PHILOSOPHICAL TRANSACTIONS.

I.—*The Defensive Spines of Fishes, Living and Fossil, and the Glandular Structure in Connection therewith, with Observations on the Nature of Fish Venoms.*

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[Plates 1–3.]

Contents.

I. Introductory and Historical.

In this century very little original work has been done on the subject of defensive spines of fish. The articles in text-books on the subject of venoms, such as Calmette's work, are largely based on the excellent treatise by Bottard (1889) ('Les Poissons Venimeux et Vénéneux'), but this author gives no account of the defensive spines of Elasmobranchs. The following pages are devoted to a review of the whole subject, with special reference to certain spines, the nature of which has hitherto been a matter

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of dispute. Previous work on the nature of fish venoms has been examined and many experiments performed which give results not entirely in accordance with those of other observers.

From early times the question of true venoms secreted in connection with the defensive spines of fish has been a disputed subject. Aristotle mentions several dangerous fish, notably Trygon (the Sting-Ray) and Scorpæna; but he was not explicit as regards the venomous nature of the sting, and he made no mention of the Weever as a stinging fish.

Bottard (1889), to whom I am indebted for most of the early historical literature, does not mention the presence of a poison apparatus in Trygon, and the 'Cambridge Natural History' (1904) merely states (p. 177) that "among Elasmobranchs the Eagle-Rays (Aetobatis) and Sting-Rays (Trygon) have barbed or serrated spines on the tail, which inflict wounds far more severe than those caused by mere mechanical laceration; but, except the mucus secreted by the gland cells of the skin, which may possess venomous properties, no special poison-forming glands in connection with the spines are at present known."

We first find precise information about fish with poison organs in the vast compilation of Pliny. He says "Aranæus" (probably Trachinus aranæus, a species of Weever found in the Mediterranean) "carries on its back a sting which is very dangerous; but there is nothing more terrible than the sting that arms the tail of Trygon, called Pastinaca by the Latins, which is 5 inches long. When driven into the root of a tree it causes it to wither. It can pierce armour like an arrow, it is strong as iron, yet possesses venomous properties."

When we come to the Renaissance we find Belon, Rondelet, Salviani (1554) and Gesner possessing exact ideas concerning Trygon, Trachinus and Scorpæna. Speaking of Trygon, Pierre Belon describes "the dart at the root of its tail, which is sometimes double and triple, with which it pricks those who touch it carelessly." Rondelet describes the "dart" at length. "Its margin is armed with teeth like the teeth of a saw, which enables the dart to enter easily, but tears the flesh as it is withdrawn by the backward slant of the teeth." Superstition and romance surrounded the spine of Trygon with mysterious attributes. "If burnt, and the cinders applied to the wound in vinegar, it acts as an antidote. It relieves toothache, and helps cases of difficult dentition. If attached to the navel of a woman it causes her to have an easy childbirth, provided it be taken from a living ray, which is then thrown back into the sea." As Sir Thomas Browne quaintly remarks "it is conceived of special venom and virtues."

Aldrovandus endeavoured to shake off the yoke of antiquity, and adopted an attitude which is found even in modern writers. "I have searched," he says, "for a poison organ in Trygon and have not found it; therefore, it does not exist," and proceeds to state: "These fish are dangerous only on account of the mechanical wounds they make and the depths to which their spines penetrate." More modern

ichthyologists—SONNINI, LACÉPÈDE and CUVIER—deny the presence of poison glands in fish. LACÉPÈDE, on every possible occasion, denies the existence in the Sting-Ray, Weever, Scorpæna, Plutosus and Muræna of any poison organ, and just as energetically CUVIER supports him, but allows that the pricks of certain fish are dangerous and produce acute pain. The opinion of these authors became almost a matter of dogma, and works on ichthyology since their time continued to deny the presence of a poison organ in fish, until towards the middle of the last century ALMAN (1841) first described the gland at the root of the spine of the lesser Weever. His discoveries were widened and confirmed by BYERLEY, GUNTHER, NEWTON PARKER, BOTTARD and SCHMIDT.

PORTA (1905) described a glandular structure in the lateral grooves of the poisonous spine of Trygon. He stated that this gland was similar to that found in Scorpæna, and in a great number of other poisonous fish. This description, as I later showed (1916–1921) and shall further demonstrate in this paper, does not correspond with the true structure of the poison gland of Trygon, which is entirely different from that of the gland found in teleostean fishes. PAWLOWSKY (1907–1909) was unable to find any gland in the spine of Trygon and attributes the venomous properties to the general skin secretion.

The most recent work on the subject of venomous animals and their poisons is the work of Madame MARIE PHISALIX. This excellent treatise was published in March, 1922. In her summary of the work done on venomous fish, she states:—"Finally BOTTARD has again examined, from the point of view of the existence of a poison gland, several dangerous species in which, however, he could not discover any gland. For example, those sharks provided with fin spines such as Cestracion, Chimæra and the Spiny Dog Fish, such as Acanthias."

Later in her treatise the same writer again quotes the negative results of BOTTARD, but is apparently not acquainted with the paper I wrote in 1920,* describing the gland of the groove of Acanthias, and the results of a wound from it observed in a fisherman under my care.

In the following pages, I give in the first place a more detailed account than has hitherto been published of the anatomy and histology of the poison glands lying in the grooved spines of *Trygon pastinaca*, *Acanthias vulgaris*, *Cestracion* (= *Heterodontus*) *philippi* and *Chimæra monstrosa* based upon a study of serial sections, in which the delicate gland tissue has been more successfully preserved than in earlier investigations, including some of my own earlier work on the spine-gland of Trygon. In the second place I describe a series of experiments and clinical observations which I have made on the physiological nature of the poisonous action of the venoms of *Trachinus draco* (the greater Weever) and *Acanthias vulgaris* (the spiny dog-fish).

* 'Brit. Med. Journ.,' February 28, 1920.

II. Anatomy and Histology.

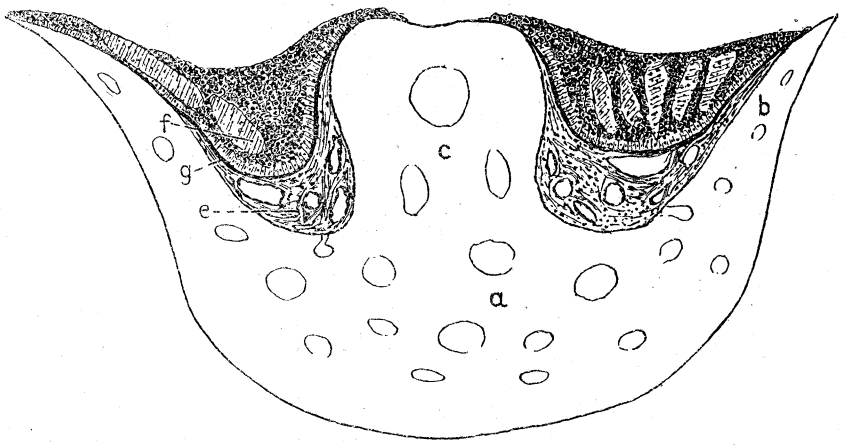
(1) *Microscopic and Naked Eye Anatomy of the Spine of* Trygon pastinaca.

In a paper read before the Zoological Society of London and published by me in 1916, an attempt was made to unravel the conflicting views of various observers on this organ. In doing so a complete series of sections was examined and certain conclusions were arrived at. This paper met with criticisms which rendered further research necessary, and the results of these further observations on the poison organ of the Sting-Ray, which seem to be decisive, are here presented.

Looking at a young spine of about an inch long, in the perfectly fresh condition the aspect facing the tail shows a longitudinal median ridge, on either side of which is a groove filled with a white glistening tissue, most marked towards the base. The outer margin of each groove is armed with a row of fine teeth, twenty teeth to 1 inch in the specimen I am describing. The teeth project towards the root of the spine and a black line of pigment separates the teeth from the white tissue that occupies the grooves.

For convenience of description one may speak of the dentate margin, median ridge and lateral grooves. The glandular tissue in the grooves has been called the "glandular triangle" (Porta (1905)) from the roughly triangular appearance it has in cross-section.

The spine in its greater portion is roughly hemispherical in section (text-fig. 1) with

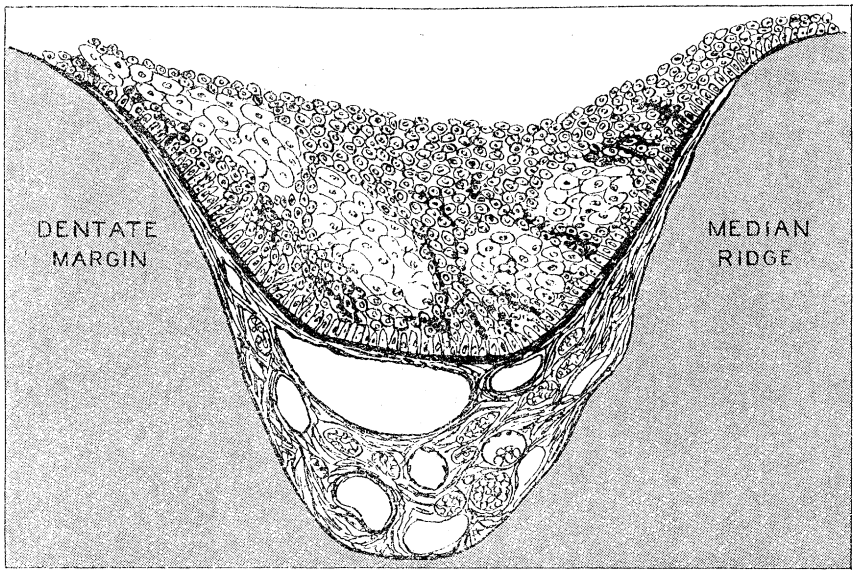


Text-fig. 1.—Transverse Section of Spine of Trygon (semi-diagrammatic). *a*, vasodentine; *b*, dentate margin; *c*, median ridge; *e*, alveolar connective tissue of lateral groove; *f*, glandular portion with columns of secretion directed towards the apex of a denticle; *g*, pigmented capillary layer.

the flat side of the hemisphere, presenting the median ridge and lateral grooves, facing the tail.

A section of the glandular triangle (text-fig. 1*a*) shows two widely different structures which are separated by a narrow layer of pigmented tissue. This lies beneath the basement membrane of the more superficial, epithelial structure, and

limits the deeper portion, which consists of alveolar connective tissue. The pigment layer is composed of capillaries, with pigment cells in their walls, and resembles the "reseau vasculo-pigmentaire," described by M. PHISALIX (1922) as surrounding the poison glands of the Salamander. The deeper portion of the contents of the glandular triangle consists of a meshwork of connective tissue, surrounding alveoli which contain small cells with a vacuolated protoplasm. These alveoli are sometimes only occupied by a homogeneous substance.



TEXT-FIG. 1*a*.—Transverse section across the Base of the Spine of Trygon to show the lateral groove, containing superficially a mass of epithelial cells, some of which are distended with secretion. Surrounding the active cells is a mesh-work of pigment granules. Deep in the groove is alveolar connective tissue, which is separated from the true gland by a pigmented capillary layer.

The nature and significance of the alveoli is not clear, but a similar appearance is to be noted in the connective tissue which forms the basal tissue of the glandular structure that lies in the spine of Acanthias.

Blood vessels course throughout the length of the gland and there are usually two large channels, appearing throughout the series of sections, lying just beneath the pigment layer and often bulging this layer outwards, text-fig. 2.

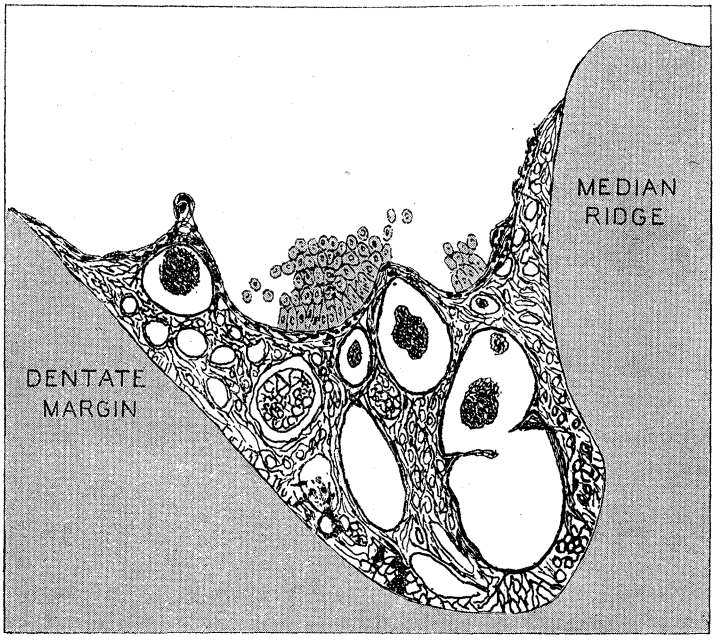
One may speak of these without committing oneself as to their function as the "central and lateral canals." Towards the tip of the spine there are frequently to be seen two lateral canals, one towards the dentate margin and one near the margins of the median ridge.

Certain peculiar structures in relation to these lateral canals will be mentioned after describing the more superficial portion of the glandular triangle.

Superficial to the pigment layer, and occupying the rest of the groove as far as a line passing from the external margin of the median ridge to the root of the teeth, is

a mass of epithelial cells. Those in the deepest layer are of a cylindrical shape, the rest of the gland consists of ovoid, elongated and round cells, which are bounded by a more cubical line of limiting cells (Plate 1, figs. 1 and 2, and text-fig. 1*a*).

In certain sections these ovoid cells are seen in an active stage of secretion. The cells become very distended, with indistinct margins, and phantom nuclei, they lose their bluish stain with hæmatine and take on a yellow stain with VAN GIESONS' solution; in other sections the cells are no longer to be differentiated, but yellow secretion takes their place. The secreting activity occurs in columns radiating from



TEXT-FIG. 2.—Transverse Section across the Middle of the Spine of Trygon, to show the lateral groove containing alveolar connective tissue and the central and lateral canals. The pigmented capillary layer projects like a nipple above the lateral canal. The true glandular portion is only represented by a few layers of cylindrical and ovoid cells.

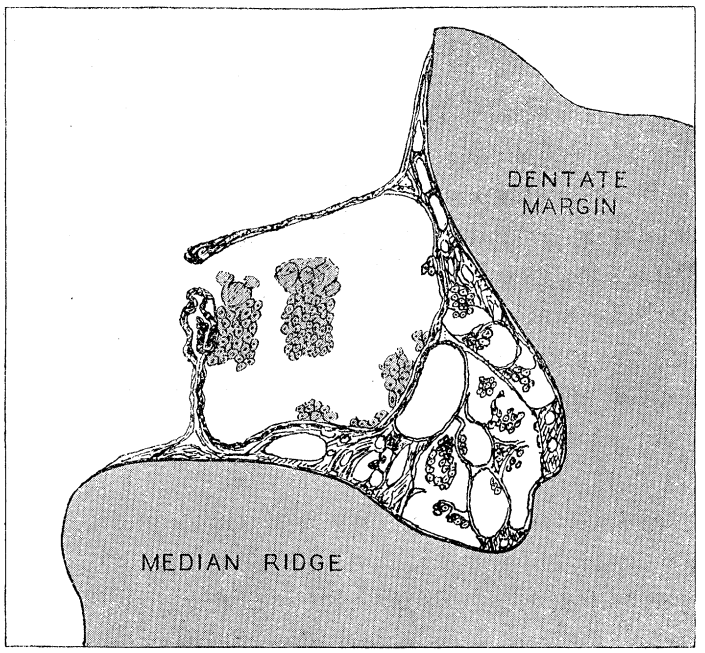
the deeper portion of the gland, and these columns tend to coalesce and cause a flow of secretion to be directed outwards towards the dentate margin (Plate 1, fig. 3).

The cells surrounding those which are active, show a peculiar appearance, being traversed by a network of fine pigment granules, which are nowhere to be seen in the secreting portion.

The origin of this epithelial structure can be seen in sections at the root. If we examine a section at a level where the spine is still attached by a narrow isthmus to the tail, we observe that there is a thick mass of epithelial tissue resting on a well-marked pigmented layer, forming here the epidermal layer of the tail. This layer of epithelium consists of cylindrical epithelium, covered with numerous layers of ovoid and round cells. It passes round the isthmus on to the tissue of the groove, so that the section gives the appearance of an invagination of epithelium on either side of the

tail. More distally the epithelium is, so to speak, split, leaving the opposing surfaces of tail and spine each covered with this special epithelial layer.

