THE ANATOMY OF THE THYROID GLAND OF ELASMOBRANCHS, WITH REMARKS UPON THE HYPOBRANCHIAL CIRCULATION IN THESE FISHES

JEREMIAH S. FERGUSON

Assistant Professor of Histology, Cornell University Medical College

TWENTY FIGURES

Introduction.....	151
Review of the literature.....	153
Material and methods.....	164
The anatomical relations of the thyroid gland.....	165
The thyroid vessels.....	171
The hypobranchial circulation and the origin of the thyroid vessels.....	175
Veins and lymphatics of the thyroid region.....	183
The histology of the Elasmobranch thyroid gland.....	187
Summary.....	207
Explanation of figures.....	209
Bibliography.....	210

INTRODUCTION

Even a casual reference to the literature of the thyroid gland is sufficient to indicate that the organ has been more carefully studied in most all other classes of animals than in the Elasmobranchs.

The organ may be said to make its first appearance in the Ascidians, Amphioxus, and Cyclostomes as a depressed groove, trough, or series of recesses in the ventral floor of the pharynx, usually known as the endostyle, or the hypobranchial or hypopharyngeal groove, which, as first shown by W. Müller ('71) who studied *Myxine glutinosa* and *Petromyzon*, is to be considered the homologue of the median thyroid of the vertebrates. In the Cyclostomes the structure, relations and development of the primitive thyroid have been more recently studied by Guiard ('96), Cole ('05), Schaffer ('06), and Stockard ('06). The structure of the organ is very simple and only partially resembles the thyroid of

higher vertebrates. Possibly the thick tenacious secretion formed by the endostyle, upon the presence of which the function of the organ very largely depends, may well be taken to bear a relation to the colloid material which is so characteristic of the mammalian gland. Inasmuch as the retention of an albuminous secretion within the glandular lumina of the animal body, a condition frequently observed by the pathologists and normally present in other glands as well as the thyroid, *e.g.*, mammary gland, kidney, hypophysis cerebri and parathyroid gland, leads to the accumulation of a colloid material bearing a more or less striking resemblance to the colloid material in the follicles of the thyroid gland, the deduction from the phylogenetic standpoint, that the retention within the follicles of the thyroid of a once free mucous secretion would account for the colloid character of the follicular content, would not seem inappropriate. The character of cells which pour forth the free secretion of the endostyle or hypobranchial thyroid of the Ascidians, Amphioxus and Cyclostomes is not so very different from the colloid secreting cells of the thyroid follicles of mammals.

In the Teleostei, Wagner ('53) has studied the form and location of the thyroid gland and directed attention to the similarity of its structure to that of mammals. Simon ('44) and Baber ('81) have given extended descriptions of the thyroid gland in several species of bony fishes; Maurer ('86) described the structure and studied fully the development of the thyroid gland of the carp and trout. In the more recent literature the structure of the thyroid in fishes seems not to have received the attention which it apparently deserves.

Extended descriptions of the thyroid gland of reptiles are found in the articles by Simon ('44) and Baber ('81). De Meuron ('86) studied the organ in *Lacerta*. Van Bemmelen ('87) described the gland in *Hatteria* and *Lacerta* as being transversely placed over the trachea near the heart, and as forming a small, thin unpaired organ. Guiard ('96) has also discussed the structure of the organ in reptiles.

In the Amphibia the work of Maurer ('88) and the excellent descriptions by Wiedersheim ('04) apparently leave little to be

desired, though the organ has been much studied in this class of animals.

In Aves the thyroid gland has been extensively studied by Simon ('44), Peremischko ('67) who considered the histology as well as the gross anatomy of the organ, Baber ('81) and De Meuron ('86). The literature of the structure and development of the thyroid in the chick is extensive.

In most of the Mammalia the anatomy of the thyroid gland is well known and its literature has acquired voluminous proportions. Its review does not fall within the scope of the present paper.

REVIEW OF THE LITERATURE

A careful study of the available literature has revealed, with the exception of the work of Guiard, only casual references to the anatomy of the thyroid gland in the Elasmobranchs. Simon ('44) studied the Selachian (*Squalus*) and the skate (*Raia*). He describes the thyroid gland as "a single organ, situated in the median line, in connection with the anterior surface of the cartilages which bind together the branchial arches of opposite sides of the body," and he states that it may lie in contact with the "lingual bone," or may be more or less distant from the mouth, but that it is "always at the spot where the great trunk of the branchial aorta distributes its terminal branches. It lies at the angle of this bifurcation . . . ; it is covered by the sterno-hyoid or sterno-maxillary muscle, and also by the myo-hyoid and genio-hyoid, when these are present." His description I find to hold good for *Raia*, but it does not entirely correspond to the position of the thyroid gland of *Squalus*, *Mustelus*, or *Carcharias*. As to its vascularization, Simon states that the gland receives its blood supply by means of a recurrent branch given off by the first branchial vein, while yet within the gill, and that "it never receives the smallest share of supply from the branchial artery with which it is in contact." The last portion of this statement is precisely correct for all the species which I have examined, though apparently at variance with the observations of some authors: the first portion, as to the origin of the thyroid artery,

