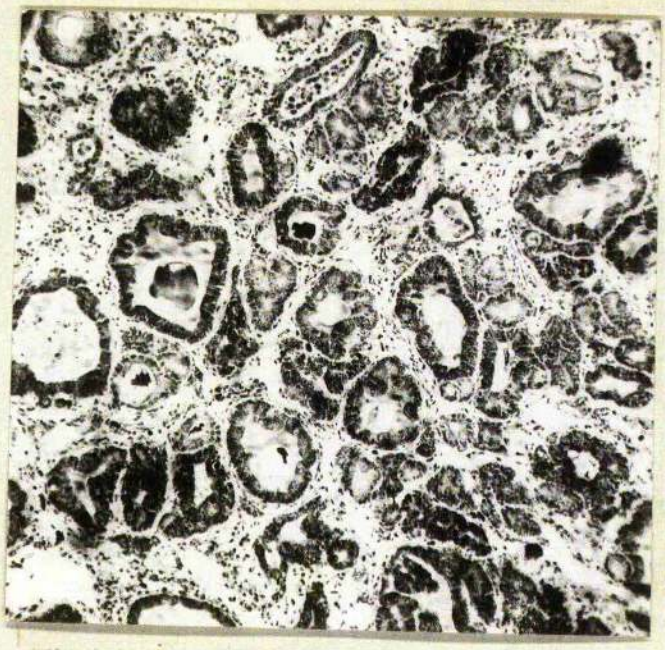
Plate 3.

Fig. 11. Thyroid D31. Follicles irregular in shape. Note large amount of inter-follicular connective tissue. x60.

Fig. 12. Thyroid D2 (control). Gland normal in appearance, shown for comparison with thyroid D1 (below). Follicles closely packed and regular in shape and size. x14.

Fig. 13. Thyroid D1. Follicles irregular in size and shape. Much of the glandular tissue has been replaced by connective tissue. x14.

Plate 3.



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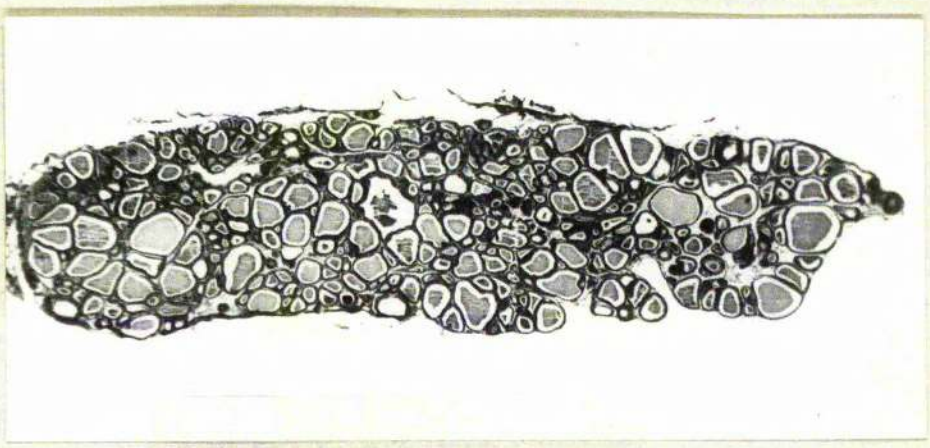

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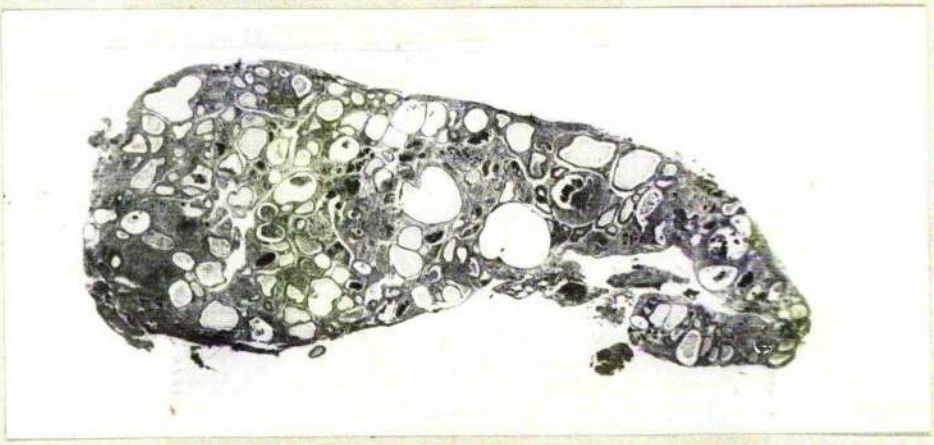

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Plate 4.

Fig. 14. Thyroid D1. Invasion of a follicle by connective tissue. Note basophil colloidal bodies in remnant of lumen and embedded around periphery of connective tissue mass. x100.

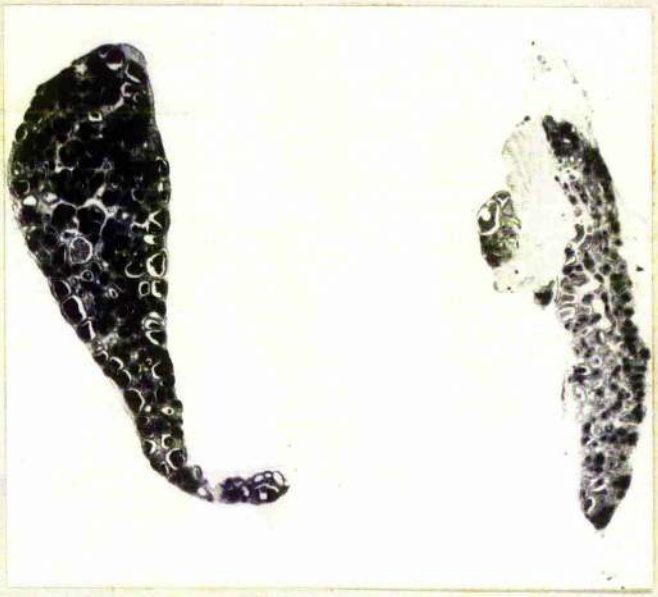
Fig. 15. Thyroid D1, showing extensive replacement of glandular tissue. Note whorled pattern of connective tissue (arrow) where a follicle has been replaced. A number of basophil colloidal masses have been isolated by the invading connective tissue. x54.

Fig. 16. Thyroid D1. Follicle containing a number of basophil colloidal bodies. The concentric structure of these bodies is clearly seen. x250.

Fig. 17. Thyroid D1. Portion of field (above) shows a group of basophil colloidal bodies lying within lumen of a follicle which is being invaded by connective tissue; latter has already surrounded a number of similar bodies in lower portion of field. Note cell at centre of colloidal body (arrow). x400.

Plate 5.