It is obvious that the epithelium of the glandular triangle is near the root somewhat protected by the opposing tail, but as the spine projects further from the tail the epithelium would be singularly unprotected if no contrivance were developed to protect it (text-figs. 2 and 3). Consequently, we find that towards the tip of the spine lateral flaps of tissue are provided, which overlie the epithelium. These flaps appear, in sections at the distal end, as two pigmented filamentous processes, which start on the inner side from the margin of the median ridge, and on the outer from the root



TEXT-FIG. 3.—Lateral Groove of Spine of Trygon (transverse section of distal portion). The deep portion of groove contains alveoli enclosing ovoid cells, the central and lateral canals and blood-vessels. It is separated from the true gland by a pigmented capillary layer which is prolonged into two processes which overlap masses of cells.

of the teeth; they are developed in connection with the lateral canals before mentioned. Their appearance in sections nearer the base is indicated by a nipple-like projection from the lateral canal. These processes and projections consist of a network of pigmented capillaries, and the filamentous processes often terminate in a glomerulus of capillaries. This appears to be the reading of a number of serial sections which give the width of successive flaps as from 2 to 3 mm. Sections showing the epithelium completely filling the space between the processes are wanting, but sections showing areas of cylindrical cell tissue and round cells, and secretion lying in the groove and covered in by the flaps, are not infrequent in the series. These sections, therefore, do not confirm PORTA'S description. He states that the poison

organs penetrate to the deepest part of the groove; this must mean the alveolar connective tissue portion. He describes his poison gland as consisting of a great many cells of various sizes and shapes, joined to one another so as to form true glandular follicles. He further speaks of a sheath of the sting, and states that the gland is similar to that found in Scorpæna, and that the poison is emitted by the pressing back of the sheath of the gland.

He does not describe the true epithelial structure lying over the pigment layer, which in the majority of specimens falls away in the processes of hardening, decalcifying, cutting, and staining.

The micro-photographs (Plate 1, figs. 1 and 2) show clearly the points which I have endeavoured to describe, and clearly do not give any support to the observations of PORTA.

At the same time they effectually nullify the views I held in 1916 as to the significance of the canals, nipple-shaped projections, and filamentous processes.

It appears to me that the specimens just described establish the following facts:—

1. That the specialised epithelium on the tail where the spine commences is the first appearance of the true glandular epithelium.

2. That the glandular epithelium occupying the more superficial portion of the glandular triangle of the groove is the essential poison-producing tissue.

3. That the mass of epithelial cells shows cells in an active state of secretory activity, and that the products of this activity are discharged towards the dentate margin.

4. That the pigmented capillary network is of the same nature as that described by MARIE PHISALIX in the Salamander.

5. That the deeper portion of the glandular triangle contains blood-vessels, and special lymph channels which supply the superficial portion with the necessary material for the secretion of the venom.

(2) *The Spines of the Dorsal Fins of the Spiny Dog-Fish* (Acanthias vulgaris). (*The "Picked Dog" or "Spur Dog."*)

I have not been able to obtain any paper dealing with this subject, although I have reason to believe that it has been worked at, as KOBERT (1905) refers to a paper about to be published on the spine of Acanthias. Acanthias has, as is well known, a sharp, strong spine in front of each of the two dorsal fins. The anterior spine is the longer, is slightly curved, and measures an inch or more in length in its exposed portion, while the posterior is nearly straight and is shorter—from half an inch to three-eighths of an inch in length; each spine tapers to a point. The posterior aspect of each spine is grooved longitudinally, the groove becoming more shallow towards its apex. The spine itself is roughly triangular in section, the sides of the triangle being convex on the two aspects which face anteriorly; the third side facing the fin margin being concave.

When the perfectly fresh spine is examined with the naked eye, each groove is seen to be occupied by a glistening, pearly-white substance, which extends to a variable distance towards the apex. Towards the base there may be seen in its centre a small linear depression, running longitudinally, about a sixteenth of an inch long, which is often discoloured, presumably by the presence of a secretion poured out by the glandular structure in the centre of which it lies. This may be looked upon as the orifice of the gland, through which its numerous follicles discharge their secretion; a similar method of glandular arrangement may be seen in the face gland of antelopes.

This pearly-white structure, removed in a fresh state from the groove, was teased on a slide. The teased gland showed follicles in which a layer of columnar epithelial cells could be demonstrated surrounding a central mass of cells and granular matter, taking up a yellow stain with VAN GIESONS' solution. Small detached portions showed columnar cells against a basement membrane, and upon these layers upon layers of round and cubical cells, some vacuolated and some distended with granular matter, in which the nuclei were just distinguishable.

The examination of 500 sections cut in series enables one to piece together the microscopic structure of this gland. It is fortunate that the spine has a large central cavity, so that the great part of the dentine can be readily filed off, leaving only the concave side of the spine, which can be further filed down without injuring the glandular structure.

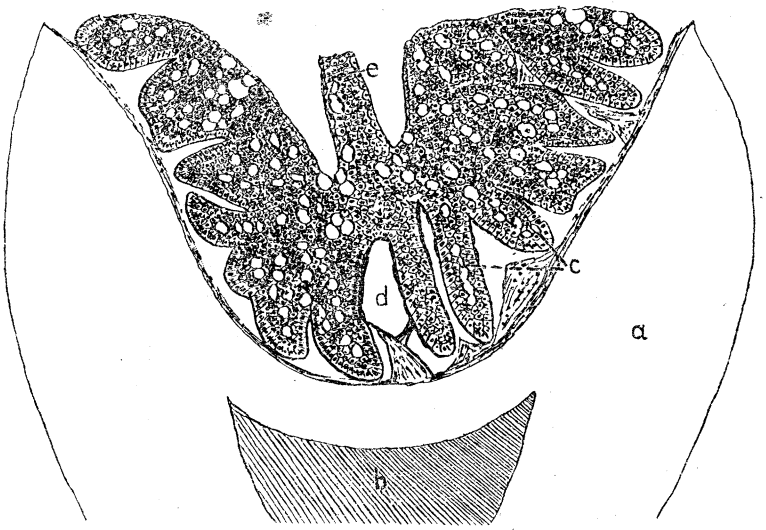
The process of decalcification is thus rendered comparatively easy, and it becomes possible to cut sections in series without having to cast aside many sections as valueless, through the tearing that results when masses of dentine have to be cut through.

If a section near the base of the spine is examined (Plate 1, fig. 4, and text-fig. 5), it will be seen that the gland is made up of follicles, large in the centre of the groove and becoming smaller towards the periphery. The follicles are arranged in a radiating manner, so that their blind, finger-like extremities rest on the floor of the groove. There is a mesh-work of connective tissue, containing one or more large blood-vessels, between the gland and the groove, which sends up supporting processes between the follicles. Each follicle has a layer of cylindrical cells resting on a basement membrane, and the rest of the follicle is occupied by masses of ovoid and round cells, which in many follicles are seen in an active stage of forming secretion. This will be more fully described later.

At this level the connective tissue sends up a median division, which divides the gland into lateral halves. This septum is acutely triangular in shape, with its narrow base attached to the centre of the groove, and consists of ordinary connective tissue, together with masses of cells arranged in alveoli; there may be two or three such alveoli, with cells showing a vacuolated appearance. This tissue is very similar to that which has been described above as the alveolar connective tissue in the deep part of the glandular triangle of Trygon; but whereas in the latter it is a very important structure, here the alveoli are few in number and less obvious.

On either side of this median septum is a clear space, probably a lymph space, which separates it from the wall of the two main follicles. The outer wall of this space is notable for the mass of pigment cells which line it. This lymph space in some sections is subdivided by partitions, but as one traces it towards the apex the median septum diappears, and there becomes one central lymph space, lined by pigment cells.

This layer of pigment cells is probably homologous to the pigment layer in Trygon, and the lymph space to the central and lateral canals of the same fish. If we now examine the secreting portion, we notice two large follicles on either side of the septum, more or less divided up by small divisions. The cells are massed together and show a few large vacuoles in the part nearest the middle line; the peripheral and



TEXT-FIG. 4.—Transection of Fin-spine of Acanthias, distal portion. *a*, dentine; *b*, central cavity; *c*, follicles of gland; *d*, central canal with pigmented walls; *e*, central column of cells separating two main ducts.

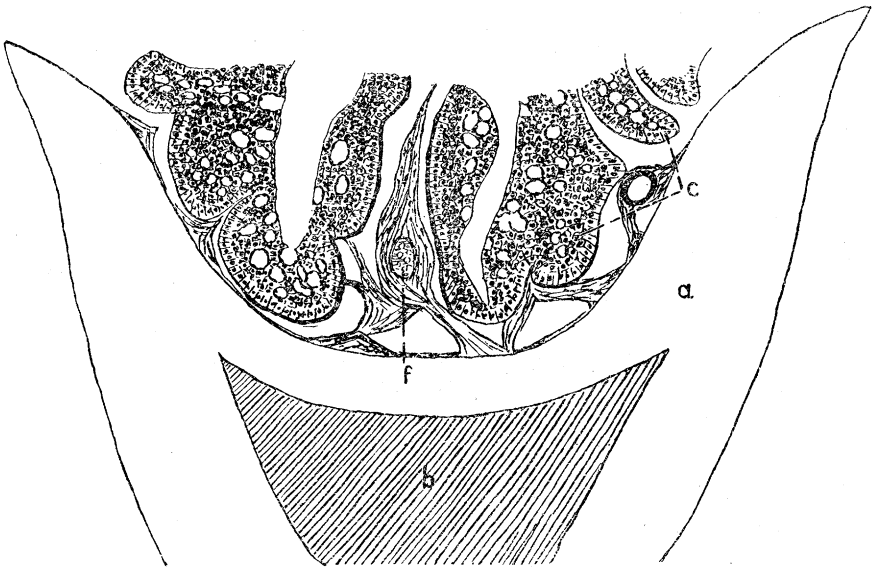
lateral portions show larger areas in which groups of cells have coalesced and become full of secretion, their nuclei remaining flattened on the margin of the vacuole.

Down the centre of each large follicle runs a well-marked duct, into which the vacuoles can be seen bursting and discharging their contents. The cells lining the duct are more or less flattened, but it seems that they are merely modified forms of the main cellular structure. On the periphery of each main follicle smaller follicles line the groove, which discharge their contents separately and directly on to the surface.

Passing towards the apex of the spine, it is noted (Plate 2, fig. 5, and text-fig. 4) that the central septum gradually gets smaller and dwindles until it is no more than the supporting fibrous tissue of the other follicles. But the lymph spaces continue to mark the separation of the gland into two lateral divisions, and they coalesce and form one large lymph space, passing to the extremity of the gland and lined through-

out with pigment. This pigment formation may also be noted in some of the supporting processes of the smaller lateral follicles. In those levels where the septum has ceased there is left a central column of secreting tissue, in which less vacuolation takes place than in the other portions of the gland. It can be seen by studying the series how the central pit, seen by the naked eye, is formed.

Passing now towards the base, the septum becomes thicker or wider in section and loses its triangular shape, in fact, becoming wider towards the surface. It contains more alveolar connective tissue, and finally it unites with, and takes part in, forming a process from the anterior margin of the fin which is inserted into the centre of the groove at the base of the spine. The epithelial structure here appears as two lateral masses spreading from the septum on to the surface of the fin in the angle where this



TEXT-FIG. 5.—Transection of Fin-spine of Acanthias, lettering as in fig. 4, proximal portion. *f*, central column of tissue dividing gland into two lateral portions.

fin joins the spine. It extends in a narrow layer on either side of this process of the fin, which gives off a number of dentinal projections, which serve to support the glandular tissue.

In the tissue beneath these processes are to be seen large masses of pigment cells; another parallel to the condition in Trygon at the commencement of the gland. In fact, if we imagine the central septum to become impregnated with dentine and enlarged, we should have an arrangement of gland on either side of a median ridge, as in Trygon. The only difference would be that, whereas in Trygon there is one epithelial mass, here there is a marked follicular gland with a primitive type of duct.

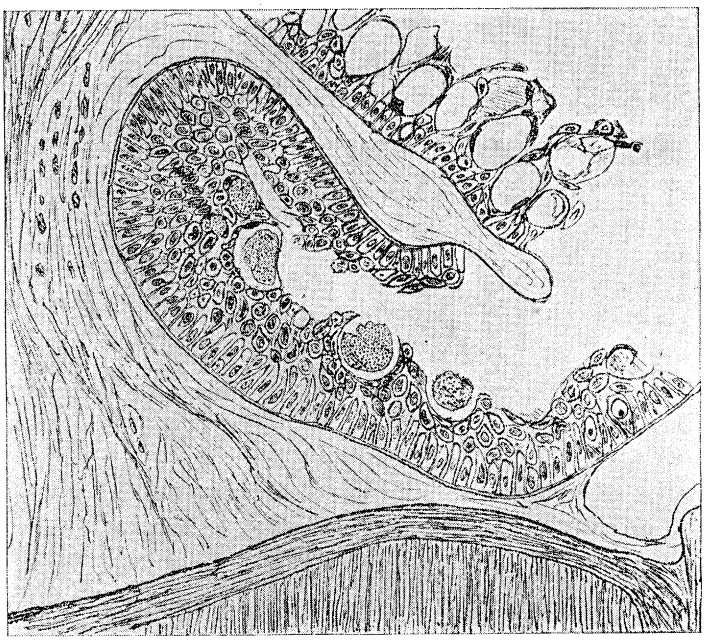
If we now examine the secreting portion of the gland under an oil immersion lens, Plate 2, fig. 6, we notice groups of ten or a dozen cells packed together, the central cells of a group showing an increase of protoplasm and deficient nuclear staining. This

stage advances until all the cells are distended and occupied with secretion so as to form a circular vacuole surrounded by flattened nuclei, eight or ten in number. These vacuoles group themselves so that a follicle may be occupied by 15 or 16 large vacuoles, which finally coalesce and discharge on to the surface.

The nuclei, it may be stated, in some sections shows marked activity, as exemplified by the presence of mitotic figures.

(3) *The Gland in Groove of the Fin-spine of* Cestracion philippi, *the Port Jackson Shark.*

The demonstration of a follicular gland in connection with the dorsal fin spines of *Acanthias vulgaris*, and the evidence to be given of the venomous properties of the secretion discharged therefrom, opens up the question as to whether the spines, with



TEXT-FIG. 6.—Transverse Section of Spine of Cestracion, before its separation from the fin, to show the commencement of the gland, which consists of follicles with cylindrical and ovoid cells and masses of secretion in the lumen thereof.

grooved or flattened surfaces facing the fin, found in other fishes, are not possessed of similar specialised secreting structures.

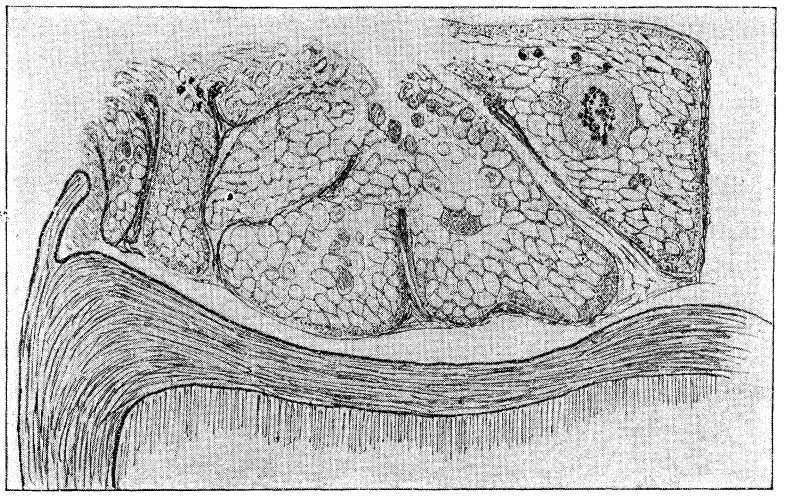
Through the kindness of Prof. GOODRICH, of Oxford University, I was enabled to obtain a specimen of the spines (dorsal) of *Cestracion philippi*, the Port Jackson shark.

The shallow groove of the spine of Cestracion is seen by the naked eye to be filled with some special epithelial or glandular structure. The spines of Cestracion are very

similar to the dorsal fin spines of Acanthias, but in the specimen I have examined they are shorter and stouter, and with a shallower groove.