would appear to be not very accurately expressed, for it never arises from the afferent branchial vessel which leaves the dorsal end of the branchial arch, but, on the contrary, arises from the ventral end of the nutrient or efferent loop. The relation of the thyroid gland to the bifurcation of the ventral aorta is so intimate as to readily suggest the error of other observers who have presumed that the organ received some blood directly from the first pair of branchial arteries. Moreover, the thyroid artery passing from its origin lies directly under, and in contact with the first pair of afferent branchial arteries so that until these latter vessels have been carefully dissected out of their sheath it is impossible to determine with certainty that they have no connection with the thyroid vessels. In the skate the gland lies directly upon the aortic bifurcation and the pulsating blood-vessels are readily seen through the transparent organ as soon as it is exposed.

Müller ('71) speaks of the thyroid gland of *Raia clavata* as a flattened brownish-red body, lying at the point of division of the branchial artery. It possesses a connective tissue capsule with trabecula which divide the organ into a small number of lobes, within which the connective tissue penetrates between the lobules. The follicles possess a thin *membrana propria* and a cylindrical epithelium; they contain a homogeneous, gelatinous yellowish mass. The epithelium possesses a shiny cuticular border and appears to send processes into the lumen. The description given by Müller holds good for *Raia*, the genus which he studied, but it does not correspond to the condition of the thyroid gland of *Mustelus*, *Squalus* or *Carcharias*, the difference being chiefly due to the fact that in the *Batoidei* the connective tissue forming the thyroid trabecula and interfollicular septa is apparently much more abundant than in the *Selachii*.

Balfour ('78) discusses very briefly the early development of the thyroid gland of *Elasmobranchs* prior to the appearance of a lumen within its follicles. He does not consider the anatomy of the organ in the adult.

Baber ('81) says that "in the skate the gland is single (with the exception of a few detached vesicles) and forms a yellow, flattened, lobulated body, occupying the median line at the bifurcation of

the branchial artery. Anteriorly it sometimes presents a narrow process of gland-tissue running forward, and behind it is limited by the bifurcation of the branchial artery." The contents of its vesicles consisted of coarsely granular masses or globules of various sizes which "correspond with the 'colloid substance' of authors." He surmises the non-existence of lymphatic vessels and says that in both the skate and the conger-eel "an extensive system of vessels lined with epithelium becomes injected by the method of puncture"; he considers that these are blood-vessels. The narrow process of glandular tissue which Baber says is occasionally present in *Raia* is more frequently seen in the *Selachii*; it is constantly present in all the examples of *Carcharias* which I have dissected. It extends forward until it comes into contact with the anterior margin of the basi-hyal cartilage (lingual bone) which presents a depression, frequently amounting to a complete foramen, for the reception of the anterior extremity of the glandular process with the connective tissue by which it is heavily invested. This process is obviously analogous to the pyramidal lobe of the mammalian thyroid, and as it extends all the way to the pharyngeal submucosa in many instances it may well be considered as indicative of a phylogenetic connection of the gland with the cavity of the pharynx, a condition which is also indicated by the ontogeny of the organ in all the orders, and which appears to be permanent in the *Ascidians*, *Amphioxus*, and the *Cyclostomes*.

Baber's suspicion of the non-existence of lymphatics within the thyroid gland appeared to the writer to be such a remarkable observation and so out of harmony with the known anatomy and physiology of the organ in the higher animal orders as to require further study. This study was pursued by means of a considerable series of careful dissections with many injection experiments and did not appear to confirm Baber's opinion. Baber's observation that the blood-vessels in these animals could be injected by the method of puncture is quite accurate, but it does not by any means disprove the existence of lymphatics. His results were apparently dependent upon the fact that the smallest veins and capillaries are of very considerable caliber and readily admit of injection, while the lymphatics are very minute and are entered

only with difficulty and not frequently when the needle is thrust into the substance of the gland.

Balfour ('81) referring to the development of the organ in *Scyllium* and *Torpedo* says that at first it is solid and attached to the esophagus. "Eventually its connection with the throat becomes lost, and the lobules develop a lumen."

Dohrn ('84) in his plate XI, fig. 5, indicates by outline the thyroid gland of *Ammocetes*, but does not illustrate or describe the thyroid gland of the *Selachii*, though in his text he includes an extended description of the thymus of the latter animals. His outline of the thyroid of *Ammocetes* does not conform to that of the gland in the *Selachii*.