18



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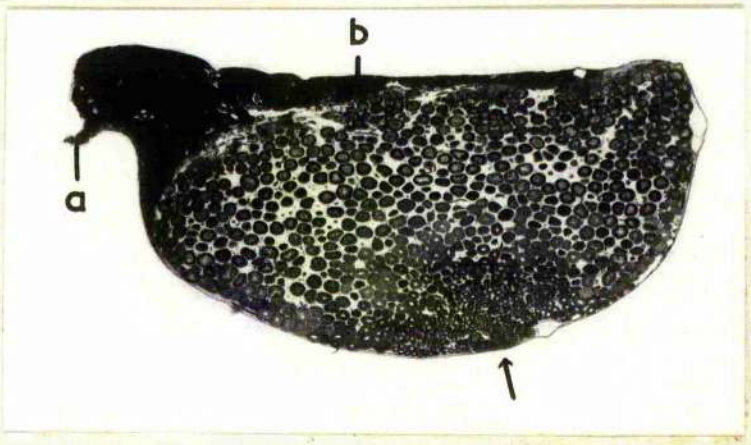

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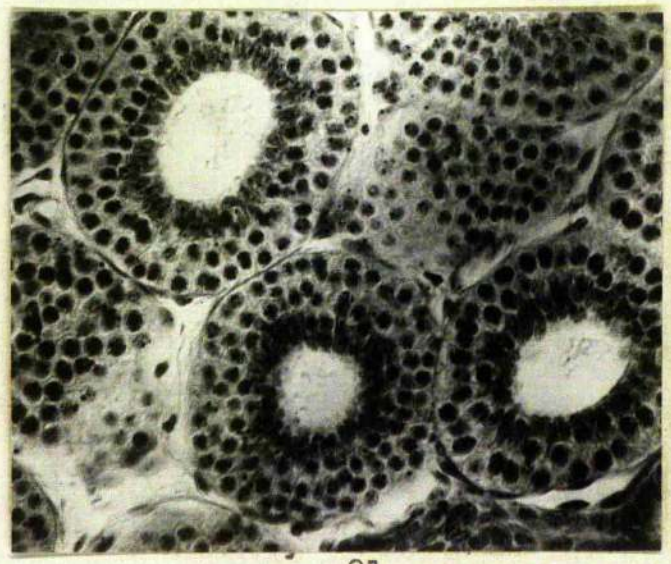

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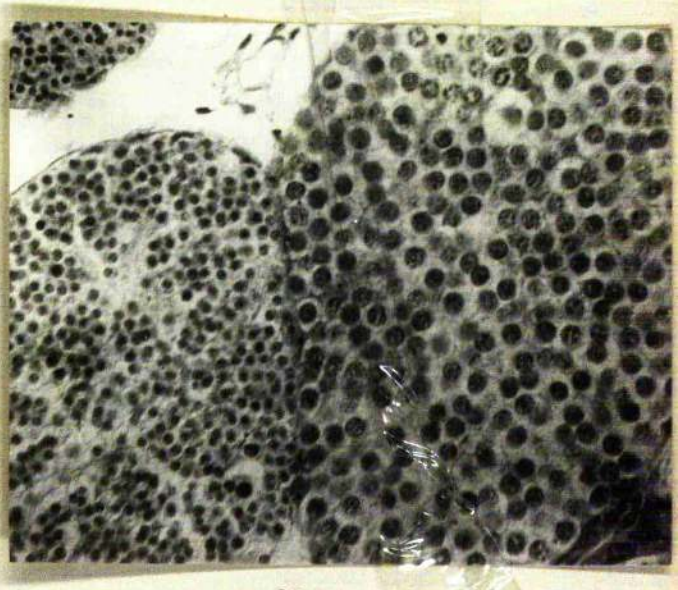

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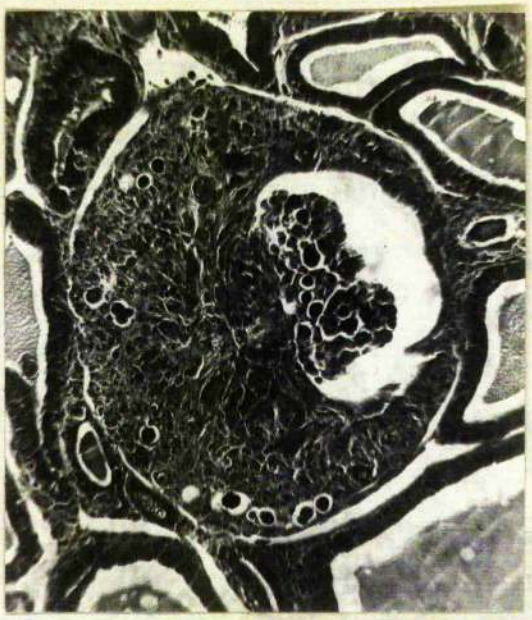
Plate 4.



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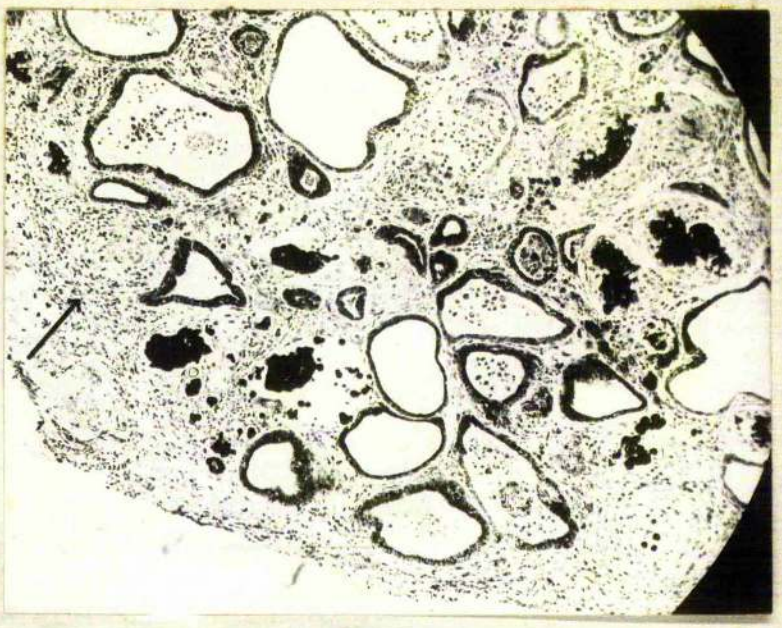

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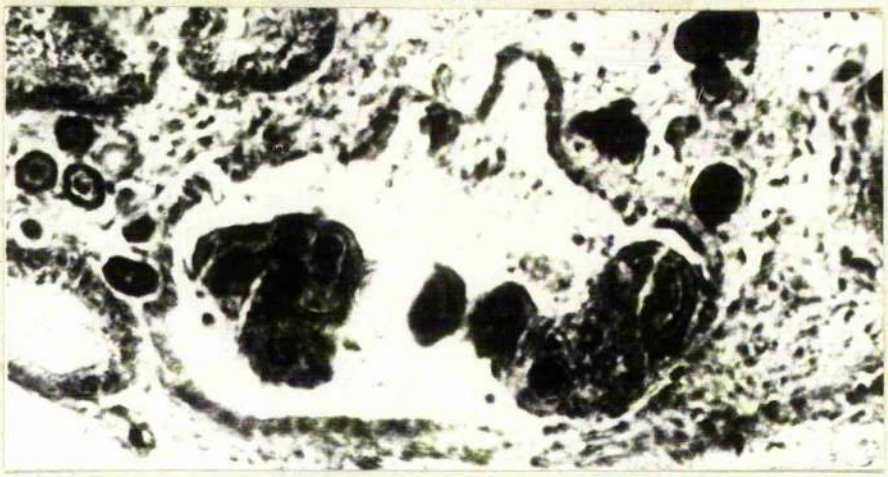