I have cut serial sections of the spine, from the attachment of the fin at the base to the free portion of the spine. Starting at the proximal portion, Plate 2, fig. 7, the fin structure shows the commencement of a gland by the presence on either margin of its anterior border of one or two follicles. As one travels distally, these follicles increase in number and size, and their orifices open laterally in the depression between the fin and the spine. The follicles are here surrounded by fibrous and muscular tissue. A high-power examination, text-fig. 6, shows each follicle to be made up of a peripheral layer of cylindrical cells, then a layer of rounded and oval cells, and towards the centre large vacuoles are formed as in Acanthias, with flattened nuclei bounding the secreting area. There may be five or six follicles on each margin of the fin, bounded by a definite fibrous partition, the cells showing all the various stages of the process of secretion.

A section at the level at which the fin is inserted into the centre of the groove of the spine, Plate 2, fig. 8, and text-fig. 6 (*a*), shows that the gland here extends com-



TEXT-FIG. 6*a*.—Transverse section of half of the groove of Spine of Cestracion near its base, showing the marginal elevation which supports the glandular tissue. Note the circular granular mass in the follicle to the right.

pletely across the spine, but that a fibrous partition divides it into two portions, and that each lateral division tends to discharge its contents towards the margin of the spine, as the orifices of the ducts of the follicles all point externally.

The microphotograph, which accompanies this description, shows four follicles in one-half. The follicle situated most centrally shows, in addition to the large number of distended vacuoles, a central mass of secretion, circular in shape, with a number of round globules staining more readily than the rest of the homogeneous secretion. Other follicles, in addition to the circular vacuoles, show larger areas of granular

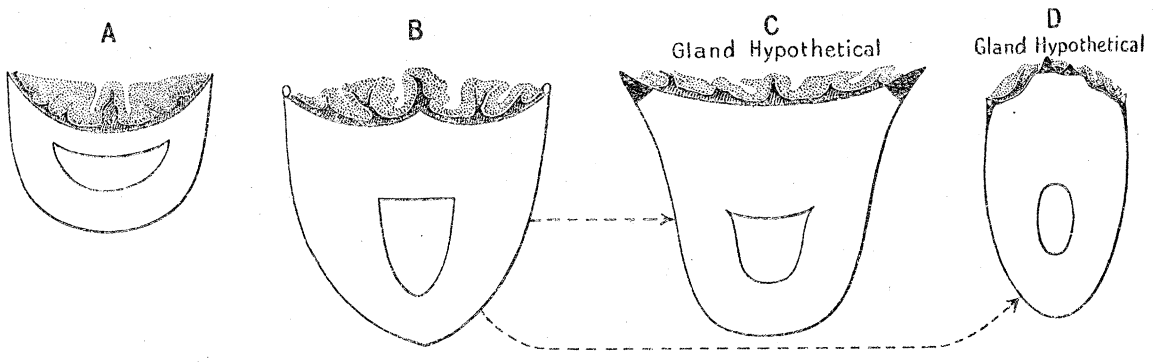
matter. The follicles are separated from each other by definite fibrous partitions. A section of the spine, about the middle of the free portion, shows that the glandular tissue is still present in a somewhat modified form. In the centre of the groove there are two fairly large follicles separated by a median partition and, laterally, there are three or more smaller glandular portions separated by dividing septa. There is a much greater proportion of cylindrical cells in each follicle than at the base, and they appear often to be spindle-shaped. These spindle-shaped cells are more numerous surrounding the tips of the septa, and at first sight might be considered the flattened cells of an epithelial layer. But a high-power drawing shows these spindle cells in various stages of distension towards the centre of the follicle, and the stages of distension, with the formation of masses of secretion, can be traced until no cell protoplasm can be distinguished.

An important point to be noticed in the spine of Cestracion is the shallowness of the groove compared to the groove in Acanthias. There is also to be noted a tendency towards a median elevation in the centre of the groove, and, finally, the presence of a small marginal elevation, a kind of bulwark of support to the cells of the glandular structure.

Comparing the gland of Cestracion to that of Acanthias, the drawing of the basal follicle shows a very similar structure, but the base of the gland in Cestracion consists of a number of follicles, while in Acanthias there is only an undifferentiated mass of epithelium. Further, the division of the gland into follicles is throughout more developed in Cestracion; the ducts are more developed, and the surface of the secreting portion, instead of being unprotected by any specialisation of cell structure, as in Acanthias, is in Cestracion protected by the flattening of many layers of epithelium around the orifices of the duct.

Finally, instead of there being a central pit in the centre of the secreting area, as in Acanthias, in Cestracion there are three or more separate orifices for the discharge of the glandular secretion on either side of the middle line.

It is interesting to apply the results of these observations to the spines of the



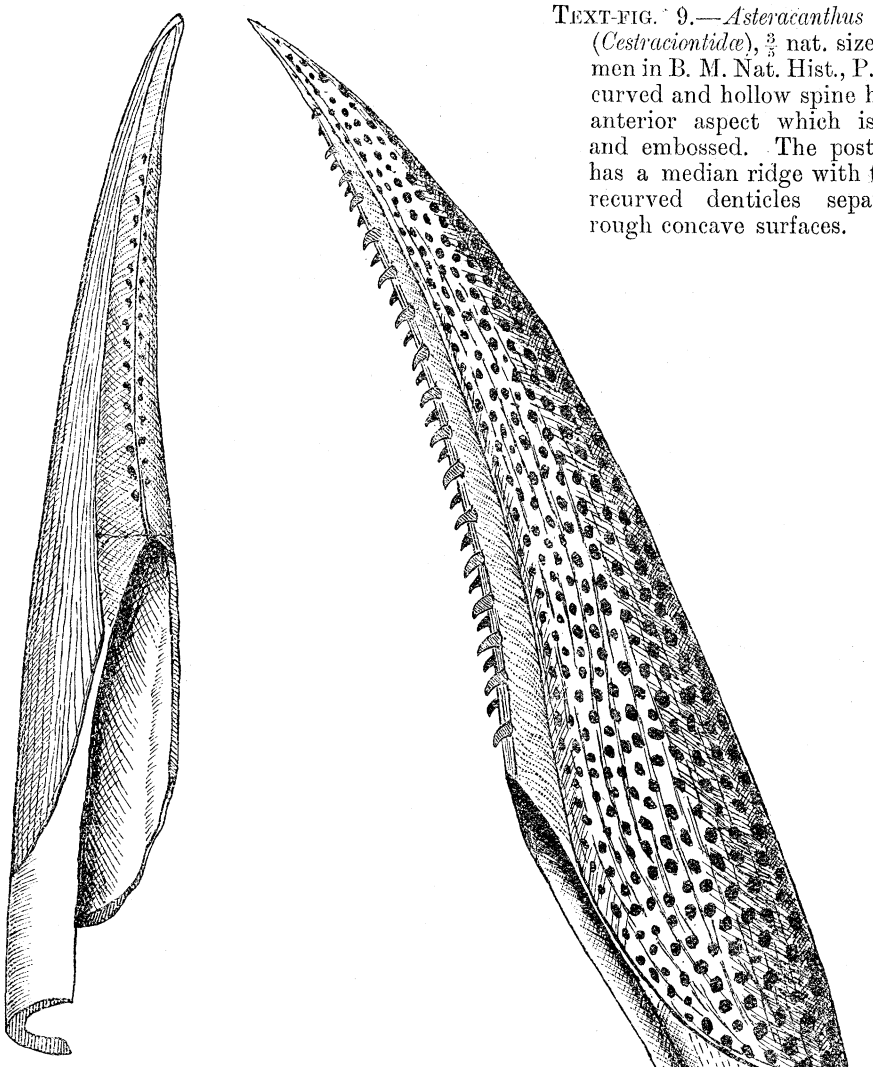
TEXT-FIG. 7.

palæozoic Heterodontidæ. I have studied the spines of the following extinct members of this group :—Palæospinax, Sphenacanthus, Asteracanthus and Hybodus.

Firstly, in Palæospinax, text-fig. 7B, we have a grooved spine with a smooth surface facing the anterior margin of the fin, just as in Cestracion.

Next, we have Sphenacanthus, text-fig. 7C, with a grooved spine, the margins of which are armed with teeth. These teeth, I picture to myself, as being rendered more formidable by the outward-flowing secretion from the follicles of the groove.

Finally, in Asteracanthus and Hybodus, text-fig. 7D, we have a median ridge on which are recurved denticles in two rows, bounded by a shallow-grooved smooth surface,



TEXT-FIG. 9.—*Asteracanthus ornatissimus* (*Cestraciontidæ*), $\frac{2}{5}$ nat. size, from specimen in B. M. Nat. Hist., P. 6867. The curved and hollow spine has a convex anterior aspect which is enamelled and embossed. The posterior aspect has a median ridge with two rows of recurved denticles separating two rough concave surfaces.

TEXT-FIG. 8.—Hybodus (Hybodontidæ), $\frac{5}{8}$ nat. size, from specimen in B. M. Nat. Hist. The hollow spine is enamelled and longitudinally ridged on its anterior convex aspect. The posterior aspect presents a median ridge with two rows of denticles separating two roughened slightly concave surfaces.

while the rest of the spine is enamelled with ribs or minute bosses in the manner of a barbaric spear. I take the median ridge to be an accentuation of the median elevation in the groove of Cestracion, which becomes surmounted by denticles, just as the margin becomes dentate in Sphenacanthus.

The drawings of the spines of Asteracanthus, text-fig. 9, and Hybodus, text-fig. 8, show the points mentioned very clearly, and the diagrams of the development of the spines of Heterodontidæ explain the hypothetical existence of a glandular structure on their posterior aspect.

(4) *The Holocephali.*

Through the kindness of Dr. RUSSELL, of the Board of Agriculture and Fisheries, I have obtained specimens of *Chimæra monstrosa* caught in the neighbourhood of Lousy Bank in the North Sea; and Prof. GOODRICH has again assisted me by presenting me with a well preserved specimen of the same fish caught in the Mediterranean Sea, near Messina. The development and structure of the dorsal fin spine in Holocephali are very different from those seen in the sharks. The spine is articulated to the neural apophysis, and both it and the fin (which closes like a fan) are received into a deep groove on the dorsum of the fish.

The spine has a cartilaginous matrix, and is covered with a layer of dentine. There is a shallow groove on the posterior aspect facing the anterior margin of the fin, and the margins of the groove are armed in *Chimæra monstrosa* with small denticles.

The spine is oval in section, with the posterior portion cut off and grooved, and the anterior aspect is keeled; in *Chimæra* this keel is smooth, but in the South Pacific genus, *Callorhynchus*, this keel is armed with small teeth, and this is also the case with the fossil *Myriacanthus*, which is thus described by Prof. SMITH-WOODWARD in EASTMAN'S translation of ZITTEL'S 'Palæontology': "Dorsal fin spine long and slender, somewhat laterally compressed, with a large internal cavity; sides ornamented with small tubercles; a series of large thorn-shaped tubercles arranged on either edge of the flattened posterior face, passing into a single median row distally, and a single series of similar denticles on the anterior border. It dates from the Jurassic period, and specimens have been obtained from the Lower Lias of Lyme Regis."

Chimæra affinis, a form closely allied to *Chimæra monstrosa*, has a less developed spine, inasmuch as the anterior surface is rounded, not keeled.

Harriotta has an immense triangular spine in front of its first dorsal fin, finely serrated upon its lateral edges.

I have cut serial sections of specimens of the spine of *C. monstrosa*. There is a layer of deeply pigmented tissue on either side of the anterior keel, which is covered with layers of epithelium.

The groove on the posterior aspect has a specialised tissue, the nature of which is obscure in its central portion, but near to each serrated margin is an area of epithelium which in some sections shows as a true follicle, and lies close to the base of a denticle.

This epithelial patch does not seem sufficient in extent or depth to justify the assumption of the possession of a special function, and further research on the point is necessary. The sections of the adjacent margin of the fin, and in fact a naked eye examination, shows this margin to be occupied by a special development of the epidermis, which appears as a narrow, glistening, white band. The sections show this to be produced by the splitting of the anterior margin by two deep longitudinal grooves which contain a mass of epithelium, containing large vacuoles, and covered by glistening patches of coagulated secretion.

Each groove faces the margin of the spine, and when the fin closes down, and the spine presses down on to the closed fin, each dentate margin fits into a recess formed by the grooves, and a channel is produced along which the secretion from the glandular structure of the recess would percolate along the dentate margin.

It is an interesting fact to note that the least specialised *C. affinis*, that is to say, least specialised as regards the absence of an anterior keel, which would appear to be the forerunner of a dentate margin, and the lesser specialised *C. monstrosa*, with a keel which is not armed, should be the surviving members of this order, while the Jurassic Myriacanthus should have possessed the more highly developed spine with the three dentate margins, just as the recent Callorhynchus.

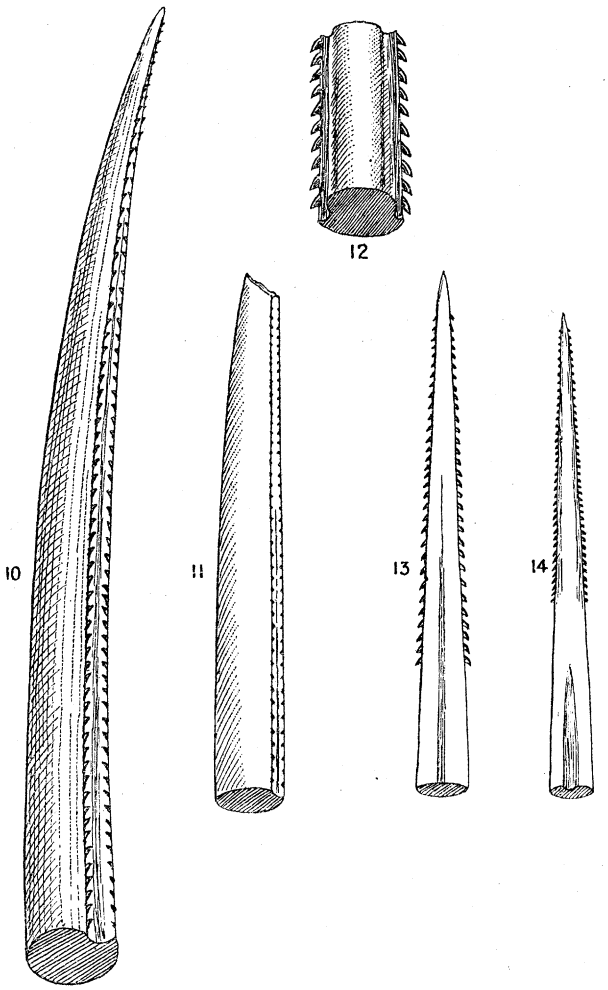
V. Head Spines of the Pleuracanthidæ.

Dr. Smith-Woodward has demonstrated to me the head spines of various members of the family of Pleuracanthidæ, which are hollow, and may be rounded or antero-laterally compressed, and bear a double longitudinal series of recurved denticles.

For example, *P. cylindricus* (P. 8113–15) from the Coal Measures has a cylindrical spine 16 inches long, text-fig. 10, with a smooth flattened area on its posterior surface, bounded by a dentate margin extending for $8\frac{1}{2}$ inches. The flattened or slightly concave area is 1/5 inch wide at the base, and there are 8—10 teeth to 1 inch. The rest of the spine has an enamelled surface finely striated with longitudinal ridges. *P. kounoviensis* (47,843) from the Lower Permian, text-fig. 11, has a spine flattened laterally to a slight extent, and there is a posterior groove with lateral denticles. *P. woodwardii* from Devonian Coal measures, text-fig. 12, is flattened antero-posteriorly, and has a groove on either side adjacent to the lateral teeth. This leads one imperceptibly to the flattened spine of *P. lævissimus*, text-fig. 13, and *P. elegans*, text-fig. 14.

There is a specimen of a spine of *P. elegans* at the British Museum in South Kensington, No. 11,366, from the Carboniferous limestone. It has an enamelled, flattened, slightly convex, anterior surface. At the proximal end of the posterior surface there is a depressed portion; on this surface, just internal to each lateral row of recurved denticles, there is a definite groove, longitudinally striated and roughened, and indistinguishable from the groove on either side of what I term the median ridge of a Trygon spine.

Now we know definitely that this groove in Trygon is occupied by a secreting epithelium, characterised by columns of secretion directed towards the denticles, and it is a fair conclusion to infer that this groove in *P. elegans* and *lævissimus* was in the



TEXT-FIGS. 10–14.—Pleuracanthidæ, ¾ nat. size. 10. *Pleuracanthus cylindricus*, Coal Measures, B. M. Nat. Hist. (P. 8113–15); almost cylindrical, but posteriorly there is a shallow groove bounded by two rows of small denticles. 11. *P. Kounoviensis*, Lower Permian, B. M. Nat. Hist. (47483); very similar to Fig. 10. 12. *P. Woodwardii*, Coal Measures (11193); on either side of a spine which is oval in section there runs a groove which is bounded by a row of sharp recurved denticles. 13. *P. lævissimus*, Coal Measures (P. 8125); ant. aspect. 14. *P. elegans*, Carboniferous Limestone (11366); post. aspect. These spines (13 and 14) are very similar to those found in the Trygonidæ.

fresh state also occupied by similar tissue ; and if this assumption is correct, we may also infer that the smooth surface between the spines in *P. cylindricus*, *P. kounoviensis*, was also covered with glandular epithelium.