De Meuron ('86) says that in *Scyllium* the thyroid is elongated, in *Galeus* and *Acanthias*, much flattened, in *Raia* pyramidal or rounded. It lies just above the terminal bifurcation of the branchial artery. In *Myelobates* it lies behind the os hyoideus, beneath the sterno-mandibularis muscle, and is triangular in shape, short, flattened, transversely elongated, and has a length of 2 cm. In *Acanthias* the thyroid gland presents an irregular contour, certain groups of follicles being even completely detached, and placed around the principal group. The observations of De Meuron would appear to be accurate as far as they go but are possibly founded upon the examination of too few individuals. Thus in *Squalus acanthias* I found the thyroid frequently broken as described by De Meuron for *Acanthias* but other individuals presented a thyroid which was perfect, not the least broken up or irregular in contour, and in the closely related *Mustelus canis* irregularity of contour is certainly the exception, not the rule. In *Scyllium* he says the thyroid is elongated and I find that superficial examination of the related species *Carcharias*, would indicate a similar condition, but if the semi-opaque white mass of connective tissue, in which the thyroid gland of *Carcharias* is heavily clothed and so closely invested that it seems to form part of the gland, be dissected out and held, stretched in its normal form, between the bright sun and the eye of the observer there is readily seen within the reddish-white connective tissue mass the outline of the yellowish-orange thyroid gland, which instead of having the elon-

gated form of the outward mass is flattened, transversely elongated, and of the same peculiar triangular or shield-like shape which is characteristic of the organ in the dogfish and closely simulated by that of *Raia*. It seems possible that the elongated gland observed by De Meuron in *Scyllium* might be susceptible to a similar analysis.

Guiard ('96) studied six species of the *Selachii* and five of the *Batioidei*. In *Scyllium* he found the thyroid gland of pyriform shape, the anterior extremity being prolonged forward as far as the anterior margin of the lingual cartilage ("copule"), where it passes between the two lateral halves of the coraco-hyoid muscle. This description, as given by Guiard, corresponds with the position and form of the gland which I find in *Carcharias* and which, as regards the anterior prolongation, appears to be analogous to the pyramidal process of mammals. But Guiard's fig. 1, in the absence of specific contradiction in his text, might be taken to indicate that the thyroid gland had been found beneath the coraco-hyoid muscle; this is not the case in any of the species which I have examined and I presume it is not the case in *Scyllium catulus*, from which species his figure was drawn. In each species I have found the gland lying, without exception on the ventral surface of the coraco-hyoideus, between it and the coraco-mandibularis, except that at the anterior portion the gland lies between the coraco-hyoid muscles of the two sides, the divergence of the two muscles exposing the ventral surface of the cartilage at this point. In the *Batioidei* the coraco-hyoidei are so widely separated that the whole thyroid gland may come to lie directly upon the basi-hyal cartilage, the aortic bifurcation and the coraco-branchialis muscles, which are successively exposed from before backwards by the separation of the coraco-hyooids, but in this case the fascia which covers the ventral surface of the coraco-hyooids dips beneath the dorsal surface of the thyroid gland.

In *Acanthias vulgaris* and *Mustelus lœvis* Guiard as did De Meuron, notes the tendency of the thyroid to present detached vesicles, its contour being very irregular. In *Galeus canis* the thyroid gland lies rather farther forward and is partially covered by a fold of the buccal mucosa. In *Carcharias glaucus* the

coraco-mandibularis muscle is relatively very broad and completely hides the thyroid gland; the gland is described as reniform and voluminous. With the exceptions recorded the position of the thyroid in the various species of *Selachii* corresponds fairly well with that described for *Scyllium*.

Of the *Batoidei*, in *Raia alba* the coraco-hyoidei are small and widely separated, and between these muscles the first pair of branchial arteries emerge. The thyroid gland is described as lying between the bifurcation of the aorta and the hyoid arch; it is a very large globular organ and its deeper surface is slightly prolonged as far as the arterial bifurcation. In *Raia oxyrhynchus* the thyroid gland is transversely elongated. In *Raia pastinaca* the coraco-hyoidei approach one another and the gland is longitudinally elongated; in this particular it corresponds to the *Selachian* type. In this last species it is a large pyriform organ with its broad end in relation with the hyoid cartilage, and its point extending nearly to the bifurcation of the branchial artery; at its point the gland presents a prolongation "which descends between the branchial sacs to a depth of about 0.5 cm." I desire to call attention to the fact that in *Carcharias* a posterior prolongation apparently also exists and is constantly present, but so far as I am able to observe it consists solely of connective tissue and contains no glandular substance; it can not, therefore, be in any way analogous to the anterior prolongation of the *processus pyramidalis*. I think it is to be connected with the fascia of the thyroid sinus, which will be discussed later on, rather than with the gland itself.

Guiard sums up his work on the morphology of the thyroid gland in the rays by saying that the organ lies beneath the coraco-mandibularis, between the coraco-hyoids, is always globous, of more or less pyramidal form, and with a prolongation backward to the bifurcation of the "branchial artery." This corresponds very well with the condition which I find in *Raia Erinacea* except that in this species, at least, the gland constantly overlies the bifurcation of the ventral aorta (branchial artery), and that it is always somewhat flattened, its ventro-dorsal axis being shortened. The organ is relatively thicker than in the *Selachians* because of the

presence of an increased amount of connective tissue between its vesicles.