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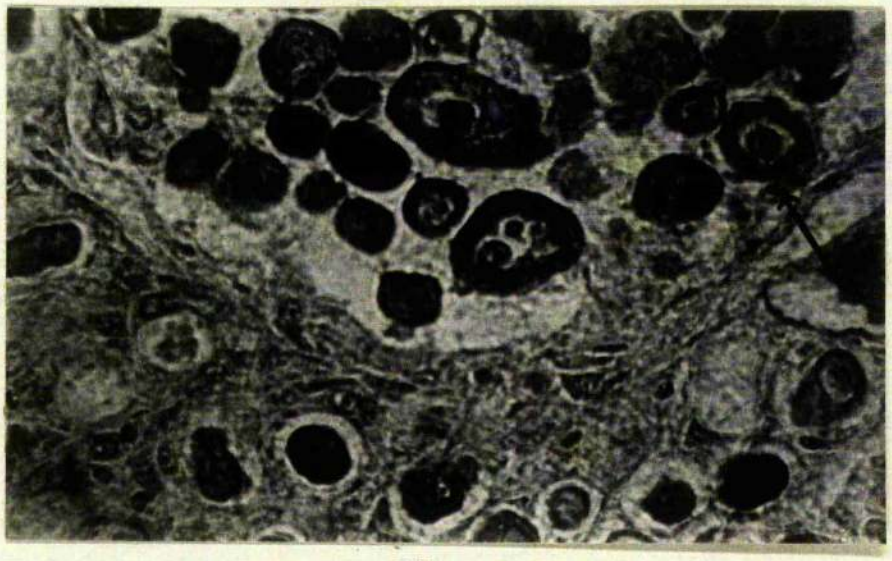

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Plate 5.

Fig. 18. Autoradiographs of thyroids P70 (control, left) and P63. There was considered to be no significant difference between these two glands with respect to the degree of blackening shown by the film. x8.

Fig. 19. Autoradiographs of thyroids D76 (control, left) and P77. Degree of blackening judged to be less in P77 than in control. x8.

Fig. 20. Cross section testis D57 (control). Germinal line indicated by arrow. (a) mesorchium; (b) epigonal tissue, lying along dorso-lateral border of testis.

Fig. 21. Spermatogonia region of testis D57 (control). Lumina of tubules bordered by primordial Sertoli cells; outside the latter are the spermatogonia. x240.

Fig. 22. Two tubules from testis D57 (control). That on the right contains primary spermatocytes; that on the left contains secondary spermatocytes. x240.

Plate 6.

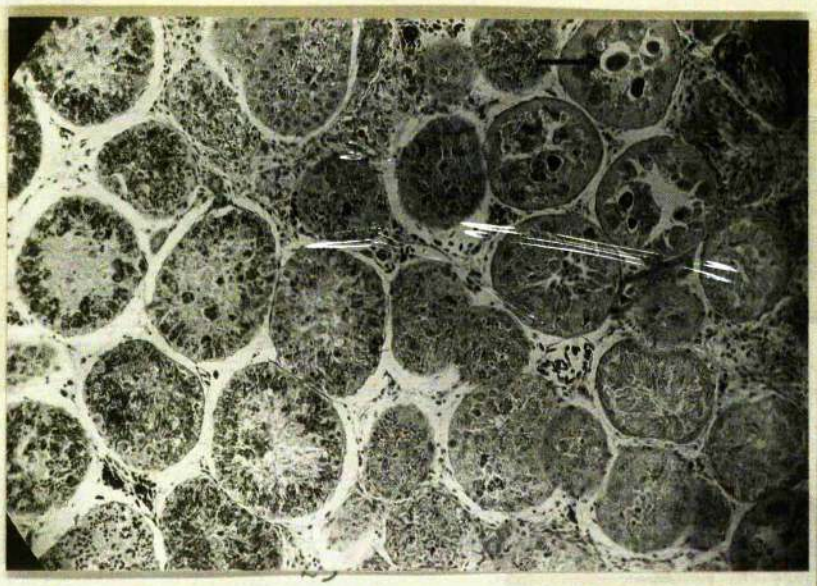
Fig. 23. Testis D57 (control). Tubule containing spermatids x240.

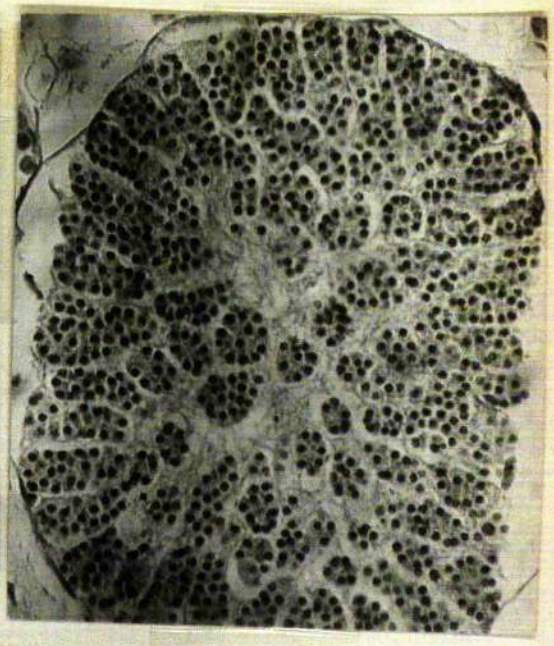
Fig. 24. Testis D57 (control). Transition zone between spermatids and spermatozoa, showing transformation of spermatids. x240.

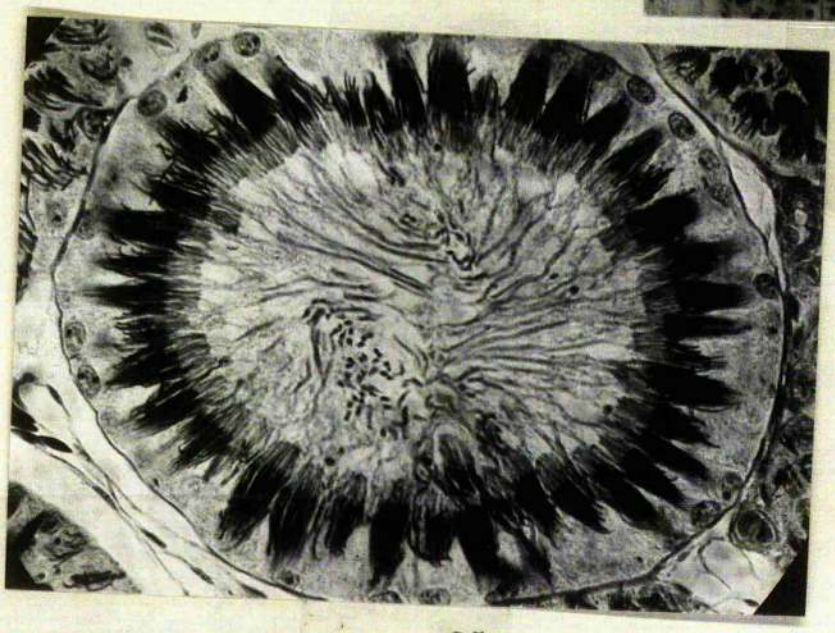
Fig. 25. Testis D57 (control). Tubule containing spermatozoa. Each cluster of spermatozoa is associated with a Sertoli cell, the nuclei of which can be seen around the periphery of the tubule. x240.