(6) *The Poison Gland of* Trachinus draco (*the greater Weever*).

The anatomy of the poison gland found in connection with the dorsal fin and operculum of *Trachinus draco* has been so often described and illustrated both in monographs and text-books that nothing further on this point need be written.

But some points in connection with the histological appearances, which are seen in a gland fixed in the state of active secretion, deserve a brief mention.

Plate 3, figs. 9 and 10 are from sections of the gland of the opercular spine at the level of what BOTTARD (1889) calls the conical cavity. The periphery of the gland in section consists of very large cells, the nuclei of which are difficult to demonstrate: these cells are filled with a substance of a highly refractile nature.

Towards the centre globules of this material are seen to be discharged into the lumen of the gland. The globules gradually become less refringent and lose their staining power, and finally become part of the granular material that is seen to pass towards the groove of the spine. This section was stained with hæmatine and counter stained with VAN GIESSONS' solution.

The contents of the cells stain a brilliant yellow, while the granular secretion appears of a bluish colour. The effect of the section is much diminished by the absence of the colour differentiation of tissue produced by staining.

III. PHYSIOLOGICAL ACTION OF FISH VENOMS.

(1) *The Venom of the Weever* (Trachinus draco).

The venom of *Trachinus draco* (the greater Weever) has been studied by GUNTHER, GRESSIN, BOTTARD, PHISALIX, and more recently by KOBERT and A. BRIOT. A. BRIOT (1902) removed the venomous spines, powdered them in a mortar and mixed the mass with pure glycerine. He obtained, after filtration through paper, a toxic fluid which kept perfectly and was neutral to litmus paper. The following is his account of its action.

A few drops of this maceration was sufficient to kill a guinea-pig. Injections into the thigh caused paralysis of the foot with tetanic convulsions; 24 hours afterwards a slough appeared and death followed in two to three days.

Two or three drops injected into the marginal vein of the ear of a rabbit caused death in 4 to 10 minutes from asphyxia. The heart continued to beat some time after respiration had ceased. The blood was not coagulated. The toxicity of the venom was completely destroyed by heating to 100° C. by chloride of lime and by chloride of gold.

The venom of the Weever dissolved the red corpuscles of the horse in the presence of heated horse serum, but it did not dissolve them in the presence of fresh serum. Unheated serum, therefore, contained, as CALMETTE had shown in connection with the action of cobra venom on the blood, a natural anti-hæmolysin. BRIOT has immunised rabbits and obtained a serum capable of neutralising the venom *in vitro*, and has also

immunised nine rabbits against doses several times larger than a fatal dose given intravenously.

GRESSIN (1884), quoted by CALMETTE, describes the acute stabbing pain the result of a Weever prick in man. Fainting and actual syncope may result. The limb swells rapidly, and inflammation and even gangrene may follow. Fever, delirium and bilious vomiting has also been noted.

Physical Properties.—If one introduces the needle of a small exploring syringe along the groove of the opercular spine, one can withdraw a drop of the venom. The appearance of this varies according to whether it is extracted from the living or dead fish. From a recently killed fish it is rather of a milky aspect; under low power one sees floating in the plasma, refringent cells, and a large number of large cells with nuclei and nucleoli mixed with granular matter; strong acids and alkalis coagulate it; and it is coagulated by heat. If mixed with distilled water, it gives it a soapy tint. If the venom is completely dried in a vacuum desiccator over sulphuric acid it keeps indefinitely, and can be redissolved in water or normal saline. Mixing it with a small quantity of pure glycerine also preserves it.

Effects on Man.—I have had frequent opportunities of studying the effects of a severe Weever sting in man. The first symptom is acute pain of a burning, stabbing character. If untreated this lasts for a few hours or throughout the whole day. Fishermen believe that the pain does not subside until the next tide. The pain is so acute that men have been known to try and throw themselves overboard when at sea.

A young fisherman was brought to the local hospital to have his hand dressed, and several hours afterwards he was brought back by the Police in a raving state. To ease the pain fishermen will scorch the injured part; they will wrap a piece of paper soaked in vinegar round the part and set it on fire, and they will endeavour to numb a finger by hammering it with a thole or belaying pin. Associated with the pain there is often a tendency to faint, while others complain of palpitation. Almost immediately after the part is stung, there is local swelling, spreading up the arm if the hand is injured. The acute inflammatory œdema may lead to an acute cellulitis with enlargement of the glands and general toxæmia. I have seen a man lose his life as the result of an acute cellulitis, and the maiming of a hand is quite a frequent result.

A rarer complication is the appearance of hæmorrhagic patches; these I have seen on the forearm of a small boy who was badly pricked on the hand by the lesser Weever (*Trachinus vipera*); they occurred 24 hours after the injury.

Effects on Animals with Artificial Injection.—The effects on inoculation of fish with Weever venom are as follows:—If inoculated in the region of the lateral line on one side, the fish at once darts about and in a few seconds the movements cease; it lies on one side, bent towards the side opposite to the injection, in consequence of the predominating action of the muscles of the unaffected side. The movements of

respiration are markedly affected, and the heart continues to beat after the respiration has ceased.

If the fish does not die from the immediate effects a local abscess forms, which is due to a pure necrosis of the muscles. The *post-mortem* appearances of a fish killed by inoculation, shows local extravasation of blood, hæmorrhage into the peritoneal cavity, congestion of the branchiæ, hæmorrhage into the pericardium, distension of the gall-bladder and bile-staining of the right lobe of the liver. The blood is not coagulated in the heart.

The lethal dose of my dried venom was 0·01 grm. for a gold-fish 4 inches long; death ensued in 2 hours. 0·005 grm. caused death of a fish of the same weight in 8 days.

A frog inoculated with 0·015 grm. of dried venom died in 2 hours with paralytic symptoms. A mouse injected with 0·02 grm. dried venom died in 2 days; the day after the injection, which was made under the skin of the back, there was marked paralysis of the hindquarters and local hæmorrhage at the site of inoculation.

The effect on birds is interesting. Smacksmen often throw live weevers overboard, and if one is seized by a gull, they tell me, the gull falls into the sea as if struck dead.

A guinea-pig inoculated with 0·05 grm. dried venom intra-peritoneally died in 3 hours. After preliminary pallor and convulsions the animal became gradually weaker and showed paralysis of the hindquarters. After two hours the heart could not be felt beating, but catchy respiration continued for another hour. Intravenous injections in the rabbit and cat, which were made by Dr. CHARLES BOLTON, with a solution of my dried venom, caused a slight preliminary rise of blood pressure, followed by a very rapid and deep fall. A cat weighing 3400 grm. died after the injection of 0·1 grm. dried venom, the heart ceasing to beat in two minutes, although respiratory movements continued for another two minutes. The details of the observations just described, with tracing of carotid blood pressure and respiratory movements are contained in a paper I wrote for the 'British Medical Journal' (1907).

Hæmolytic Action.—BRIOT (1902–3–4) has repeated CALMETTE'S experiments on cobra venom with Weever poison. In a series of test-tubes he put a drop of washed corpuscles suspended in 2 c.c. of normal saline. He then added serum heated to 60° C. for one hour and varying doses of his solution of Weever venom. Complete hæmolysis took place in $1\frac{1}{2}$ hours. Two controls were made, one without the venom and the other without heated normal serum. In neither tube did solution take place, nor did it take place when unheated serum was added. BRIOT also states that the hæmolytic effect of the venom remains intact after heating for an hour at 75° C., and that even after it had been heated for 20 minutes at 100° C. dissolution of the red cells took place, though slowly.

My first experiments were made with the blood of roach and perch. I took 2 minims of a solution of equal parts of normal saline and Weever venom, extracted with a syringe from the opercular spines, and normal saline, in which was suspended

five drops of the nucleated red cells of the roach, which had been washed and centrifugalised three times with normal saline. Solution of the red cells began in from one to two hours, and no formed elements remained a few hours after commencing the experiment. There was no hæmolysis in the control.

The same result was obtained with perch blood. I then proceeded to test the blood of pigeon, guinea-pig, sheep, horse, ox, and man, and I employed the more exact methods as described by MORGENROTH in his article in EHRLICH'S volume of 'Collected Studies on Immunity.'

The blood, having been defibrinated, was centrifugalised to remove the serum, which was carefully pipetted off; 10 c.c. of normal saline was added to 0·5 c.c. of corpuscles and well mixed, centrifugalised, and the clear solution pipetted off. This was repeated three times and finally a 5 per cent. emulsion of red corpuscles was made in 0·85 per cent. salt solution. Either 2 c.c. or 1 c.c. of this solution was put in a series of tubes, the poison added in varying doses, and the mixture put into the incubator for two or more hours at 37° C. They were then allowed to settle in a cool place for several hours.

With pigeon blood one drop of a 50 per cent. solution of fresh Weever poison was sufficient in two hours to produce marked hæmolysis. The poison solution used was obtained by extracting the venom with a needle and syringe previously sterilised. In order to get all the milky fluid from the syringe, an equal quantity of salt solution was added to wash it out, so that a 50 per cent. solution of fresh poison was obtained. This was kept in a cellar on a block of ice in the dark, and its virulence remained intact. From a great number of experiments I found that uniform results were obtained if the poison was removed from freshly-caught fish and extracted at once. It appears, as the fishermen state, that the poison loses its effect soon after death. With one specimen of venom I got most contradictory results, due to my using a batch of 100 fish which were not quite fresh, although they had been kept on ice.

With guinea-pig corpuscles, two drops of 50 per cent. Weever poison were added to one tube containing 2 c.c. emulsion, and to two other tubes the same quantity of Weever poison, with in one tube some normal serum, and in another serum heated for an hour at 62° C. In all three tubes marked hæmolysis took place; in the control, none.

A similar experiment was made with sheep's blood. In this case, with 5 per cent. emulsion and two drops of 50 per cent. Weever poison there was advanced hæmolysis, and with the same quantity of poison, with both normal serum and serum heated for an hour at 62° C., there was complete hæmolysis after 2 hours in the incubator at 37° C. We have here a very marked example of the amboceptor of Weever poison combining with the endocomplement of the sheep corpuscles, resulting in rapid hæmolysis. The increased hæmolysis with the added serum was not affected by heating to 62° C.

With human blood we also find that there is hæmolysis of the washed corpuscles

without the addition of serum. But, unless the venom is quite fresh, one drop of the 50 per cent. solution is not sufficient to produce hæmolysis, although two drops suffice. It seemed important, in the light of BRIOT'S investigations previously referred to, to be quite sure of this. I have made seven separate experiments with human blood. The following experiment was very conclusive :—

Six tubes, each containing 1 c.c. of 5 per cent. washed human corpuscles, were numbered 0, 1, 2, 3, 4 and 5 ; 0 was the control—

1 contained 1 drop of 50 per cent. Weever poison.
2 ,, 2 drops of 50 ,, ,, ,,
3 ,, 3 drops of 50 ,, ,, ,,
4 ,, 4 drops of 50 ,, ,, ,,
5 ,, 2 drops+0·1 c.c. normal serum.
0 underwent no change after 2 hours at 37° C.
1 showed medium hæmolysis with dark red sediment.
2 showed well-marked hæmolysis with dark pink sediment.
3 ditto.
4 showed very marked hæmolysis, almost complete, the sediment being merely a faint pink.
5 showed complete hæmolysis ; sediment yellow.

There seems no doubt that Trachinus venom can dissolve human corpuscles without serum, and that normal unheated serum does not delay, but rather increases, the solution. Experiments were made to endeavour to activate venom, insufficient in quantity to dissolve the human corpuscles, by means of sheep serum and ox serum. A venom of which one drop did not dissolve washed human corpuscles was mixed with 0·3 c.c. sheep serum, and a control was made with sheep serum only. The result was that with one drop of Weever poison in 0·3 c.c. sheep serum there was marked hæmolysis ; the control showed agglutination but very faint hæmolysis.

Horse's blood gives the hæmolytic reaction with washed corpuscles alone. Seven tubes were filled with 1 c.c. of 5 per cent. emulsion. 1 w.p. = 1 drop 50 per cent. Weever poison :—

With 1 w.p. slight hæmolysis.
With 2 w.p. well marked hæmolysis.
With 2 w.p.+0·05 c.c. normal serum, strong hæmolysis.
With 2 w.p.+serum heated for half an hour at 62° C., slight. The controls were all negative.

Ox blood gave similar results :—

With 2 w.p. well marked hæmolysis.
With 2 w.p.+0·5 c.c. 1/10 normal serum, well marked hæmolysis.
With 2 w.p.+0·5 c.c. 1/10 heated serum, strong hæmolysis.

The results of these experiments establish (1) that Weever venom has a hæmolytic property capable of dissolving washed corpuscles of all the bloods, so far experimented on, without the addition of serums. (2) That the addition of the serum of the same animal does not inhibit, but rather increases, the activity of the venom. (3) That in most cases, heating the serum for an hour to 62° C. diminishes its activating power. (4) That minimal doses of the venom can be activated by the serum of other animals in the case of man, or the corpuscles are rendered more susceptible to the poison. (5) That probably the poison acts as an amboceptor, which unites with the endocomplements of the blood cells.

It seemed important to investigate this matter further, as I did not feel content with the explanation of PRESTON KYES in regard to the phenomenon of hæmolysis by venoms without added serums—namely, that the toxin (in his case of cobra venom) was an amboceptor, which was complemented by the endocomplements of the red cells. PRESTON KYES (1902) explained the different action of the same venom on the blood of different animals by assuming that there were two groups of blood cells: (1) those that are in themselves dissolved by cobra venom; (2) those that are only affected by venom after the addition of other substances, complements, etc. In the case of Weever venom, according to my experiments, there was but one group of blood cells, belonging to Group 1, and therefore we must assume, according to his amboceptor theory of the nature of venoms, that the blood of pigeon, guinea-pig, sheep, ox, horse and man contained endocomplements to Weever poison.

The difference between my results and those of BRIOT (1904) might depend on the method of preparation of the toxic solution, or on its freshness, as Weever venom seems to be of a very unstable nature. As was pointed out at the time, it seemed that my method of extracting the venom from the opercular glands with a syringe was more likely to yield a pure solution than the removal of spines and glands with scissors, maceration in glycerine, and filtering through filter paper, the method employed by BRIOT.

In order to throw further light on the difference between my results and those of the last-named observer, I have made a series of experiments, mixing my extracted venom with glycerine, and filtering it through filter paper or through a Berkefeld filter. In this way a toxin giving similar reactions to BRIOT's was obtained.

I will quote one experiment, made with glycerinated venom filtered through paper, and another filtered through a Berkefeld filter.

In each tube, 2 c.c. 5 per cent. human red corpuscles, washed with normal saline three times and centrifuged. The solution of venom was made by extracting the poison with a syringe from 100 fresh Weevers, and mixing it well with 30 minims pure glycerine (four drops of the unfiltered glycerinated venom gave immediate complete hæmolysis). The mixture was then filtered through paper.

The tubes (see Plate 3, fig. 11, photographic record of this experiment) were numbered I to V :—

I. Control after incubation. *No hæmolysis.*
II. +fresh Weever venom after 2 hours' incubation at 37° C. *Complete immediate hæmolysis.*
III. 1 minim filtered glycerinated venom after incubation. *Slight hæmolysis.*
IV. 1 minim filtered glycerinated venom+a trace of normal serum after incubation. *Very faint hæmolysis.*
V. 1 minim filtered glycerinated venom+a trace of serum heated to 60° C. for half an hour after incubation. *Almost complete hæmolysis.*

This experiment is in almost complete accord with BRIOT'S results. It was also found in other experiments that a solution of lecithin was able to take the place of heated serum. The following experiment was made with glycerinated venom filtered through a Berkefeld filter.

The venom of 100 Weevers was mixed with 100 minims pure glycerine ; this was passed through a Berkefeld filter. The first filtrate of 20 minims gave negative results. The further filtrate was tested with normal serum, with serum heated half an hour at 60° C., and with a solution of lecithin.

After Incubation Two Hours at 37° C.