On page 26 Guiard says that the thyroid gland "of fish" is always unpaired; it is quite obvious that this remark should apply only to the Elasmobranchs, the only order of fishes which Guiard appears to have studied.

Bridge ('04) passes the thyroid gland with the brief statement that "in adult Elasmobranchs the thyroid is represented by a moderately large compact organ, situated near the anterior end of the ventral aorta." Although he describes the gland as one of the "blood glands" in connection with the vascular system, he does not mention, nor indicate in any way, the source of its blood supply. The statement of its intimate relation with the aortic bifurcation might well lead one to erroneously suspect a supply from this source. In quite another place (page 332) he speaks of "a remarkable system of arteries for the supply of nutrient blood to the gills and heart," which takes origin from the ventral ends of the loops about the gill slits, the commissural vessels forming by their union the "median longitudinal hypobranchial artery which lies beneath the ventral aorta." He fails to mention the ultimate ramifications of this system of vessels or its relation to the thyroid gland, falling into the same error in this particular as T. J. Parker, from whose plates Bridge takes his figures, and upon whose description he appears to have largely based his text.

The literature upon the blood supply of the Elasmobranch thyroid begins with Hyrtl ('58) who first described the hypobranchial arterial system in the Batoidei, if we except the very incomplete description by Monroe (1787). Hyrtl described the thyroid artery as the "Ramus thyreoideus seu submentalialis" which takes origin from the "vein" of the second gill sac, and which gives off muscular branches to that part of the oral mucosa which lies between the inferior maxilla and the tongue bone as well as the Glandula thyreoidea." Hyrtl did not at this time describe a median hypobranchial artery, this vessel being represented in his description by two anastomosing vessels on either side of the median line which arise from the second and third arches and which

pass backward to supply the anterior coronary vessels. Hyrtl very clearly pointed out that the posterior coronary vessels arise from the subclavian artery in the Batoidea and in 1872 he showed that these vessels (posterior coronaries) were absent in the Selachii, a point emphasized at considerable length some years later by G. H. Parker and Davis ('99). In 1872 Hyrtl extended his description of the hypobranchial arterial system to the Selachii. He found the thyroid gland to be supplied by the "Arteria thyreo-maxillaris seu submentalis" which supplied the floor of the mouth and thyroid gland as in the Batoidei but which took origin from the "veins" of the first gill sac, rather than from the second as he had previously described for the Batoidei. He also described the "Arteria cardio-cardiaca," called later the "commissural" and "longitudinal commissural" (T. J. Parker) and the commissural and "lateral hypobranchial" (G. H. Parker and Davis), which fused in the median line to form a median vessel (median hypobranchial) and from which the coronary vessels were derived. At this time Hyrtl emphasized the absence of the posterior coronary branches of the subclavian in the sharks and called attention to an anastomosis from the subclavian forward to the median vessel from which the coronary arteries arose. This anastomotic vessel has since been called by T. J. Parker the "hypobranchial artery."

Turner ('74) injected the conus arteriosus and studied the course of the afferent and efferent branchial vessels; the course of these vessels is now well known. He neither mentioned nor excluded any relation to the thyroid gland, apparently not recognizing this organ, nor did he work out the ultimate connections of any of the smaller cervical vessels.

T. J. Parker ('80) described the venous system of *Raia nasuta* and called attention to "the extraordinary number of transverse anastomoses it [the venous system of the skate] presents, the results being to produce numerous 'venous circles,' comparable to the circle of Willis in the arteries of the mammalian brain, and the circulus cephalicus in the arterial system of bony fishes. There is also a direct passage from the sinus venosus and back again, in four different ways, namely: (1) by the hepatic sinus; (2) by the anterior part of the cardinal vein and the cardinal sinus; (3) by the whole length of the cardinal veins and their posterior anastomosis;

(4) by the lateral veins and the prolongation of the cardinal sinus into which they open."

I would like to direct attention to the presence of similar venous anastomoses in the Selachii as well as in the Batoidei. These anastomoses in the cervical region are frequent and voluminous. A complete circular anastomosis surrounds the mouth close to the maxillary and mandibular cartilages. Though not so readily seen in the Selachii, it follows the same course as in the skate in which fish it is visible through the skin and oral mucosa; it ends in a maxillary sinus at either angle of the mouth, which is connected with the orbital sinus and with the jugular vein. The hyoid sinuses are similarly connected across the median line near the ventral surface, two anastomotic vessels, the anterior the larger, connecting the opposite sides. This anastomosis bears a most important relation to the thyroid gland. The anterior vessel is so large as frequently to almost envelop the gland as in a capsule, the vessel is subdivided by fibrous partitions, or consists, rather, of a mass or series of vessels within a common sheath, and from its relation to the thyroid gland, in its more or less dilated condition it is more truly a sinus than a vein; it is conveniently designated the *thyroid sinus*. It fills and empties with each movement of the mouth and gills as water is forced through the branchial clefts, thus functioning with the aid of extrinsic muscles after the manner of a venous heart. When the fish is examined out of the water the violent movement of the gills so distends the sinus as often to wholly obscure the thyroid gland by the volume of its contained blood. It is almost impossible to reach the gland by dissection from the ventral surface without cutting the sinus or some of its numerous tributaries. The thyroid sinus receives the veins from the thyroid gland, most of these vessels leaving the dorsal surface or posterior margin of the organ.