Fig. 26. Testis D48 (control). Resorption phenomena affecting spermatids and spermatozoa. On the left is the transition zone, in which the spermatids are undergoing transformation to spermatozoa; nuclear debris is a conspicuous feature of this zone. The spermatozoa tubules (right) are empty. Note the large hyaline inclusions (arrow). x60.

Plate 6.







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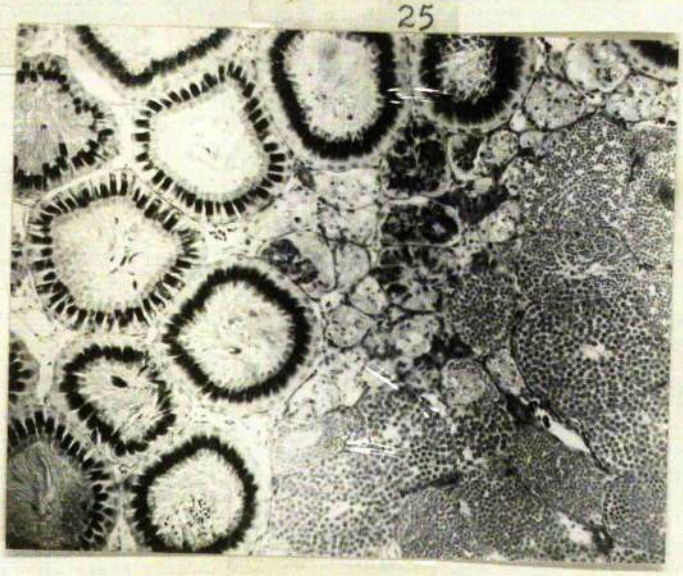

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Plate 7.

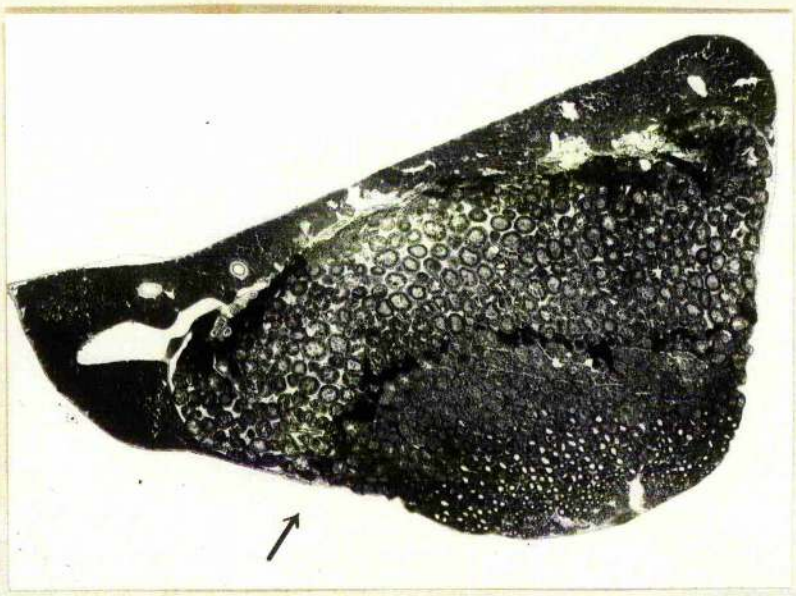
Fig. 27. Cross section testis D3 stained with Sudan Black. Zone of degeneration (arrow) is strongly sudanophil, and stretches in a well-defined band across the testis. x9.

Fig. 28. Testis D4 (control), showing transition region between spermatids (left) and spermatozoa (right). x60.

Fig. 29. Testis D3. Similar view to that shown in fig. 28. Transition region between spermatids (left) and spermatozoa (right) shows shrinkage of tubules and resorption of germinal elements. x60.

Fig. 30. Testis D3. Portion of field seen in fig. 29. Some tubules contain nuclear debris; in others resorption of the germinal elements is complete. x240.

Plate 7.



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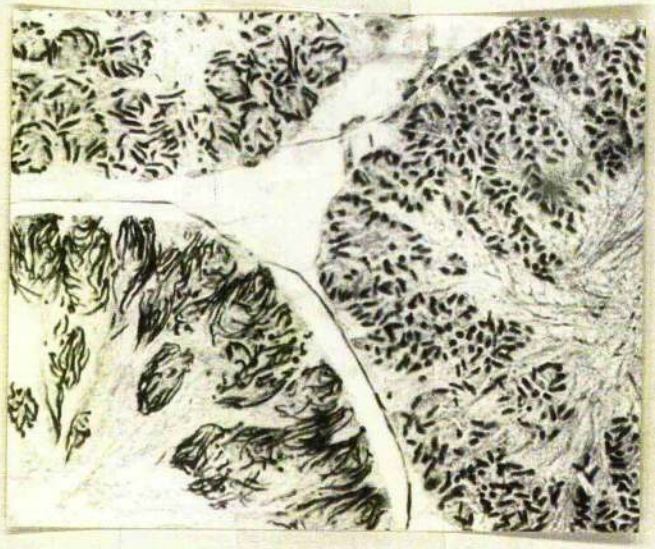

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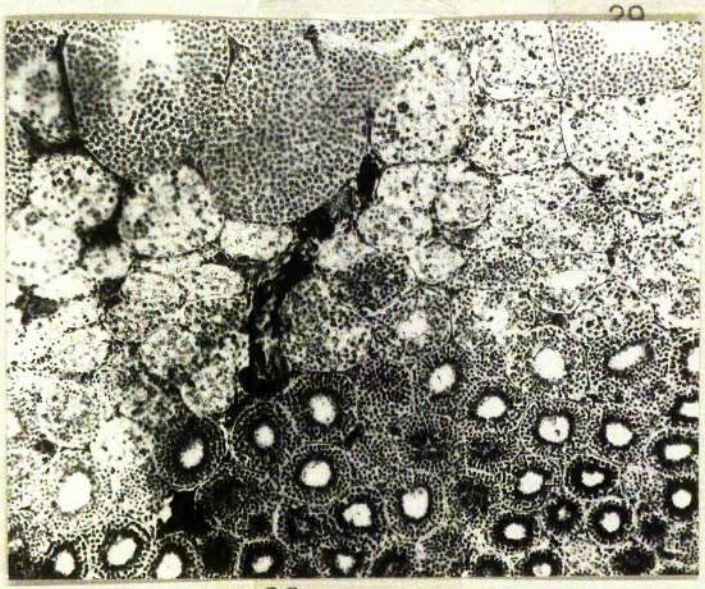

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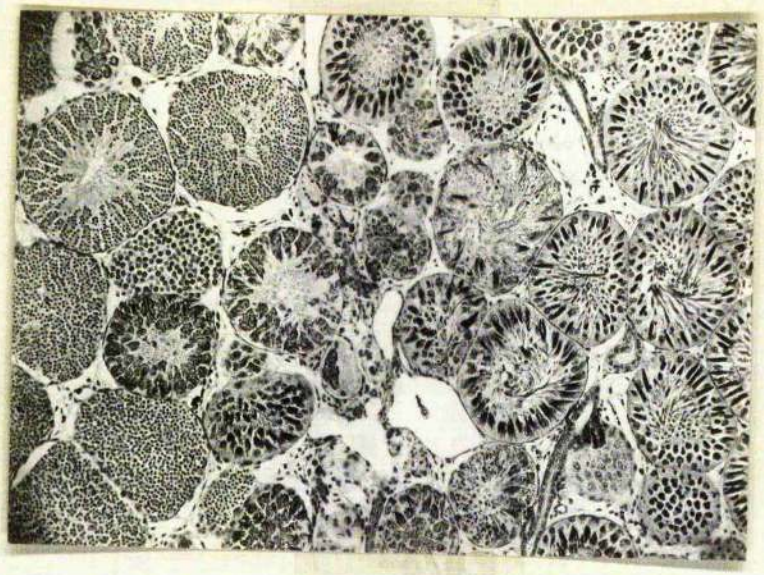
Plate 8.