5 minims alone.	5 minims + normal serum.	5 minims + serum heated at 60° C.	5 minims + solution of lecithin.
Slight hæmolysis	No hæmolysis	Marked hæmolysis	Marked hæmolysis.

This result is in complete accordance with those published by BRIOT, and show the activating effect both of heated serum and of a solution of lecithin.

BRIOT (1902-1904) in his article stated that snake venoms dissolve the red corpuscles of those animals which are sensitive to the poison. But if the corpuscles are washed repeatedly in physiological salt solution, hæmolysis does not take place. It is necessary to add normal serum. CALMETTE (1902) has shown it is further necessary to heat the serum to 62° C. in order to produce hæmolysis, because normal serum contains a natural anti-hæmolysin, capable of protecting the red cells against the solvent action of the venom. According to BRIOT, the same phenomenon occurs with Weever venom, namely, that no hæmolysis takes place without the addition of heated serum or a solution of lecithin. FLEXNER and NOGUCHI (1902) state that snake venoms are made up of a number of substances acting after the manner of amboceptors, which are activated by certain complements of the serum.

PRESTON KYES (1903) describes two groups of blood cells, those that are dissolved

by cobra venom, and those only affected by venom after the addition of other substances, such as serum heated to 60° C., or a small quantity of a 1 per cent. solution of lecithin in methyl alcohol, such as to make 1 in 10,000 solution of lecithin in the normal saline. The lecithin, or the lecithin-content of heated serum, combines with the venom to produce a hæmolysing lecithid, more resistant to heat than its components. It can withstand heating to 100° C. Certain kinds of red cells, very sensitive, although washed and deprived of serum, are hæmolysed without any added substance. This is because these corpuscles contain sufficient lecithin to unite with the venom and so produce a hæmolysing compound.

It has been held that lecithin in natural conditions, whether it is found normally in activating sera, as that of the horse, which can be heated to 65° C., plays the part of complement according to EHRLICH'S theory, or of alexin after BORDET'S theory, while the venom acts as an amboceptor or *substance sensibilatrice.* But, as CALMETTE points out, one cannot thus explain the phenomenon, because one cannot admit the identification of heated serum or lecithin with complements or alexines, the essential character of which is thermolability (heating to 58° C. destroys them, or simply exposure to air and light for several days).

One must therefore admit with PRESTON KYES that there exist in red cells themselves substances playing the part of complements, and that it is with these that the venom combines when it is activated by heated serum or lecithin. These theoretical considerations have a great interest in the light of the experiments which I have made in order to explain the difference between my results and those of the French observers.

It will be remembered that the French observers always used a filtered venom, and were unable to obtain hæmolysis without the addition of heated serum or lecithin, while my venom hæmolysed all the types of red cells without any addition.

Accordingly I tried the effect of filtering my venom through filter paper, and through a Berkefeld filter. In this way I was able to obtain a venom similar to that of BRIOT and PHISALIX which did not hæmolyse without added serum, heated to 60° C., or a solution of lecithin, as described in the experiments above detailed.

It is clear from these experiments that filtration removes from the venom a substance which enables hæmolysis to take place without any addition, and that the *rôle* of this substance can be played by the lecithin-content of heated serum or a solution of lecithin.

I have not been able to isolate this substance, as the precipitate which is obtained by washing out that, which is held back by the filter, with normal saline, and adding an excess of alcohol, proved to be difficult to re-dissolve in water or normal saline.

All one can say for the present is that unfiltered venom acts like a lecithid, and combines with the endocomplements of the red cells to produce hæmolysis. Considering the ease with which large quantities of Weever venom can be obtained, further investigation should be a fruitful field of research.

Action on White Corpuscles.—In studying the destructive effect of Weever venom

on white blood corpuscles, or the leucotoxic effect, I have modified WRIGHT'S method for the determination of opsonin. In making these experiments I took a volume of washed corpuscles, plus serum, plus a bacterial emulsion of *Staphylococcus aureus*, and a volume of salt solution in one capillary tube, and in the other the washed corpuscles, serum, bacterial emulsion, and salt solution containing a drop of venom. After incubating for a quarter of an hour, the white cells in the poisoned tube, when stained in a film by JENNER'S stain, showed vacuolation, deficient staining, and a marked diminution in the phagocytic action, 25 per cent. fewer bacteria being present in the count. As the bacteria stained equally well in each film there was no reason to assume that this was due to bacteriolysis, and not to a paralytic effect on the white cells. Considering how frequently secondary septic inflammations result from the sting of these fish, the experiments would show how readily the barrier of phagocytosis may be broken down, so that the pathogenic organisms from the dirty skin may find an entry into the system.

(2) *The Venom of* Acanthias vulgaris.

That there is some doubt about the poisonous nature of the dorsal fin-spine of Acanthias is evident from the following quotations from 'Les Animaux venimeux,' by M. PHISALIX (1922) :—

"Lastly, BOTTARD has again examined from the point of view of the existence of a poison organ those sharks provided with spines such as *Cestracion*, *Chimæra* and *Acanthias*" (p. 496) with negative results and on p. 515 "BOTTARD has not found a poison gland in connection with the spines of *Acanthias*."

"Nevertheless DAMPIER has seen at Santa Clara, near Guayaquil, some members of the family which possess spines which may cause fatal injuries."

Effects on Man.—An intelligent fisherman has shown me the scar on the last phalanx of the thumb which resulted from the prick of a dog-fish in October, when he was 12 years old. The pain was as bad as that from a Weever, but was of a duller and more numbing character. The pain lasted 2 to 3 hours. The thumb gathered, and he was ashore a month as the result of the prick. The smacks usually carry a dog, more often a retriever, and he tells me that "dogs always go for dog-fish."

He has seen a "black retriever attacked by a dog-fish, and the spine broke off right in his nose: this caused the dog acute pain and inflammation ensued."

Other fishermen have related very similar experiences.

Towards the end of October, 1919, a fisherman came up to the Lowestoft Hospital complaining of a poisoned hand. There was a punctured wound at the base of the thumb. Six hours previously he had been pricked by a dog-fish. The injury was followed by acute stabbing pain in the part which lasted 4 or 5 hours; the hand then began to swell, and when he arrived at the hospital there was great swelling and œdema of the back of the hand, and the front of the wrist and forearm were painful, tender, red and œdematous.

This acute inflammatory œdema lasted for 4 days and for a time it seemed that suppuration would occur. On the fifth day the œdema at the back of the hand had subsided, but it was not until 7 days had elapsed that the tenderness and swelling over the wrist had disappeared and the patient was convalescent.

Experiments on Fish.—The injection into roach of filtered glycerin extract of the gland substance obtained by scraping the groove of a dog-fish's dorsal spine gave the following results :—In most cases there was a period varying from 10 to 30 minutes in which the fish lay quiescent, and during this period the respirations became very rapid, from 120 to 140 per minute. The general symptoms usually then subsided, but locally swelling and œdema occurred at the site of injection and the scales became erect over this area ; no suppuration occurred. The fish, however, seemed ill, as it lost its pale colour and became dark and dull-looking, like fish allowed to remain too long in a live-bait can. I am continuing my investigations into the nature of this venom, and its effects on animals, as opportunities arise ; but there is some difficulty in obtaining recently caught dog-fish.

(3) *The Venom of Trygon.*

I have no personal observations on the poisonous nature of the spine of Trygon ; but there is plenty of good evidence in the literature of the subject.

The evidence that the gland is really a poison organ depends on the following points :—

i. That the nature of the wounds produced is not such as would be produced by a simple laceration ;

ii. That the symptoms of acute pain and inflammation and paralysis are similar to the symptoms produced by the stings of the other venomous fish, particularly the Weever ;

iii. The observations of Dr. Lo Bianco, quoted by Dr. Porta (1905).

The observations of Dr. Lo Bianco are very interesting. He himself saw a young man become extremely pale and fall down almost senseless for a few minutes, from having received only a very small puncture while he was in the act of passing a Trygon, weighing 3 kgrm., from one person to another. Besides this, he relates the following most interesting fact : In the month of September there were in the great tank of the Aquarium of the Zoological Station of Naples four *Trygon violacea* and three *Thalassochelys caretta.* One of the Trygons died, and on examining it he found that the sting was broken and entirely gone. After a few days one of the Thalassochelys would not eat any more, unlike the others who ate with great appetite, and remained in a corner of the tank. It lived thus for four days and died on the fifth. On examining it, he found the sting of the Trygon buried quite 6 cm. under its right fin, piercing only the skin and muscles ; in the part where the sting

was buried the tissue was of a violet colour. The wound was about 3 to 4 cm. in length and breadth, and contained a putrid liquid with a most offensive smell.

In addition to the examples already given of the dangerous effects produced by the spine of Trygon, M. PHISALIX (1922) describes the results of wounds of *Trygon garappa*, a species common in Guiana, the symptoms being compared by SCHOMBURG to those caused by snake-bite. He relates the case of a colonist of Demerara, who died in violent convulsions, and of the two Indians who accompanied him, who, wounded in the feet, became seriously ill, and only recovered the use of their feet after a long period of suffering.

This species is the same as that usually known as *Trygon hystrix*, of the upper reaches of the Essequibo. The wound is very painful and tetanus often supervenes.

The following notes on Malay Trygonidæ have been kindly given me by Dr. GIMLETTE :—

"*Urogymnus asperrimus.*—A Chinaman, aged 20 years, was attacked and wounded in the thigh. He fainted, and on regaining consciousness had complete numbness and paralysis of limb affected. Wound remained unhealed. He was brought to hospital a fortnight later; on admission he had a peculiar stiff look and unusual glassiness of the eyeball; extreme weakness bordering on collapse, pallor, feeble heart, but ravenous appetite. Leg not swollen, but sensibility lost. There was a jagged, irregular, sloughing wound, $2\frac{1}{2}$ inches deep, with a copious, fœtid, thin, dark grey discharge. Sloughs separated, exposing bone; knee-joint also became infected with the same fœtid pus.

"Certain dangerous fishes, *pari*, are used as a poison by Kelantan criminals. The Bishop Ray, *Ætobatis narinari*, causes violent pain and a tendency to syncope, rapidly forming local swelling at site of puncture, which becomes acutely inflamed and terminates in gangrene. Some Malays at Cherang Jelor, Northern Kelantan, in 1912, fastened a couple of Trygon spines to a pole and stabbed a horse. Horse became delirious and seemed likely to die. Wounds enlarged and treated with a 2 per cent. iodine solution."

(4) *The Venomous Action of* Chimæra.

Evidence is to be found in the literature of the dangerous nature of wounds inflicted by the spine of *Chimæra*. RICHTER, quoted by M. PHISALIX (1922), refers to a *Chimæra* very much feared by the Spanish fishermen, the wounds from which are sometimes followed by death.

The histological findings are again very suggestive, and I think that a groove containing glandular tissues, and lying between serrated margins, is some evidence of the venomous nature of these spines. But, as I have already stated, further research, both histological and physiological, is necessary, and might be readily undertaken by observers who live in South-Eastern Alaska and near the wharves at Esquimalt, where *C.* (*Hydrolagus*) *colliei* is plentiful and swims at the surface of the sea.

(5) *Treatment.*

The treatment of wounds produced by venomous fish is simple and efficacious. If untreated the pain will last for several hours, the period among fishermen being often spoken of as until the tide turns. From practical experience I have come to the conclusion that the most useful method is to inject into the puncture a few minims of 5 per cent. solution of permanganate of potash.

Messrs. Brady and Martin, of Newcastle, have made a useful outfit which consists of a small glass hypodermic syringe with rustless needles, and some small ampoules of a solution of potassium of permanganate. I am convinced that if this outfit was made compulsory on our smacks and drifters a considerable amount of suffering and often permanent disability might be prevented. Two patients I treated in this way, having been stung by the lesser Weever in the foot, had immediate relief from pain and were able to walk away from my house in perfect comfort, and no inflammation or gangrene resulted.

A simpler method is the use of a LAUDER-BRUNTON snake-bite lancet; but this has certain disadvantages. The lancet is apt to get rusted at sea and the solution does not get in contact with the venom in such a satisfactory way as by injection. A 1 per cent. solution of chloride of gold is probably better still as a solution for injection, but I have had no experience of its value. For the resulting inflammation in untreated cases, cooling lotions or hot fomentations must be applied.

In conclusion, I wish to record the valuable help I have received from numerous scientific men. In my first researches Dr. SIDNEY MARTIN put the laboratory at University College at my disposal, and before I had a licence for inoculation experiments, Dr. STEVENSON made the necessary injection of the dried venom I had collected. In the study of sections of the Weever poison-gland Dr. BORLEY, of the Ministry of Agriculture and Fisheries, gave valuable help.

Mr. TATE REGAN has taken great interest in the question of the poison organ of Trygon. Dr. RUSSELL, of the Board of Agriculture and Fisheries, has given me specimens of Chimæra from the North Sea, and Prof. GOODRICH, of Oxford, specimens of Cestracion and Chimæra, the latter from the Mediterranean.

Prof. HANS GADOW has given me valuable help in examining specimens in the Museum at Cambridge, and finally in the palæontological work I have received every help and facility from Dr. SMITH WOODWARD, of the British Museum of Natural History. I am also indebted to Dr. H. H DALE for the encouragement he has given me, and for assistance with regard to the arrangement of this paper.

I have again to thank my colleague, Dr. DONALD HUTCHINSON, for taking the micro-photographs of my preparations, and Dr. CLARIDGE, of Norwich, for making suggestions as to the technique of decalcifying the specimens and for his trouble in imbedding them for me.

(6) *Summary.*

The work I have undertaken confirms :—

1. The existence of specialised glandular structures—

   (*a*) In the lateral grooves of the serrated caudal spine of *Trygon pastinaca.*
   (*b*) The groove of the dorsal fin-spine of *Acanthias vulgaris.*
   (*c*) The groove of the spine of *Cestracion philippi.*
   (*d*) There is some evidence of a similar structure in connection with the spine of *Chimæra monstrosa.*

2. The examination of fossil fin-spines suggests that they also in the fresh state had a glandular structure of a similar nature.

3. A study of the nature of fish venoms most readily undertaken with the venom of *Trachinus draco,* establishes the facts that the venom is both neurotoxic and hæmolytic in its action ; the latter character seems to be of the greater importance. The hæmolytic action of fresh unfiltered venom takes place without the addition of heated serum or lecithin : nevertheless filtered glycerinated venom requires the presence of heated serum or a solution of lecithin in order that hæmolysis may take place.

4. Wounds envenomed by fish poisons are best treated by the hypodermic injection of 2—5 per cent. solutions of permanganate of potash or 1 per cent. solution of chloride of gold into the site of the punctured wound.

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DESCRIPTION OF PLATES.

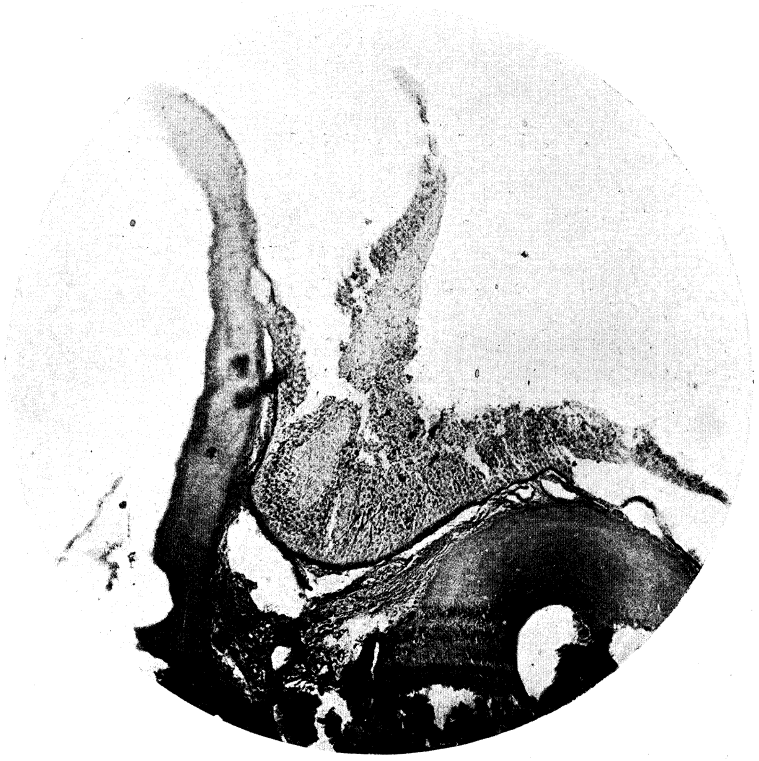
PLATE 1.