T. J. Parker ('86) offers a description of the larger blood-vessels of *Mustelus antarcticus*, which is, however, deficient as regards the ultimate distribution of the smaller arteries. Exceptions may also be taken to his statement of the distribution of the arteries which constitute the rather remarkable hypobranchial arterial system, which as already mentioned, bears an important relation to the thyroid gland. Parker's description of the venous system is quite

accurate. The hyoid sinus is shown to empty into the jugular vein with a valve at the orifice. The anastomosis between the hyoid sinuses is considered, but its relation to the thyroid gland is not discussed; since no mention of the thyroid gland is made it would appear that this important organ was either overlooked or ignored. Unless one is specially looking for the gland, in the effort to separate the muscles without injury to the venous channels the organ is easily broken up, and once disintegrated its particles are readily lost amongst the mass of muscular tissue.

The tributaries of the hyoid sinuses are stated by Parker to include the submental, posterior facial, internal jugular, and the nutrient veins from the first hemibranch.

Parker's description of the hypobranchial system follows that of the ventral and dorsal aorta and begins with the subclavian artery, which, he says, gives off the branchial and hypobranchial arteries. Apparently he omits to mention the large lateral or epigastric artery, whose course parallels that of the lateral vein, though the beginning of the vessel is indicated but not named in some of his figures. The hypobranchial artery described and figured as a continuation of the subclavian, after giving off the antero-lateral artery—which I find to be distributed to the pericardium and adjacent muscles—unites with its fellow of the opposite side, passes forward 2 cm. in front of the conus arteriosus, and forms a plexus from which are given off the coronary arteries posteriorly, and anteriorly the median hypobranchial artery. The plexus communicates laterally by two commissural arteries on either side with the longitudinal commissural vessels uniting the ventral ends of the efferent branchial loops. One gathers from the description that the course of the circulation is from the subclavian artery through the hypobranchial to the efferent branchial loops, a direction which may be thus tabulated:

- branchial
- hypobranchial
 - coronary (paired)
 - median hypobranchial (azygos hypobranchial)
 - commissural (two pairs)
 - antero-lateral (paired)

Mention is not made of the lateral nor of all the coronary arteries. Parker's observations were made on *Mustelus antarcticus*. I have dissected three species of the Selachii and one of the Batoidei, I have not only been unable to confirm the course of the circulation as indicated but I find that beyond the so-called hypobranchial artery the course of the circulation is in the opposite direction, viz., from the efferent branchial loops to the coronary vessels and systemic capillaries, and the hypobranchial artery serves as a relatively unimportant anastomosis which, in these species is not even constantly present. In addition to the pair of coronary arteries distributed to the ventricle I have in my specimens observed a dorsal artery which ramifies largely in the wall of the auricle. The mandibular artery as described and figured by Parker, is the one from which in my preparations the thyroid artery is sometimes, though not constantly, derived. His description leaves one somewhat in doubt as to the origin of this vessel, but he has figured it correctly as coming from the first efferent branchial loop. His coraco-mandibular artery, derived from the mandibular, is a vessel which apparently corresponds with that which distributes its main branches, in my preparations, within the thyroid gland and only incidentally gives small branches to the coraco-mandibular and coraco-hyoid muscles; I have therefore called this vessel the *thyroid artery*.

G. H. Parker and Davis ('99) in an article on "the blood-vessels of the heart in *Carcharias*, *Raia*, and *Amia*" repeated the work of Hyrtl ('58 and '72) so far as it immediately concerned the origin of the coronary vessels, but being concerned only with the cardiac vessels they made no mention of the thyroid artery or other derivatives of the first hemibranch, nor of the gastric and pharyngeal branches which arise in close relation to the anterior coronaries. They described "the irregular longitudinal artery by which the ventral ends of some or all of the efferent branchial arteries of a given side are brought into communication," hitherto referred to as "longitudinal commissural" vessels (T. J. Parker) or as part of the *Arteria cardio-cardiaca* (Hyrtl), and called the vessels the "lateral hypobranchial artery," reserving for the name commissural "those arteries which leave the lateral hypobranchials on their median

sides and, after more or less tortuous courses, unite with one another in the median plane" to produce by their union the median hypobranchial artery. The ventral continuation of the subclavian artery they call the "coracoid artery." Concerning the anastomosis of this vessel with the median hypobranchial formed by the hypobranchial artery of T. J. Parker they speak as follows: "Moreover neither of these vessels [median and lateral hypobranchial] can be properly considered a dependency of the subclavian, for the branch which leaves that artery, and which T. J. Parker regarded as their root, may be connected with them, as Hyrtl ('58, p. 17, Taf. 2) has shown, by only a relatively small vessel. The union, then, is not in the nature of a continuous trunk, but an anastomosis, and the vessel posterior to this union must be considered in the light of an independent artery. This we have called the coracoid artery."