Fig. 31. Testis D45 (control). Spermatids (left) and spermato:
:zoa (right) are intact, but resorption phenomena can
be detected in the transition region. x60.

Fig. 32. Cross section testis P36. Zone of degeneration be:
:tween spermatogonia and primary spermatocytes
indicated by arrows. x6.

Fig. 33. Testis P36. Zone of degeneration lying between
spermatogonia (below) and primary spermatocytes
(above). x60.

Plate 8.



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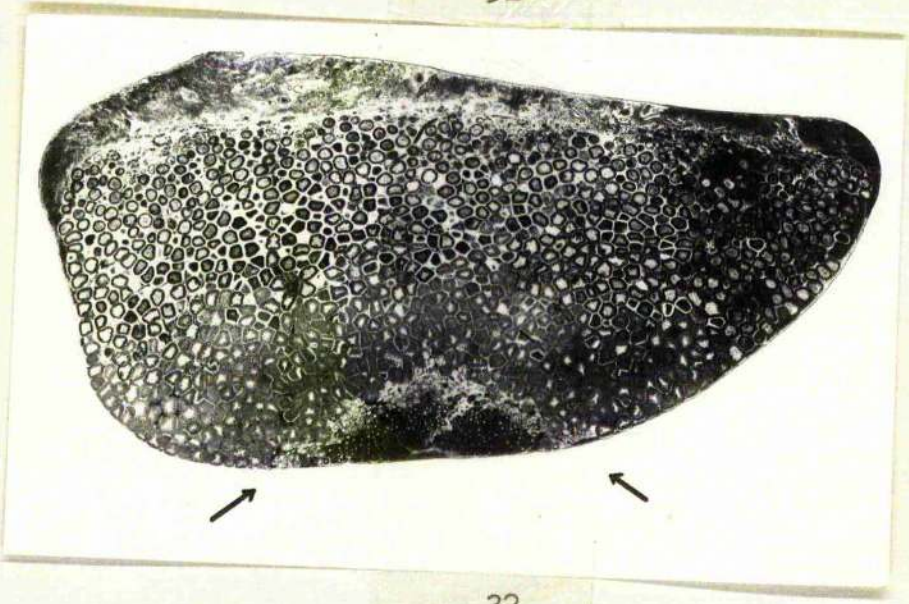

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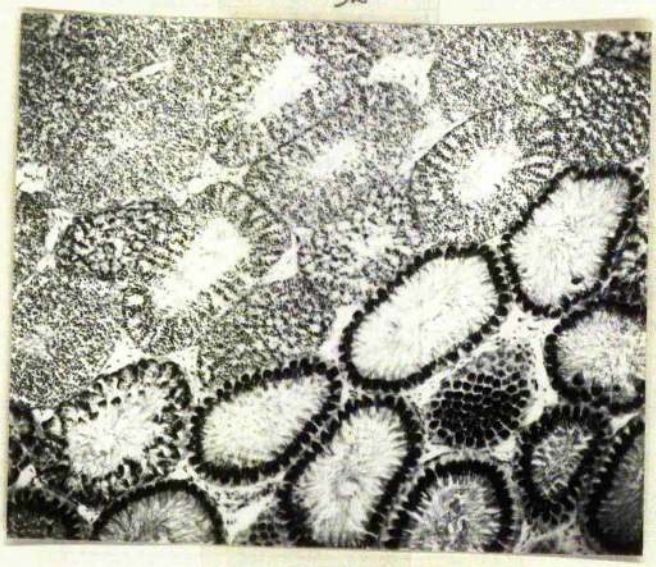


33

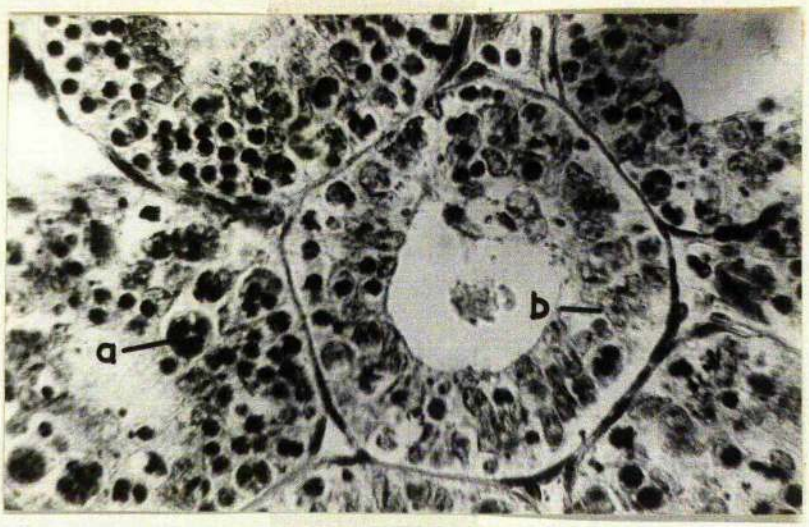
Plate 9.

Fig. 34. Testis P80. Resorption of spermatogonia. Note clumping of spermatogonia nuclei (a); nuclei of primordial Sertoli cells dispersed among degenera: :ting spermatogonia (b). x270.

Fig. 35. Testis P36. Resorption of germinal elements further advanced in distal tubules (right) than in proximal. On extreme right the zone of degeneration is suc: :ceeded by tubules containing primary spermatocytes. x240.

Fig. 36. Testis P80. Zone of degeneration encroaching on primary spermatocytes (right). Note clumping of primary spermatocyte nuclei in tubule occupying centre of field. x125.

Plate 9.