Fig. 1.—Microphotograph of lateral groove of the spine of *Trygon*. Portions of median ridge and dentate margin are shown. The groove is occupied by a mass of glandular tissue separated by a pigmented layer from the alveolar connective tissue lying in the deepest part of the groove. The cells covering the dentate margin have become separated from the tooth. A column of secreting cells are seen discharging their contents laterally. (Mag. × 40.)

Fig. 2.—Microphotograph of lateral groove of spine of *Trygon*, to show structure of the deep alveolar portion. The central canal is large and lies just beneath the pigment layer. The superficial glandular portion shows several columns of secreting cells. (Mag. × 40.)

Fig. 3.—Microphotograph of a portion of the glandular tissue shown in Plate 1, fig. 1. A layer of columnar cells lies upon the pigmented basement membrane. Above them are masses of ovoid and round cells. To the left, more superficially, the cells are seen distended with secretion, with indistinct margins and phantom nuclei. Bordering the secreting portion there is a fine meshwork of pigment granules surrounding the ovoid cells. (Mag. × 250.)

Fig. 4.—Microphotograph of the gland in the groove of the spine of *Acanthias*. This section is near the base of the spine and shows the central column of connective tissue which divides the gland into two lateral portions. The two main ducts are shown with the distended vacuoles bursting and discharging their contents into the lumen. (Mag. × 80.)



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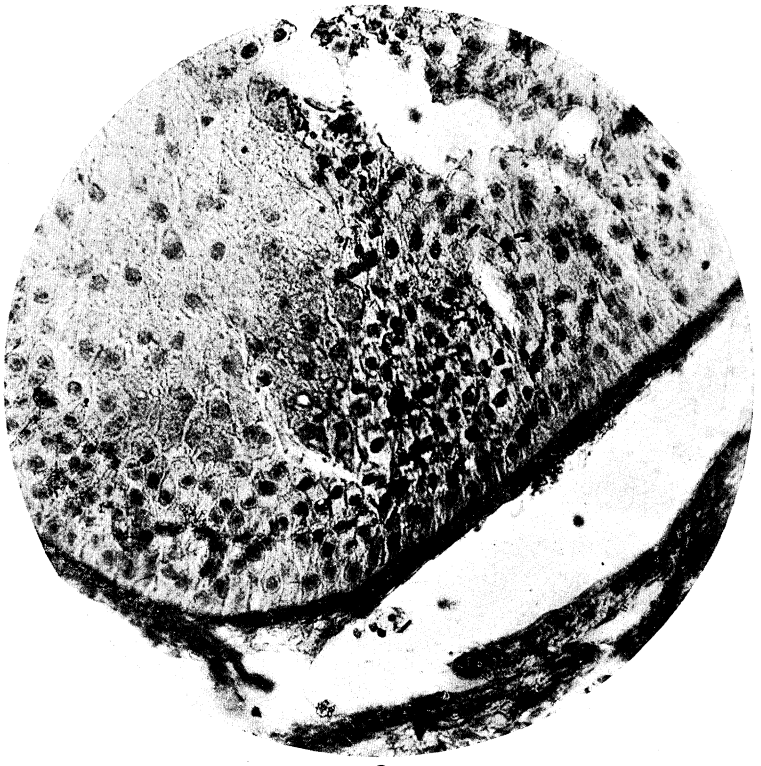

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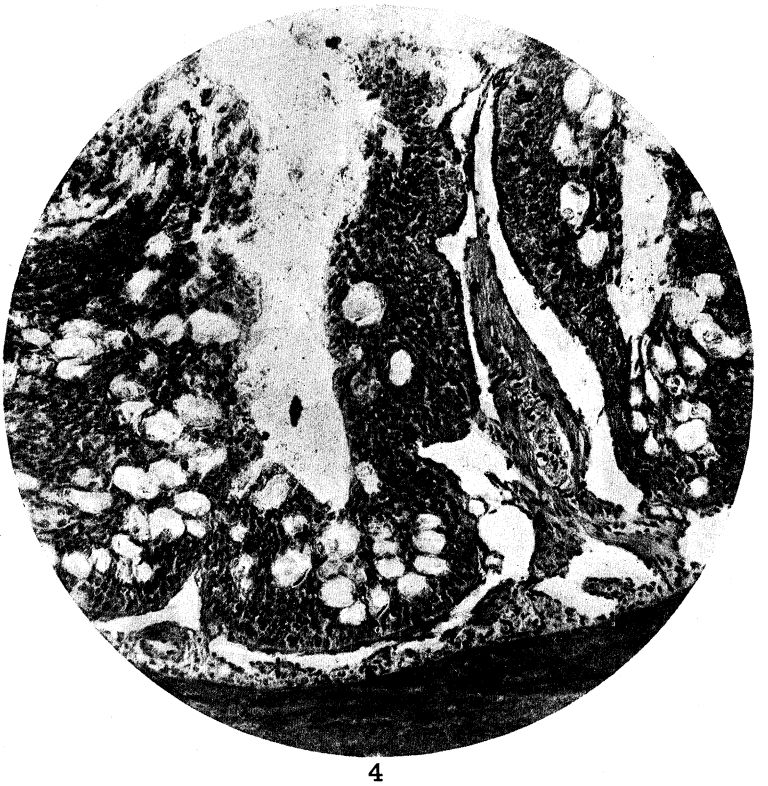

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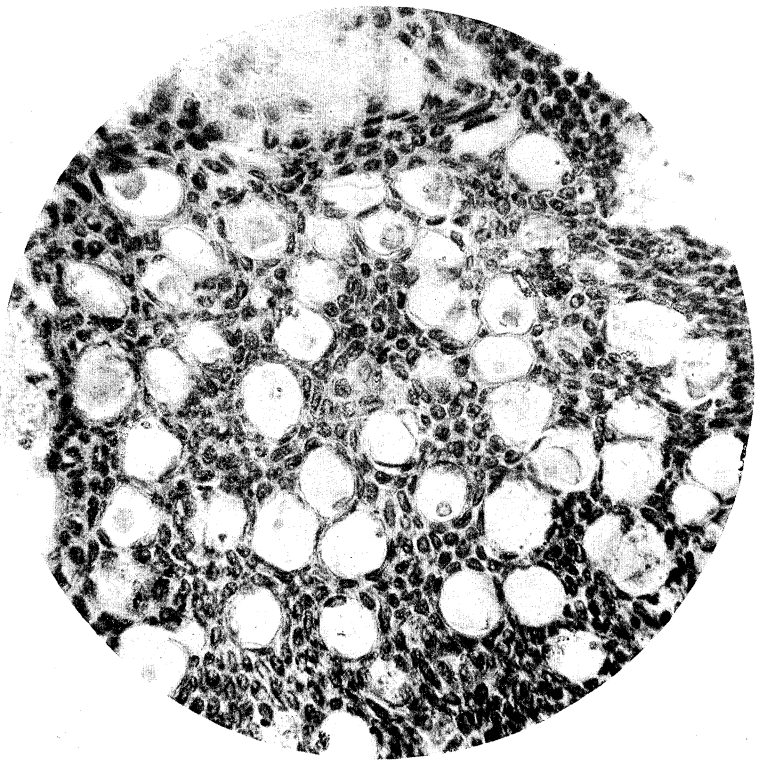

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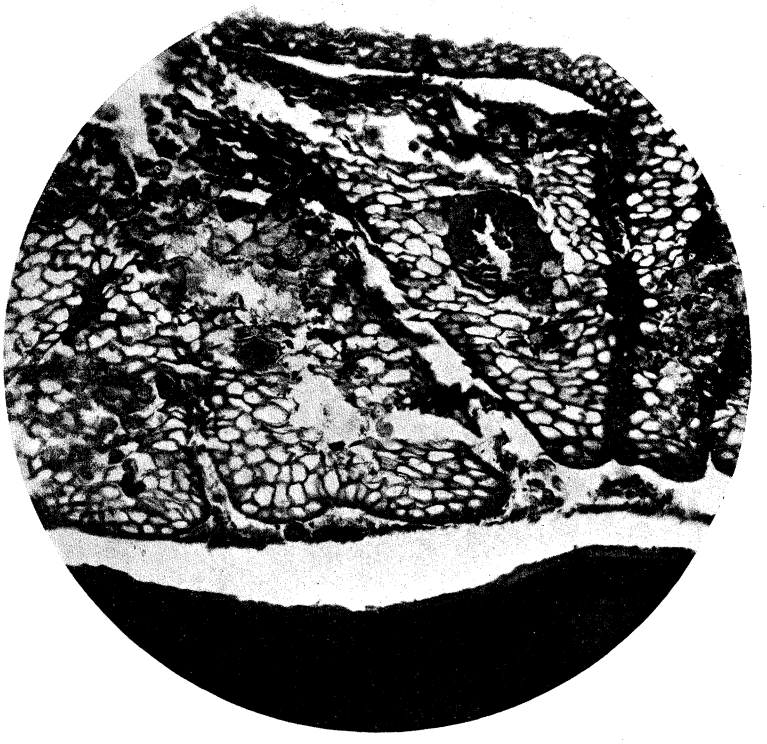

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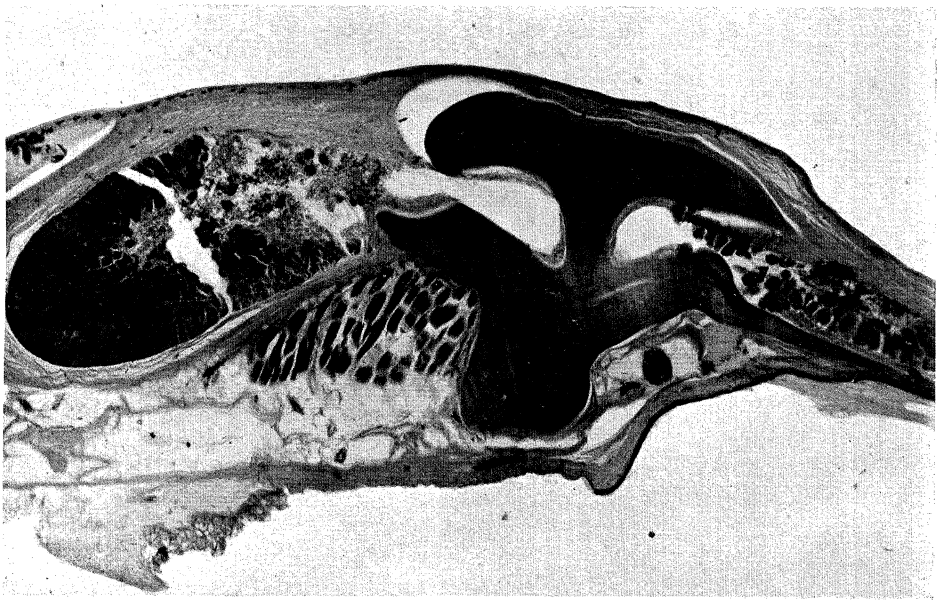

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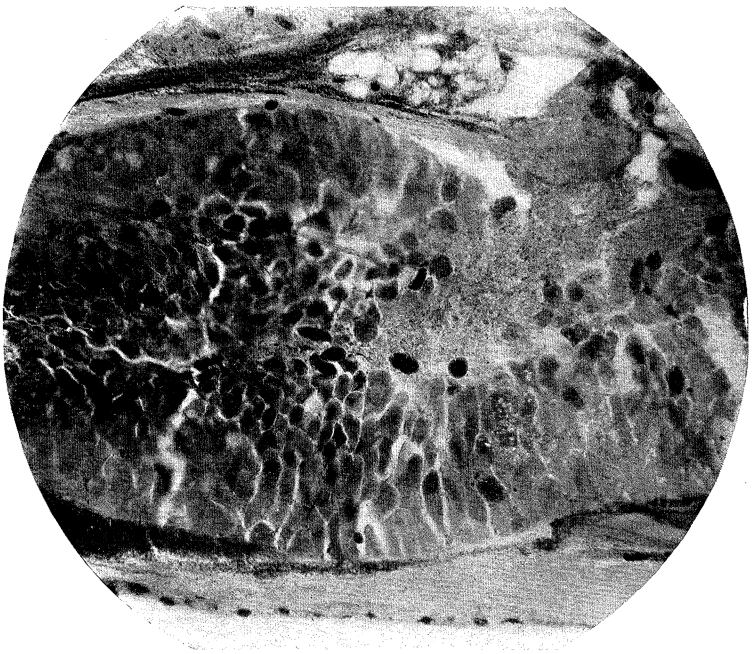

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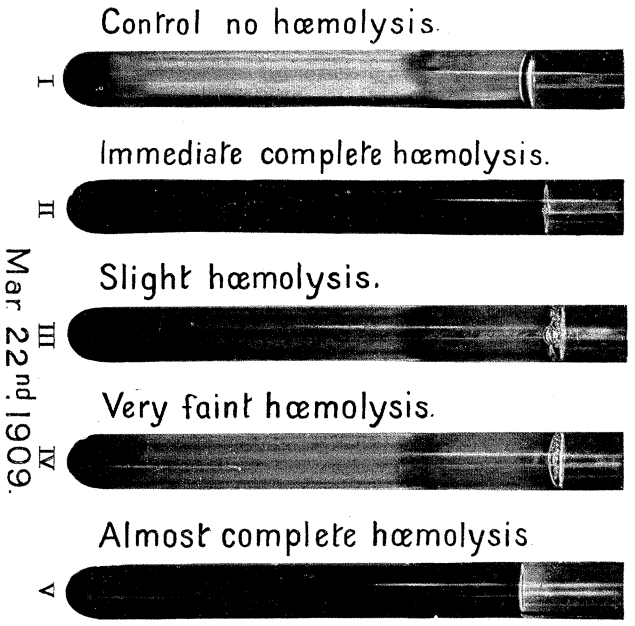
Control no hæmolysis.
I
Immediate complete hæmolysis.
II
Slight hæmolysis.
III
Very faint hæmolysis.
IV
Almost complete hæmolysis
V
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Plate 2.

Fig. 5.—Microphotograph from the same specimen as Plate 1, fig. 4, but this section is taken from the distal portion of the spine. There is one central duct into which numerous follicles discharge. A central canal with pigmented walls passes through the base of the gland. (Mag. × 60.)

Fig. 6.—Microphotograph of a portion of Plate 2, fig. 5, to show the process of secretion and the formation of vacuoles by the contents of neighbouring cells coalescing. (Mag. × 300.)

Fig. 7.—Microphotograph of base of spine of *Cestracion* before the separation of fin from spine. Three follicles are shown full of secretion and three in a resting stage. (Mag. × 150.)

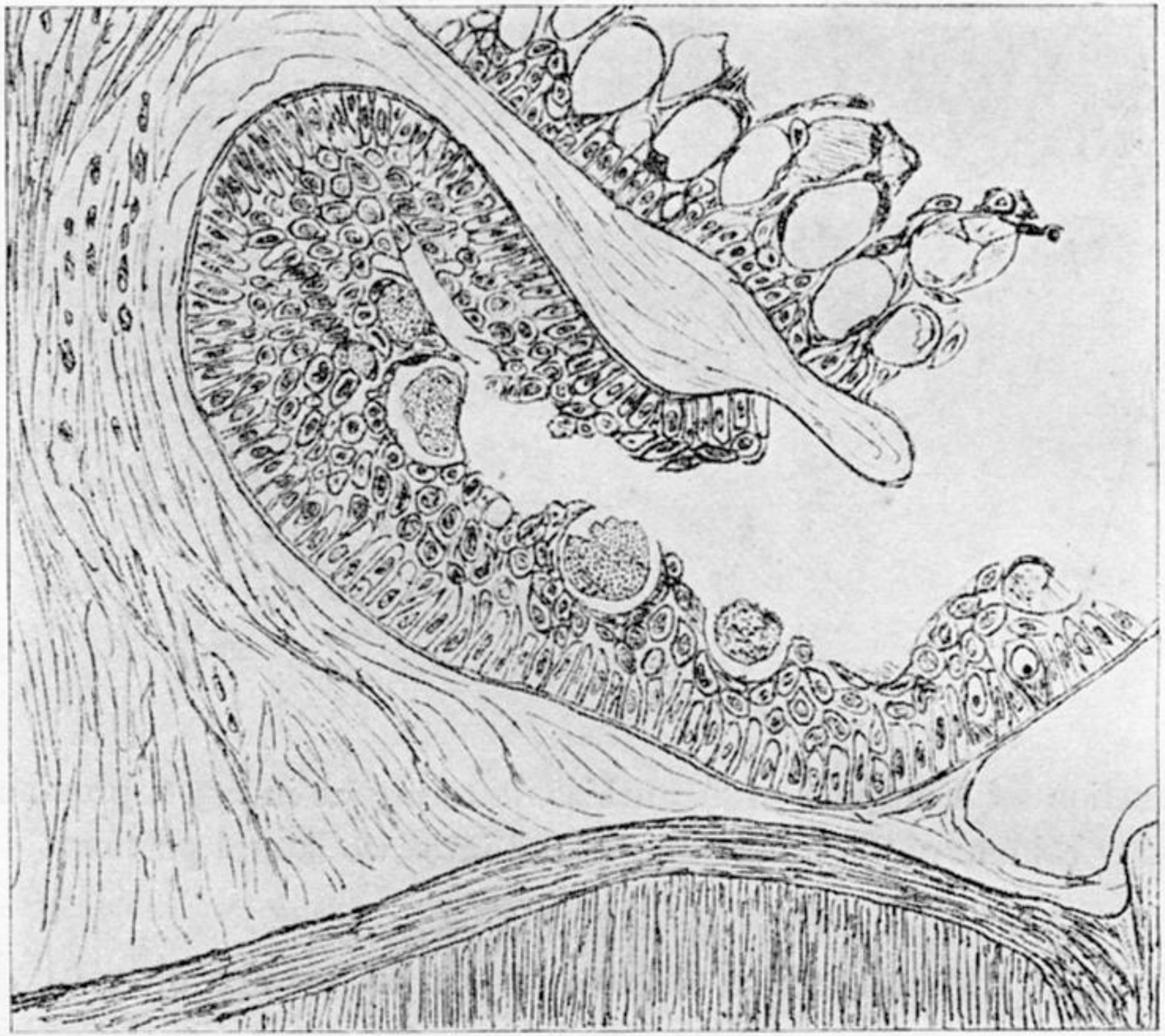
Fig. 8.—Microphotograph of portion of groove of spine of *Cestracion* near the base. The follicles are distended with secretion. In the centre of the main follicle is a circular granular mass in the middle of which are numerous small globules which coalesce and form larger dark staining masses of secretion. (Mag. × 150.)