MATERIAL AND METHODS

For the purposes of the present study I have dissected 32 specimens of *Mustelus canis*, 10 of *Carcharias litoralis*, 3 of *Squalus acanthias*, and 14 of *Raia erinacea*. In addition to these I have had access to a number of sections from various Elasmobranchs prepared by my late assistant, Dr. Guy D. Lombard. The most of these animals were dissected through the courtesy of The Wistar Institute of Anatomy at the Marine Biological Laboratory at Wood's Hole, Massachusetts. My thanks are due these institutions for the opportunity afforded.

The form and position of the thyroid gland was carefully observed in each instance and its vascular connections determined both by dissection and by various methods of injection. The injections were made chiefly with a hypodermic needle of very fine caliber, though finely drawn-out glass tubes were used with some success. For pressure an aspirating syringe was used for routine work and served very well; air pressure was also used at times. For tracing the lymphatics, injections were frequently made into the substance of the thyroid gland and into the connective tissue about the thyroid blood sinus and the other cervical blood vessels. For tracing

the blood-vessels injections were made into both remote and near-by vessels, the points selected including the hyoid and thyroid sinuses, the thyroid artery, the efferent branchial loops and commissural arteries, the median hypobranchial artery, the coronary arteries, the ventral aorta, conus arteriosus, cardiac ventricle and auricle, the caudal artery and vein, the mesenteric artery and the dorsal aorta. Injections from these various points were made not only because of expediency in a given species but for the special purpose of determining the direction of flow and the relation of the vessels to the thyroid circulation; hence, injections were made from both sides of the branchial circulation, in the direction of the flow in the veins and the arteries while other injections were made in a direction opposite to the usual course of the circulation on the arterial side, though this was, of course, impossible in the veins because of the presence of valves.

Many of the thyroid glands were cleared and mounted *in toto*. This was best accomplished with those from *Mustelus*, in which species the gland is very thin. Some of the others were cut free-hand into thick sections. These preparations gave very good pictures of the lymphatics and blood-vessels except in the case of the very thick glands. Still other thyroid glands were sectioned for histological study.

THE ANATOMICAL RELATIONS OF THE THYROID GLAND¹

The thyroid gland is more or less closely related to most of the structures of the ventral cervical region, a region included between the mandible in front, the coracoid arch or shoulder girdle behind, and the branchial clefts on either side. This region forms the ven-

¹ The fact that the thyroid gland may be readily overlooked in the *Selachii* is amply demonstrated by the frequency with which this region has been studied and the almost entire absence of any adequate description of the gland. A brief description of the methods of dissection which may be relied upon to locate and expose the gland is offered in the hope that it may materially aid future investigation of this organ.

The thyroid gland of *Elasmobranchs* can be readily reached from either the oral or the cutaneous surface. By the cutaneous route two methods are especially serviceable, the one by longitudinal, the other by transverse incision.

tral wall of the pharynx and contains the whole course of the ventral aorta and its immediate branches. In *Raia* the heart is contained within this area at its posterior margin, lying in the median line just in front of the cartilaginous arch, but in *Mustelus*, *Squalus* and *Carcharias* the heart has been pushed backward and lies just beneath the coracoid arch.

The skin of the ventro-cervical region is thin but very tough; laterally it is folded upon itself at the branchial clefts, on the inner surface of which it becomes continuous with the mucosa covering the gills. The fibers of the mylohyoid and geniohyoid muscles are attached to the derma over the greater part of the ventral cervical area. These muscles form a thin sheet, thickest and most prominent in *Carcharias*, thinnest and frequently almost wanting

The first method is the more applicable in *Raia*, where the skin is loosely attached and the coraco-mandibular muscle is small, thin and easily lifted. With some variations I have followed the method outlined by Lombard ('09). A longitudinal incision is made in the median line through the skin and membranous constrictor pharyngis. The skin and adherent muscle are dissected away and retracted laterally, exposing the coraco-mandibularis. (Fig. 1 B.) A probe is passed beneath the muscle which, after being well freed, is divided midway between the mandible and the coracoid arch. The anterior flap is grasped and reflected forward, at the same time dissecting away from its dorsal surface the deep cervical fascia in which the thyroid gland is embedded. The gland is easily recognized by its deep yellowish orange color and its peculiar rounded or triangular form. In those exceptional instances when the thyroid gland is displaced forward in *Raia* the anterior division of the coraco-mandibularis will have to be reflected forward all the way to its mandibular insertion before the gland is fully exposed; ordinarily the organ will be found directly over the aortic bifurcation about midway between the mandible and the point at which the muscle was bisected.