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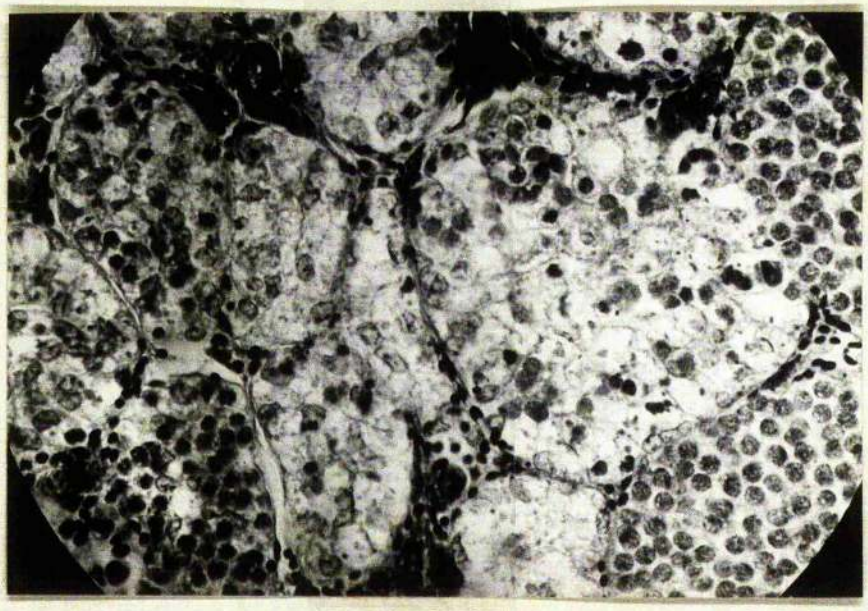

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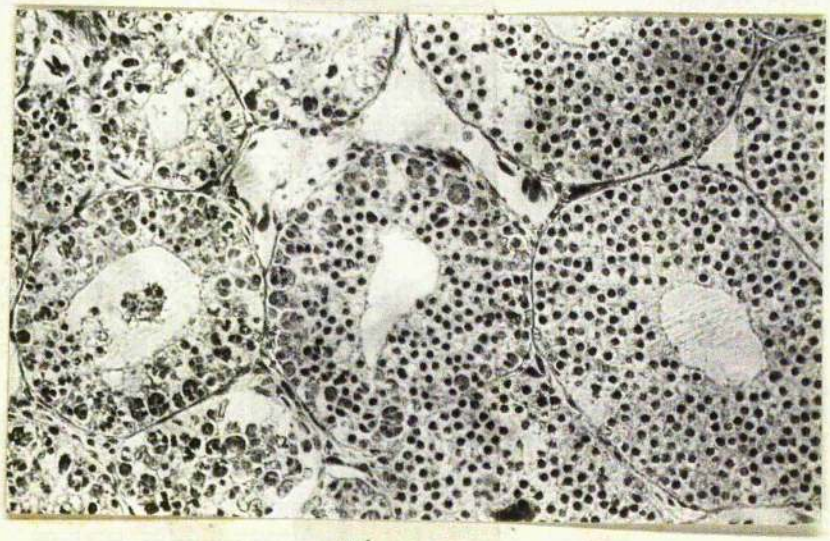


36

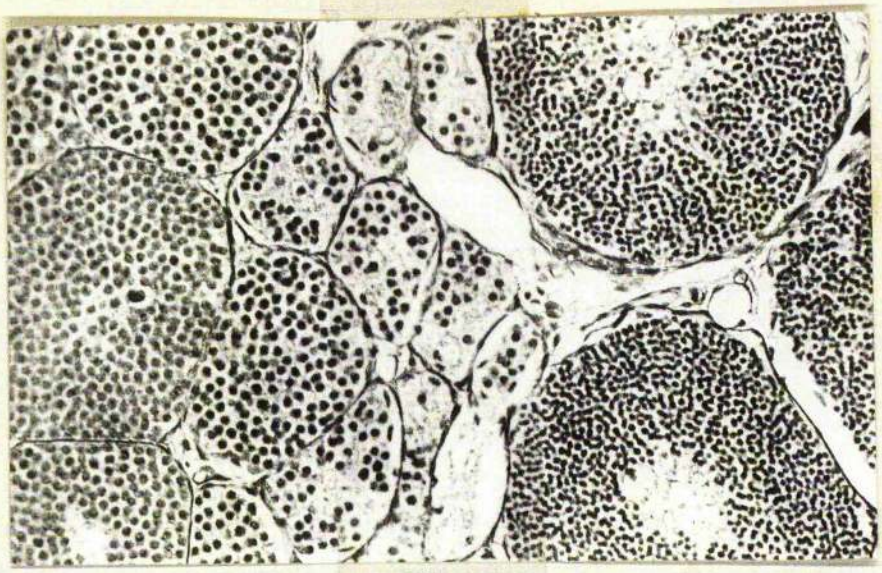
Plate 10.

Fig. 37. Testis P60. Zone of degeneration lying between primary spermatocytes (left) and secondary spermatocytes. x125.

Fig. 38. Testis P71. Zone of degeneration bounded by spermatogonia (left) and secondary spermatocytes (right). Primary spermatocytes are absent. x125.

Fig. 39. Testis P66 (control). Signs of degeneration in tubules lying between spermatogonia (left) and primary spermatocytes (right). Note clumping of germinal nuclei. x125.

Plate 10.



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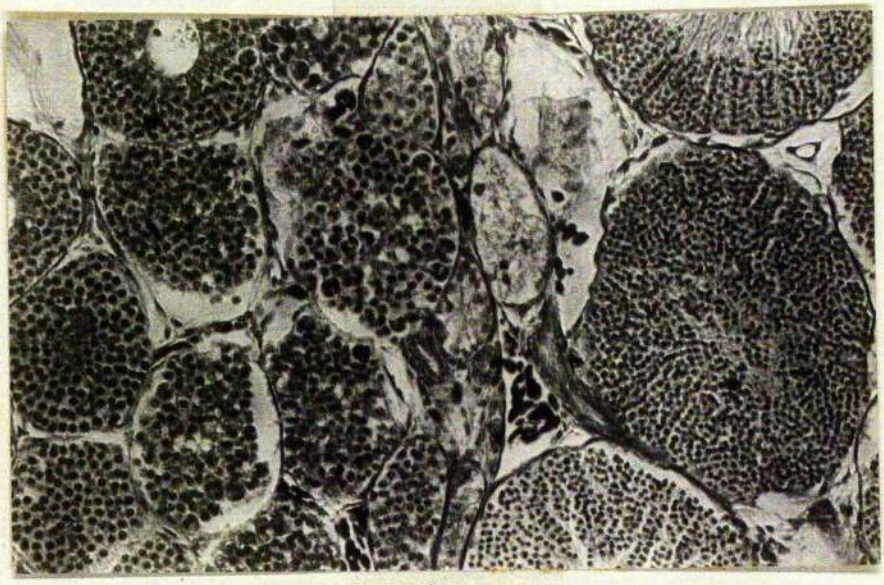

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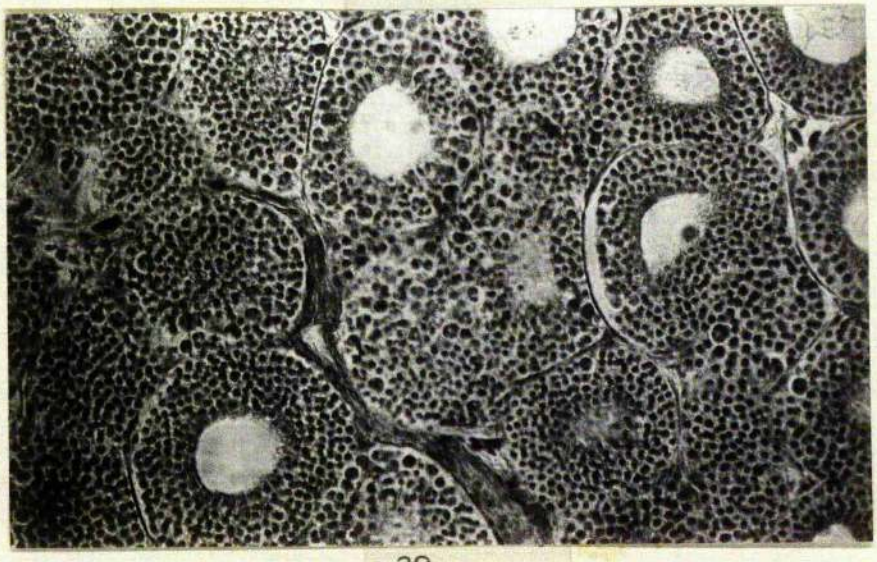


39

Plate 11.