Plate 3.

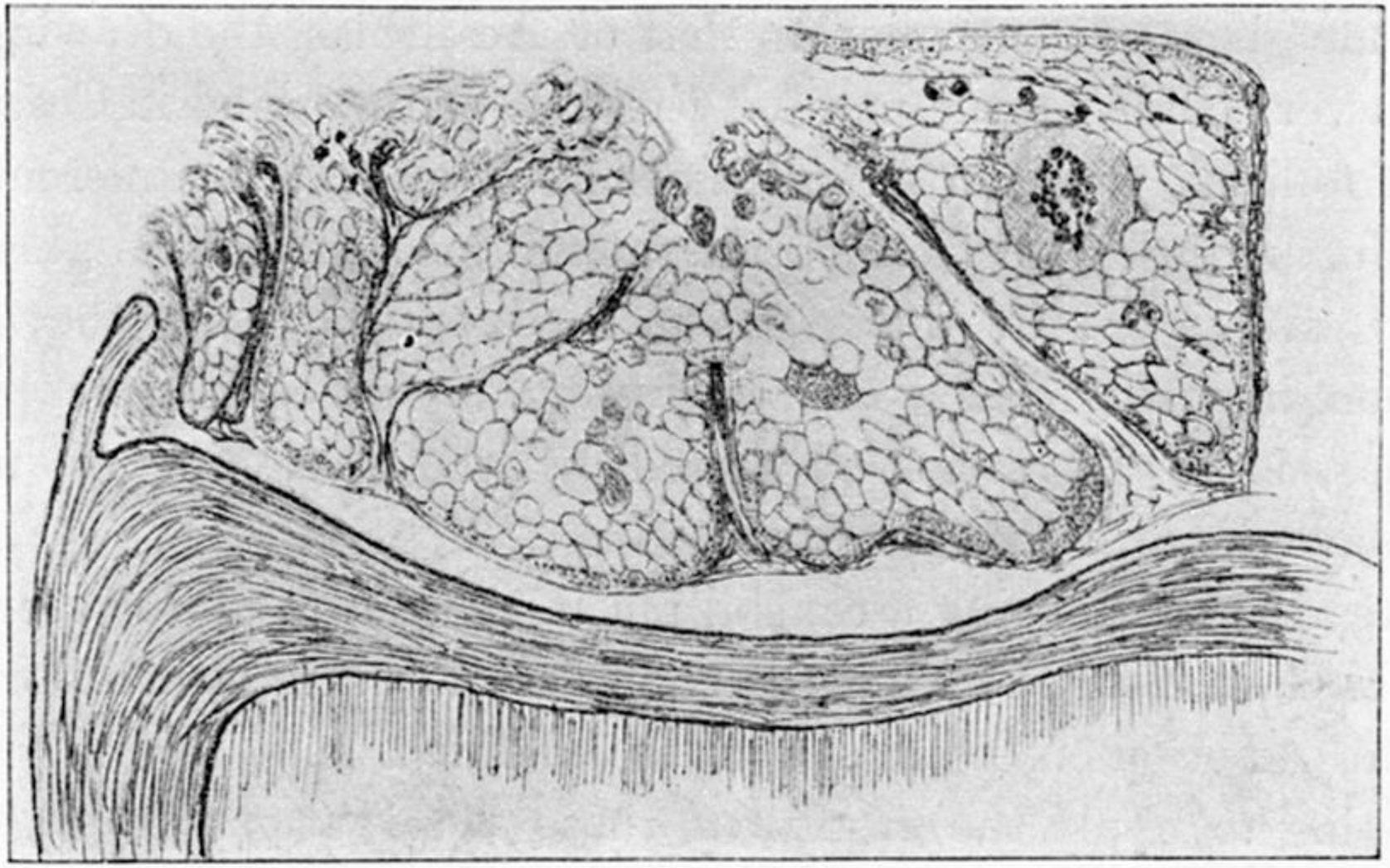
Fig. 9.—Section of the double-grooved opercular spine of *Trachinus draco*, showing the gland on either side discharging secretion into the groove. (Mag. × 40.)

Fig. 10.—Section of gland of *Trachinus draco* under high power, to show the large columnar cells containing masses of refringent material. This is discharged into the lumen in spherical masses. These masses lose their dark stain, and finally form a granular secretion. (Mag. × 280.)

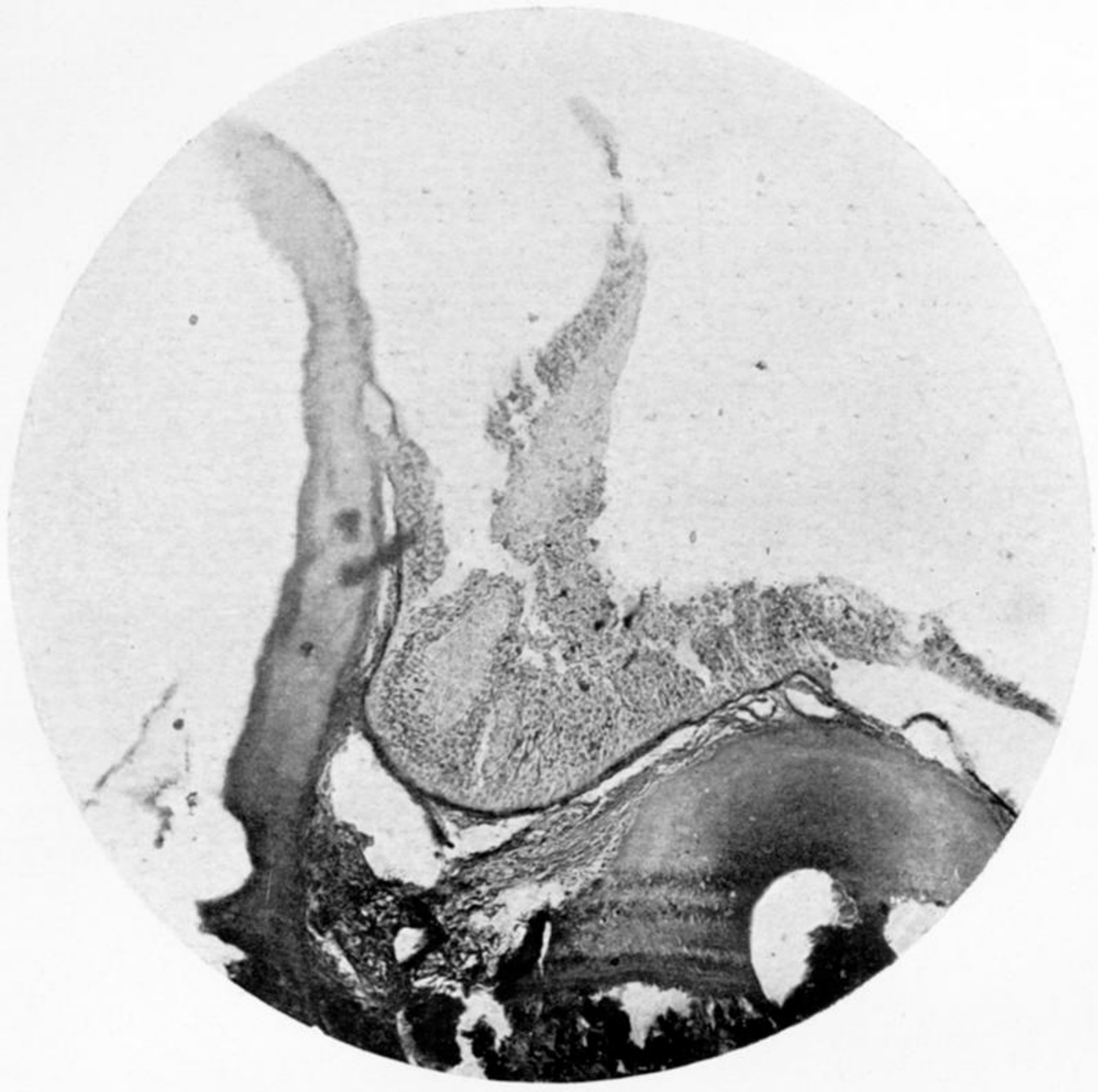
Fig. 11.—Photos. of five hæmolysis tubes.



TEXT-FIG. 6.—Transverse Section of Spine of Cestracion, before its separation from the fin, to show the commencement of the gland, which consists of follicles with cylindrical and ovoid cells and masses of secretion in the lumen thereof.



TEXT-FIG. 6*a*.—Transverse section of half of the groove of Spine of Cestracion near its base, showing the marginal elevation which supports the glandular tissue. Note the circular granular mass in the follicle to the right.



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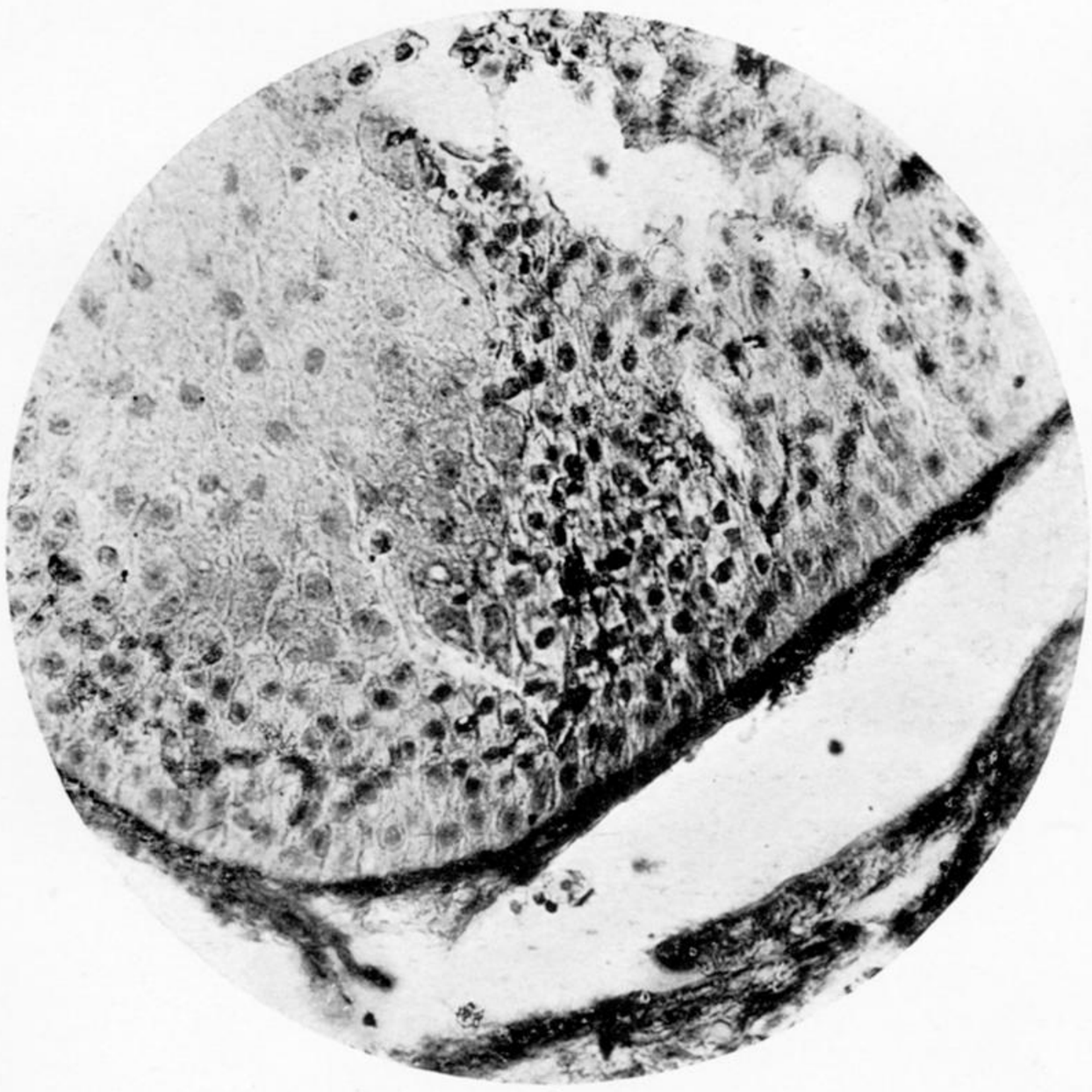

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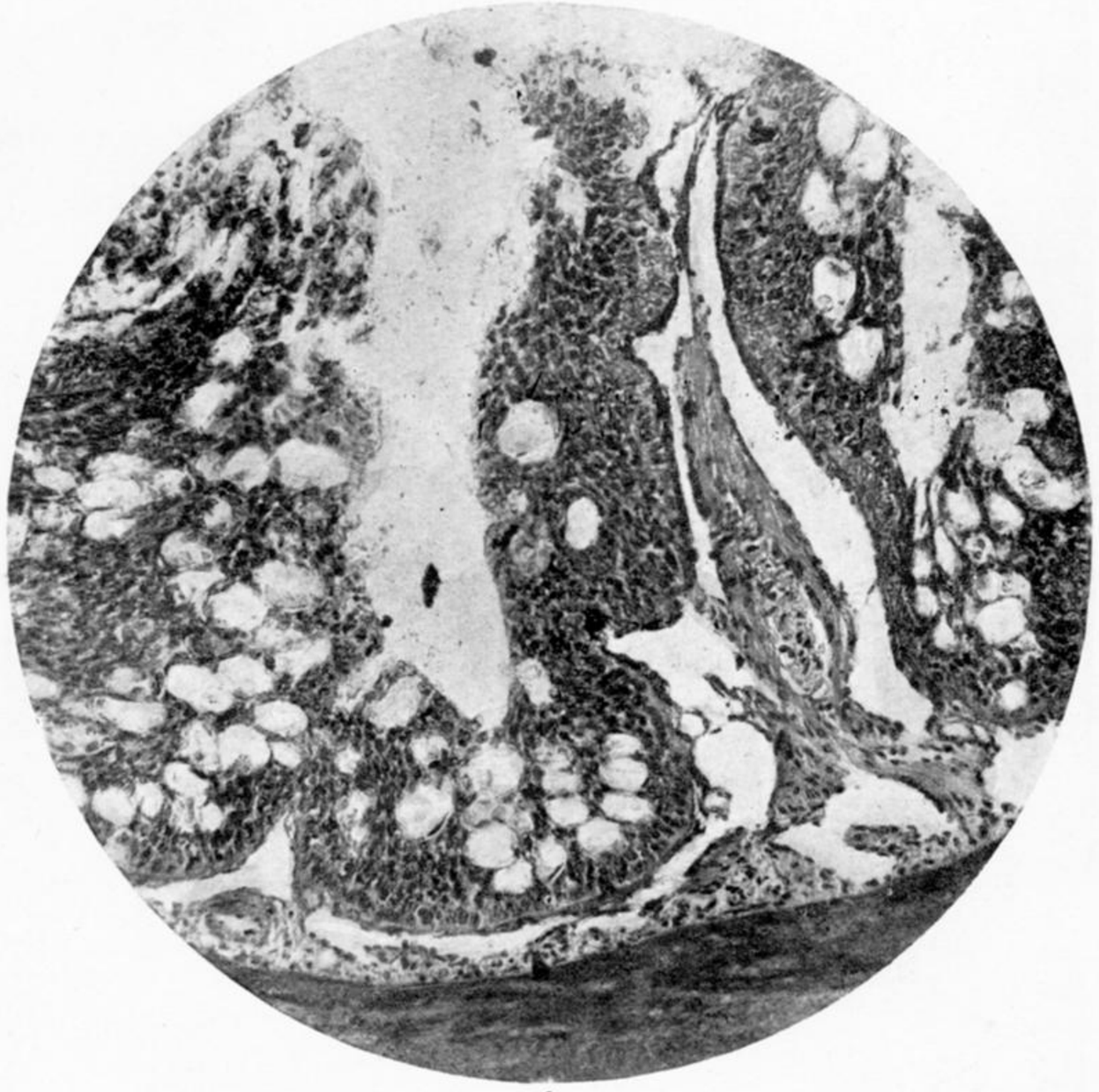