The above method is less easily applied to the *Selachii* for the reason that the skin and the constrictor pharyngis are much more firmly adherent to the underlying structures than in *Raia*; moreover, in reflecting forward the anterior division of the coraco-mandibularis one is almost certain to injure the thyroid sinus, deluging the part with blood, before the gland can be exposed. In *Carcharias* one encounters the added disadvantage that the thyroid gland is quite firmly united to the coraco-hyoideus and the surface of the basi-hyal cartilage, and the gland is buried in a mass of connective tissue by which it usually is entirely obscured even after the coraco-mandibularis has been completely reflected away from its surface. The second method is, therefore, the more applicable in *Mustelus* and *Squalus* and is very much more certain in *Carcharias*. One blade of a blunt scissors is inserted into the first branchial cleft on the right and then on the left side and the clefts lengthened to their extreme ventral limits, the ends

in Raia, and subject in all species to great individual variation in volume. The muscles take origin posteriorly from the coracoid arch, anteriorly from the hyoid arch and mandible, and laterally from the outer surfaces of the branchial arches. Acting from these "fixed points" upon the more movable, but tough and inelastic skin, these muscles form a very powerful constrictor of the pharynx and collectively are very properly termed the "constrictor pharyngis" (fig. 1, A). In addition to this constriction the muscular contraction at the same time tends to draw open the branchial clefts, thus permitting the more ready passage of water during the rhythmic pharyngeal contraction or respiratory movement. The muscular fibers of the constrictor pharyngis are intimately adherent to the derma.

of the incisions exposing the margins of the coraco-hyoideus muscle. One blade of the scissors is then pushed beneath the skin where it readily passes between the coraco-hyoideus and coraco-mandibularis muscles (fig. 1); the incision is continued across the median line from side to side. This divides the coraco-mandibularis; its anterior portion is grasped with the forceps, lifted, and a longitudinal incision through the skin and fascia carried forward along either margin of the muscle. In the dogfish the divided muscle with the attached skin is easily raised and the loosely attached deep cervical fascia dissected away from its dorsal surface, exposing the thyroid gland. In *Carcharias* it is better to dissect the deep cervical fascia away from the ventral surface of the coraco-hyoideus muscle, rather than from the coraco-mandibularis; the thyroid gland is then raised with the latter muscle and dissected out from the mass of connective tissue which envelopes it. Finally, the gland must be dissected away from its anterior attachment to the margin of the basi-hyal cartilage, or, in *Carcharias*, to a median depression, in the ventral surface of this cartilage, which corresponds to the foramen caecum linguae of mammals; occasionally this depression is a true foramen, in which case the thyroid process becomes obviously analogous to the lobulus pyramidalis of the mammalian thyroid gland. This lobule is represented in *Mustelus* by a short triangular projection, not constantly present, which overlies a shallow median groove in the anterior margin of the cartilage. In *Raia* a similar condition is much less frequently present.

The thyroid gland is readily accessible from the oral cavity. A needle passed through the oral mucosa just in front of the basi-hyal cartilage—"lingual bone"—enters the substance of the thyroid gland if directed backward in *Mustelus* and *Squalus*, well backward and close to the cartilaginous surface in *Carcharias*, or backward and slightly ventralward in *Raia*. A transverse incision through the oral mucosa, parallel to and just in front of the basi-hyal cartilage, exposes the anterior margin of the thyroid gland and it may then be readily dissected out from *Mustelus* or *Raia*, though with greater difficulty from *Carcharias* or *Squalus*.

Removal of the integument with the adherent constrictor muscles exposes the coraco-mandibularis (fig. 1, B), a slender paired muscle, its two sides intimately fused in the median line, which takes origin by a tendinous fascia from the ventral surface and anterior margin of the coracoid arch. The paired muscle passes forward to its insertion, ending in short, rounded and slightly divergent tendons which are attached to the posterior margin of the inferior mandible. The muscle is inclosed within the folds of a superficial cervical fascia, which forms its aponeurosis and extends laterally to the surface of the branchial arches, but on either side of the muscle, the aponeurosis fuses with the deep cervical fascia with which it is in more or less close contact.

On lifting the coraco-mandibularis with its superficial cervical fascia the coraco-hyoid muscle (fig. 1, C) is exposed; it is similar in shape and appearance to the coraco-mandibular, but is much broader, its lateral margin projecting from beneath the coraco-mandibularis, and in *Mustelus*, *Squalus* and *Carcharias* extending laterally almost to the ventral ends of the branchial clefts, or even overlapping them somewhat. In *Raia* the gills are more widely separated, leaving a broad portion of the floor of the pharynx exposed at the side of the coraco-hyoideus in the anterior portion of the cervical region. Posteriorly the several divisions of the coraco-branchialis muscle cross this exposed portion of the pharyngeal floor to be inserted into the branchial arches.

The ventral surface of the coraco-hyoideus is smooth, its dorsal surface separates into several muscular processes, the musculus coraco-branchialis (*M. c. br.*, fig. 5), to be inserted into the membranous floor of the pharynx by a tendinous fascia overspreading and firmly adherent to the fibrous pharyngeal submucosa and the surfaces of the cartilaginous branchial arches. The divergent portion of the coraco-hyoidei, on either side of the median line, are similarly inserted into the movable basi-hyal cartilage (lingual bone), so that the combined coraco-hyoid and coraco-branchial muscles, arising from the anterior border of the coracoid arch, form a very powerful dilator of the mouth and pharynx.