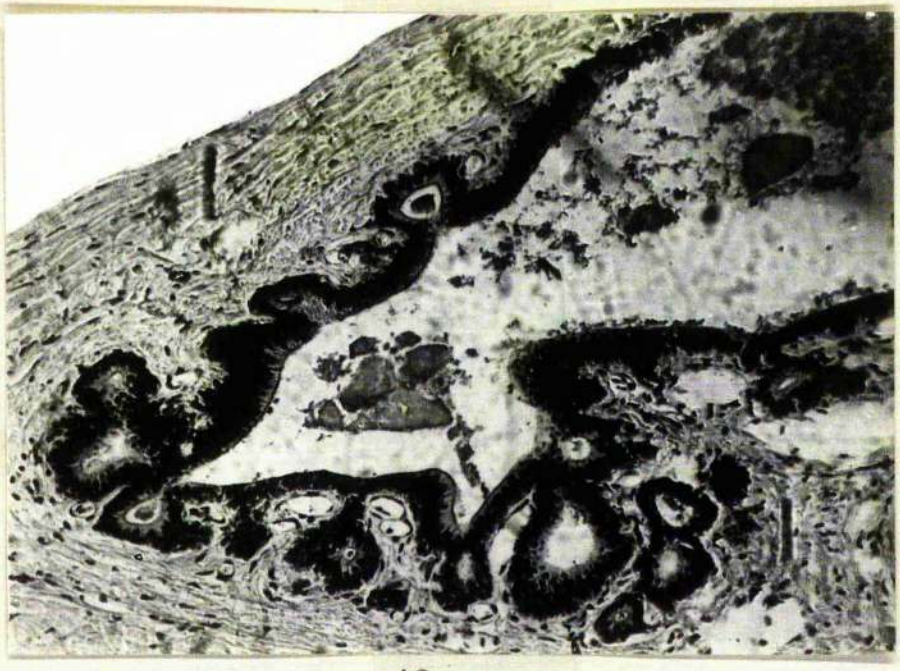
Fig. 40. D4 (control). Parasagittal section through ventral lobe of pituitary. Epithelium is high columnar; large lumen and peripheral diverticulae contain colloid. x125.

Fig. 41. D3. Parasagittal section through ventral lobe of pituitary. Lumen almost totally occluded; epithe: :lial cells pyknotic. x125.

Fig. 42. P80. Parasagittal section through floor of cranium, showing complete destruction of ventral lobe of pituitary in this region. x26.

Fig. 43. P80. Extreme lateral section through floor of cranium showing remnant of ventral lobe. x26.

Plate 11.



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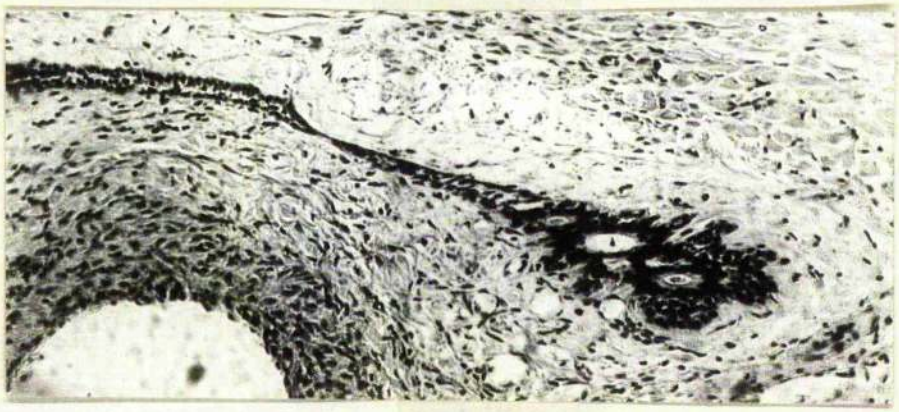

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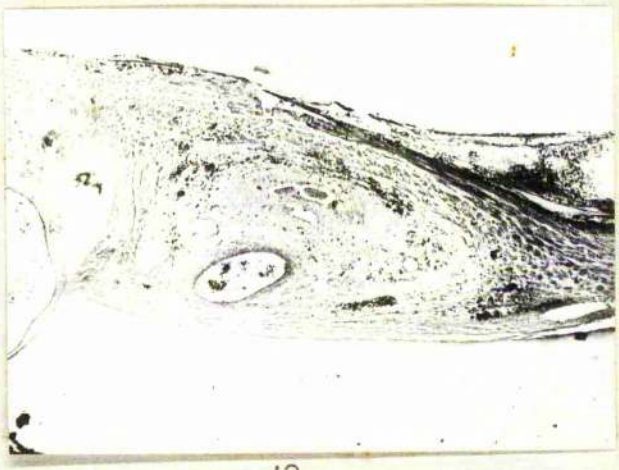

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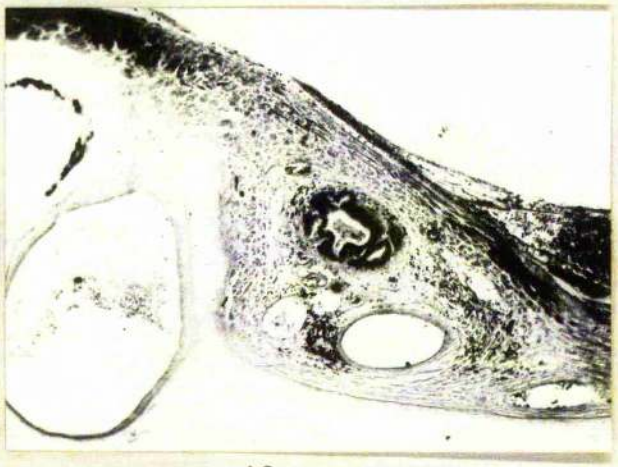


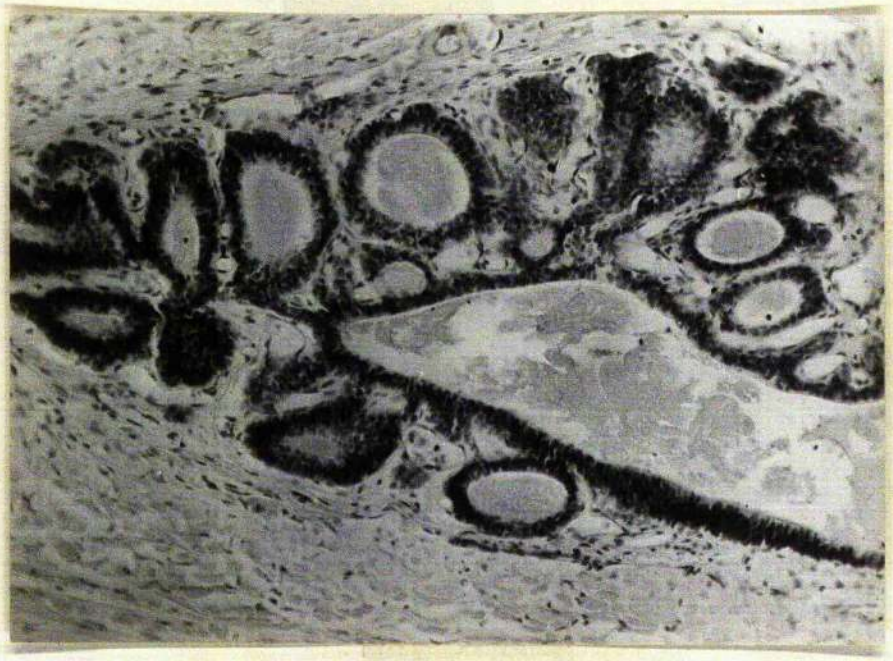
43

Plate 12.