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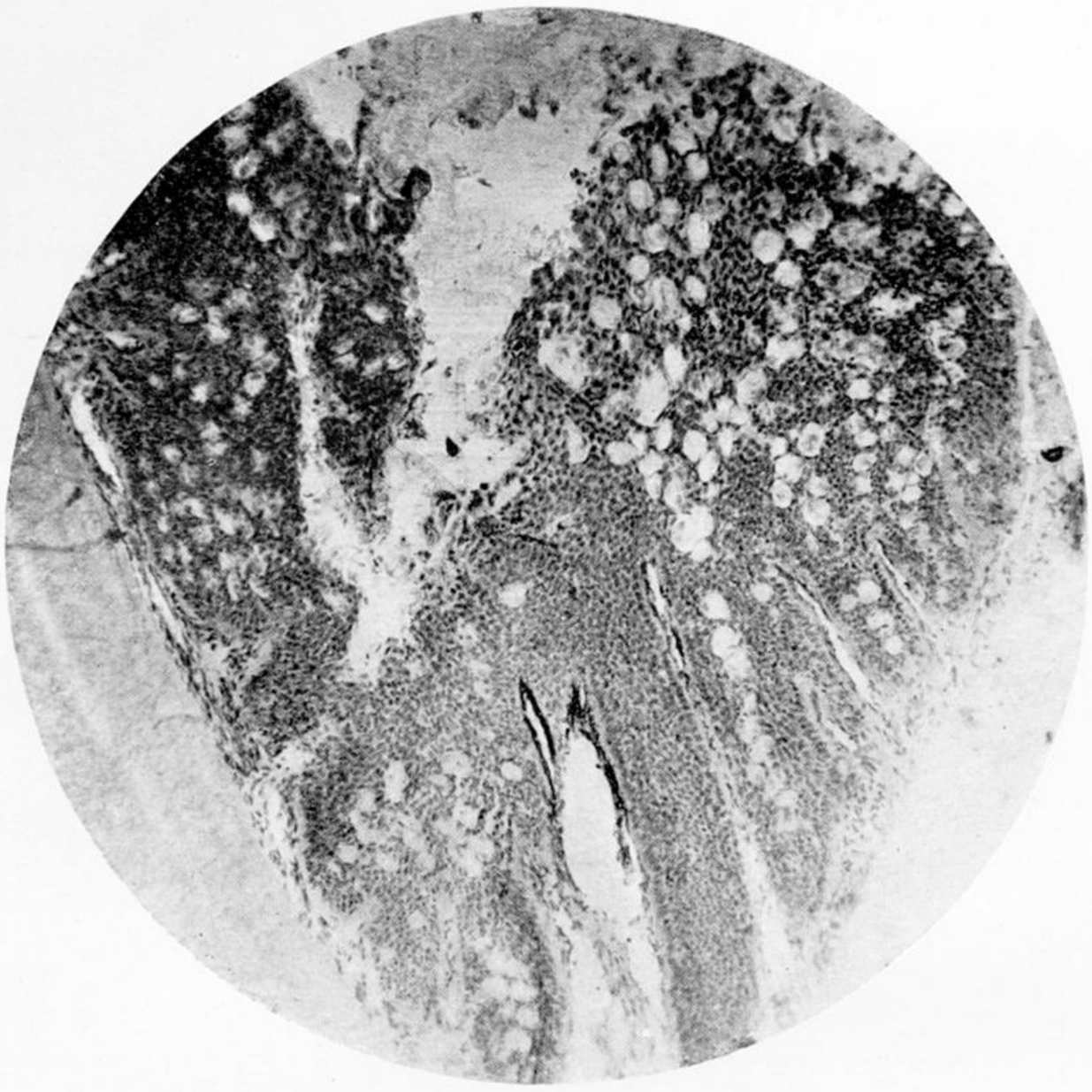

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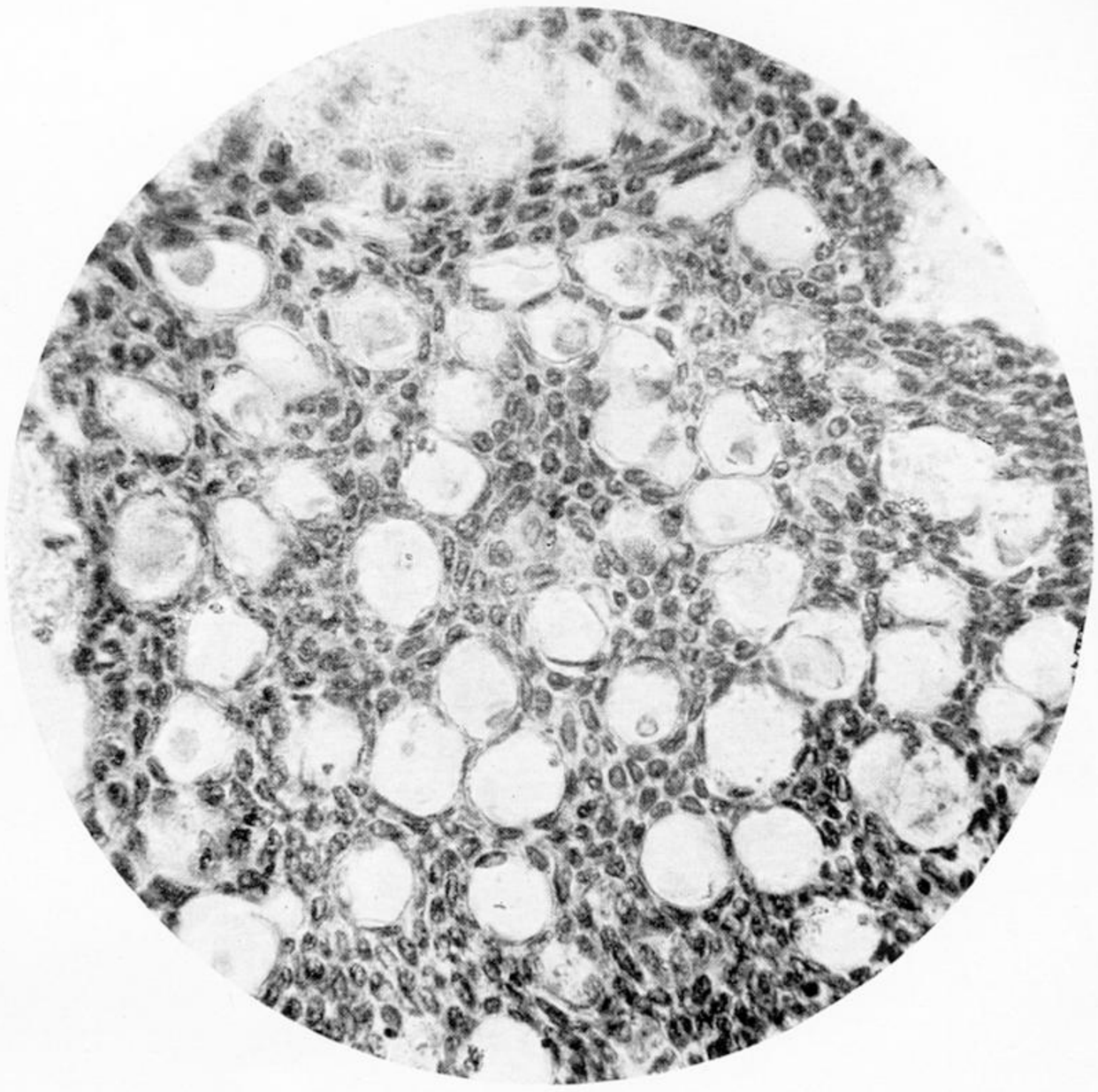

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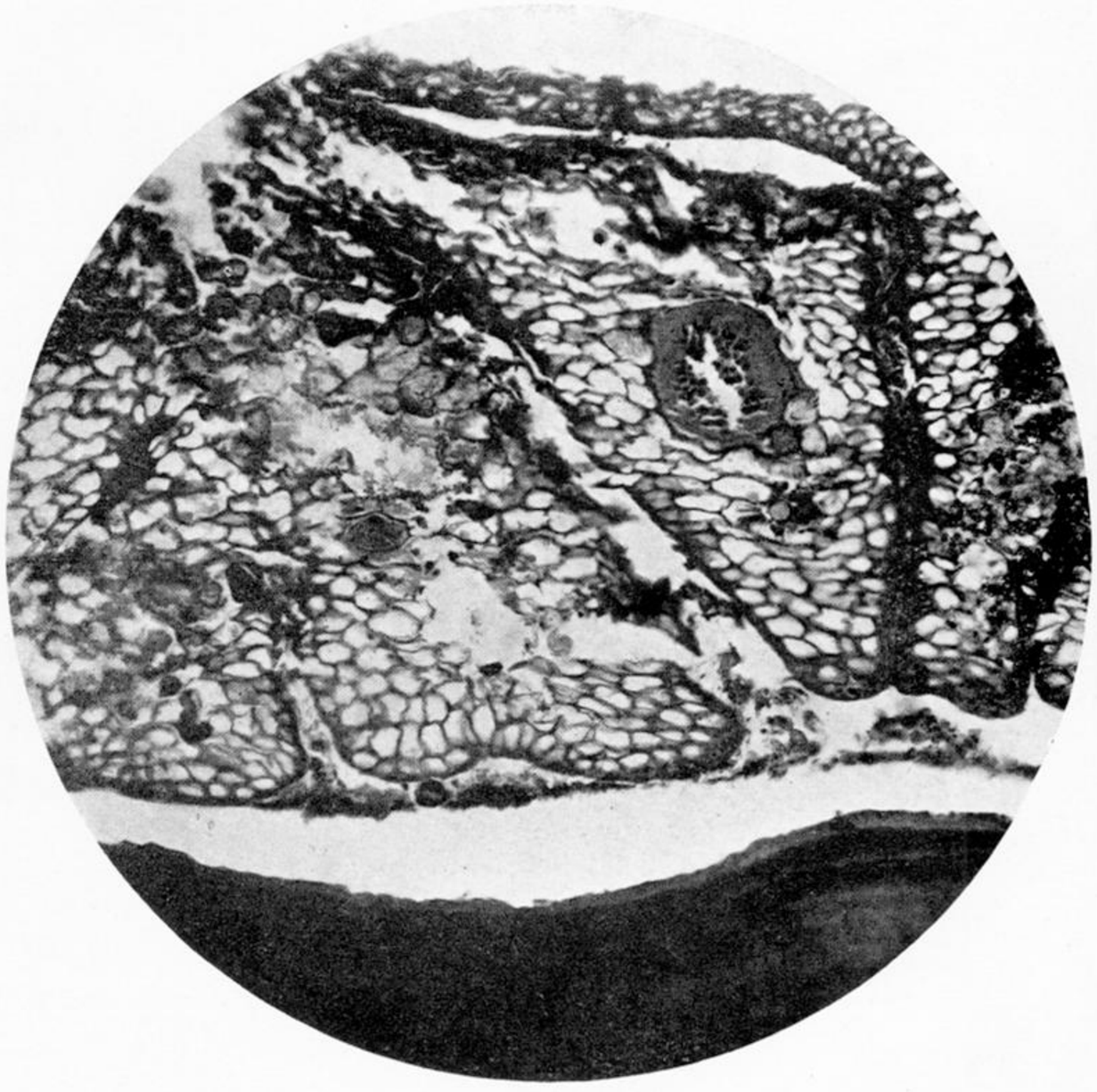

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PLATE 2.

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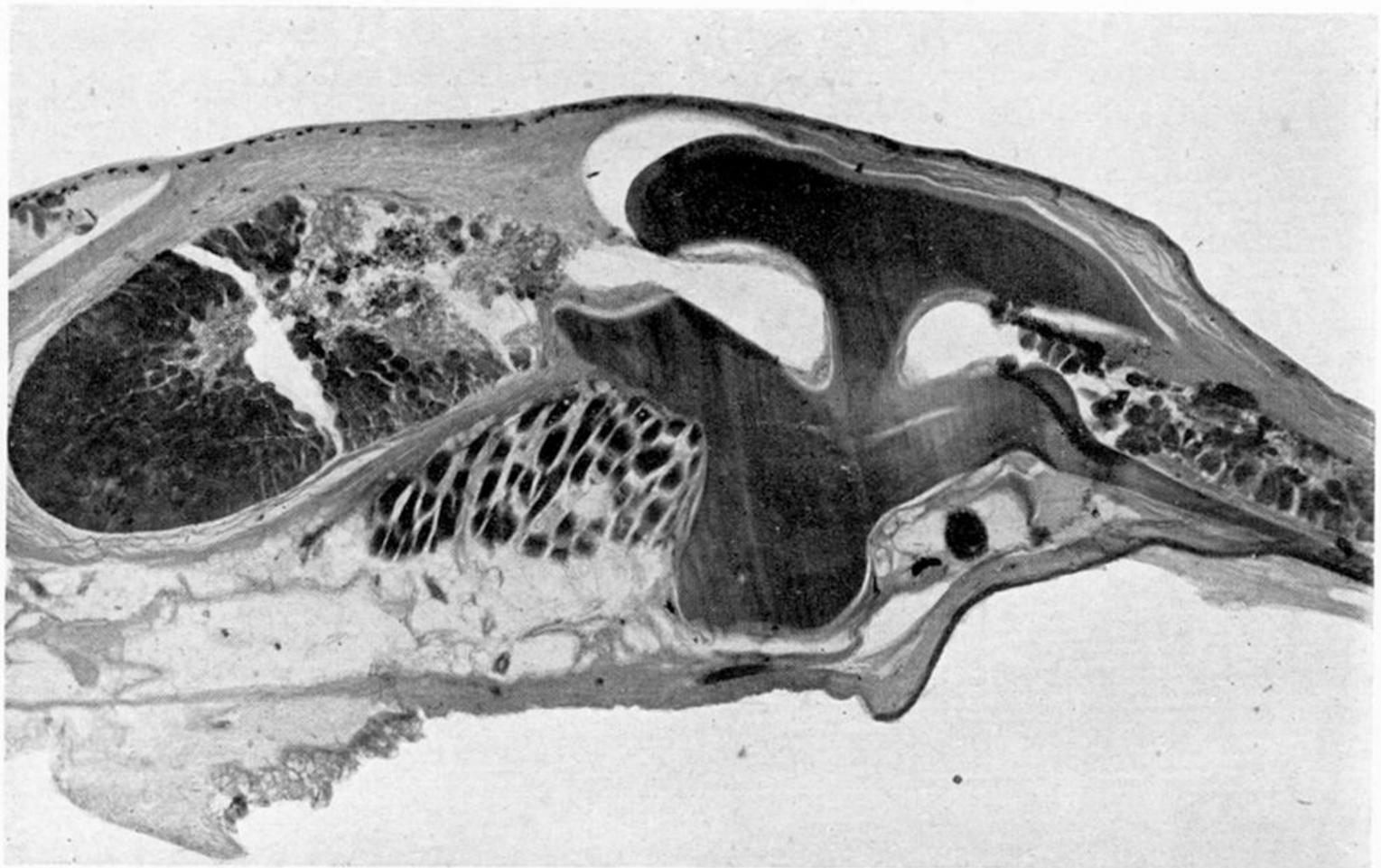

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