Fig. 44. D1. Parasagittal section through ventral lobe of pituitary. Ventral lobe normal in appear: :ance (compare fig. 40). x125.

Fig. 45. Testis D1. Appearance normal. No sign of degenerative changes between spermatogonia (left) and primary spermatocytes (right). x60.

Plate 12.



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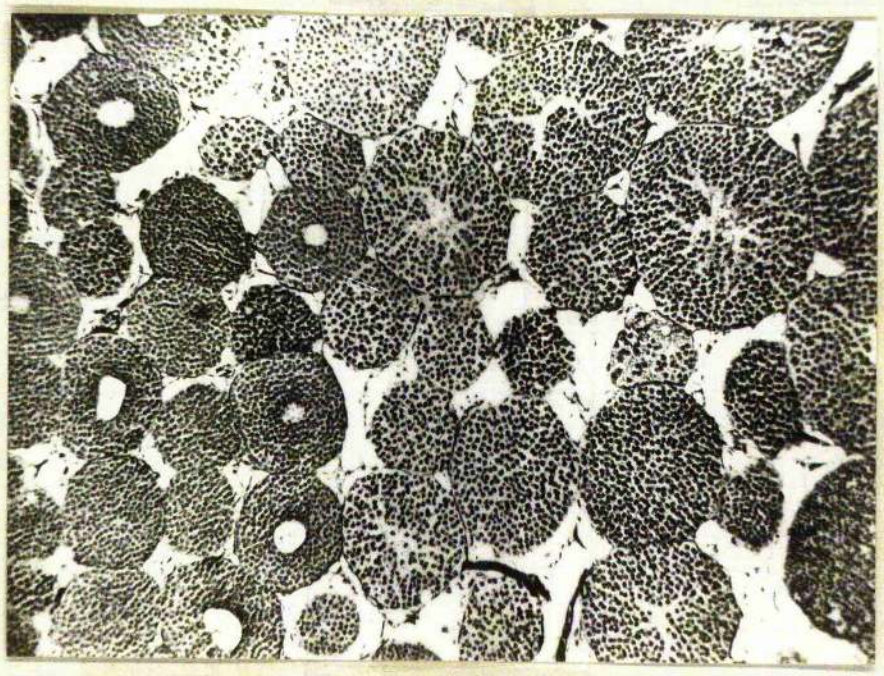

45

APPENDIX

(A) Males. Post-mortem dissections indicated thus: (x)

* Remnant of lobe remains in situ.

- Lobe absent.

Fish	Time after opern. (weeks)	Pituitary at dissection			Thyroid cell heights (Micrometer divisions)	
		Rostral l.	N-int. l.	Ventral l.	Mean	Standard Deviation
D 1	55	-	-	present	63	22
D 2	50	present	present	present	72	22
D 3	29	-	-	*	67	20
D 4	29	present	present	present	70	14
D28(x)	10	-	-	present		
D44	35	present	present	present	91	18
D45	37	present	present	present	77	16
D46(x)	27	-	-	present	58	13
D47	35	-	*	present	85	21
D48(x)	38	present	present	present	85	16
D50	20	*	*	present		
D51	37	*	present	present	90	19
D52(x)	5	-	-	present		
D55	6	-	*	present		
D56	34	present	present	present	100	18
D57	37	present	present	present	83	12
D60(x)	29	-	*	present	132	23

(A) Males.

Fish	Time after opern. (weeks)	Pituitary at dissection			Thyroid cell height (Micrometer divisions)	
		Rostral l.	M-int. l.	Ventral l.	Mean	Standard Deviation
D63(x)	4	*	*	present		
D64(x)	9	present	present	present		
D65(x)	3	present	present	present		
D66(x)	3	present	present	present		
D67(x)	5	-	*	present		
D70(x)	6	present	present	present		
D71(x)	8	present	present	present		
D72(x)	17	present	present	present		
D78	31	present	present	present	77	12
D79(x)	6	present	present	present		
D80(x)	15	present	present	present		
D82	27	*	present	present	80	15
D84	30	*	present	present	75	20
P 7(x)	14	-	-	-		
P 9(x)	25	present	present	present	85	14
P26	25	present	present	present	84	15
P36(x)	8	present	present	-		
P37(x)	15	-	*	-		
P39	18	present	present	present		

(A) Males.

Fish	Time after opern. (weeks)	Pituitary at dissection			Thyroid cell height (Micrometer divisions)	
		Rostral l.	N-int. l.	Ventral l.	Mean	Standard Deviation
P40(x)	24	present	present	present	85	14
P42(x)	24	present	present	-	82	19
P48	13	present	-	-		
P49	23	present	present	present	82	15
P52	26	*	present	present	66	10
P54	24	*	present	present	80	14
P55(x)	23	present	present	present	85	11
P56(x)	9	*	-	present		
P58(x)	19	present	present	present		
P60	24	*	present	*	73	11
P61	27	*	present	present	74	10
P62	24	*	present	present	82	10
P63	25	*	*	present	82	11
P64	27	-	-	present	74	12
P66	6	present	present	present		
P70	25	present	present	present	85	12
P71	23	-	present	-	84	25
P74(x)	7	present	present	present		
P77	26	-	present	present	81	11

(A) Males.

Fish	Time after opern. (weeks)	Pituitary at dissection			Thyroid cell height (Micrometer divisions)	
		Rostral l.	N-int. l.	Ventral l.	Mean	Standard Deviation
P80	5	-	-	*		
M56	9	present	present	present		
M57	9	present	present	present		

(B) Females. Post-mortem dissections indicated thus: (x)

* Remnant of lobe remains in situ.

\- Lobe absent

Fish	Time after opern. (weeks)	Pituitary at dissection			Thyroid cell height (Micrometer divisions)	
		Rostral l.	N-int. l.	Ventral l.	Mean	Standard Deviation
D12(x)	42	-	*	present	47	10
D13	42	-	-	present	78	25
D19(x)	38	-	-	present	73	10
D20(x)	24	*	present	present	81	13
D22(x)	24	*	present	present	70	12
D29	45	present	present	present	77	13
D31	45	*	present	present	81	4
D34	37	present	-	present	83	21
D39	38	present	present	present	77	13
D40(x)	23	-	-	present	65	19
D43	30	present	present	present	122	31
D77	32	present	present	present	86	13
M58	9	present	present	present		
P81	-	present	present	present		
P82	-	present	present	present		

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