As the coraco-hyoideus does not extend forward beyond the hyoid arch it exposes the membranous floor of the oro-pharynx

Fig. 1. Dissection of the thyroid gland of *Mustelus canis*. In *A* the skin has been reflected to show the superficial "constrictor pharyngis" muscle. In *B* the "constrictor pharyngis" has been removed and the coraco-mandibularis exposed. In *C* the coraco-mandibularis has been divided, exposing the thyroid gland lying upon the coraco-hyoideus. (See page 209 for explanation of abbreviations used in all figures.)

between this point and the inferior mandible. The cartilages of the hyoid arch except only the dorsal surface of the basi-hyal, are loosely adherent to this membranous floor so that when the mouth has been closed and the pharynx contracted the tongue-like basi-hyal cartilage is pushed forward, producing a deep fold in the oral mucosa. A needle thrust through this fold in the median line, passing dorsal to the cartilage, penetrates directly into the thyroid gland.

Not all of the ventral surface of the basi-hyal cartilage is covered by the insertion of the coraco-hyoideus muscle; the portion of the cartilage thus exposed varies in different species and to some extent in individuals. In *Carcharias* and *Raia* the muscle is inserted into only a small portion of the cartilaginous surface, while in *Mustelus* all but a narrow anterior margin is covered by the muscle fibers. The thyroid gland typically lies upon this exposed cartilaginous surface, extending backward for a greater or less distance upon the ventral surface of the coraco-hyoideus (fig. 1, C). In *Mustelus* and *Squalus* the ventral surface of the basi-hyal cartilage has a raised arciform anterior margin with a very slight medial depression, in *Raia* it is nearly flat, and in all these species the thyroid gland overspreads the cartilagelike a thin membrane whose convex anterior border nearly corresponds with the outline of the cartilage; in *Carcharias* the cartilage presents a deep median groove or furrow into which the thyroid gland sinks, lying there in a gelatinous mass of connective tissue so voluminous that the gland is partially, sometimes wholly, obscured.

In the dogfish and shark the thyroid gland is rarely pushed forward beyond the margin of the basi-hyal cartilage; in *Raia* the organ may extend farther forward so that it rests in part upon the membranous floor of the oro-pharynx. In one of the skates I found the gland carried so far forward that it lay wholly in front of the cartilage. In *Mustelus* and *Carcharias* individual variations are much less frequent than in *Raia*, but in *Raia* in the majority of individuals the gland lies directly upon the bifurcation of the ventral aorta (fig. 7).

THE THYROID VESSELS

The anterior margin of the thyroid gland is firmly attached by a dense fibrous fold of fascia to the antero-ventral margin of the basi-hyal cartilage. Its ventral surface is in contact with the coraco-mandibular muscle, to which it is firmly united by a fascia. Its dorsal surface rests upon the basihyal cartilage and the insertion of the coraco-hyoid muscle as described in the preceding section. The lateral angles and posterior margin of the thyroid gland are continuous with a fold of the deep cervical fascia which is placed between the coraco-mandibular and coraco-hyoid muscles, loosely attached to the opposed surfaces of these muscles, but more firmly fixed to their lateral margins, so as to form an aponeurosis for each. This fold of the fascia is much thickened anteriorly where it approaches the margin of the thyroid gland: at this point it incloses a transverse anastomosing vein which connects laterally with the hyoid sinuses.

At the posterior margin of the gland the fascia splits, a thin layer passing dorsally between the gland and the coraco-hyoid muscle, a thicker portion extending forward over the ventral surface of the organ, between it and the coraco-mandibular muscle. This ventral division is of special importance; it contains the large thyroid sinus consisting of an intricate net of veins and lymphatics, which connects laterally with the hyoid sinus and in the median line spreads over the ventral surface of the thyroid gland so that when distended with blood it entirely obscures the organ.

The thyroid sinus is surrounded with connective tissue containing a network of lymphatic vessels. Ink injected in the living animal into the space between the lateral margin of the thyroid sinus and the ventral end of the first branchial cleft will after a few minutes be found filling many of the lymphatic vessels of the thyroid sinus as well as many other perivascular lymphatics in relation with most of the cervical veins and the arteries of the hypobranchial system (fig. 8). The lymphatics of the thyroid plexus are so numerous and anastomose so freely that when filled with ink they form a sac-like investment entirely obscuring the sinus and the thyroid gland (fig. 2). An attempt to inject with

a fine needle directly into the thyroid sinus may force the fluid into either the venous or the lymphatic plexus, according as the one or the other system of vessels happens to be entered. I am convinced, as a result, of my injection experiments, that the lymphatics open freely into the veins of this part, after the manner of the "vasa lymphatica" of Favaro (*vide infra*). These observations are of interest in connection with Baber's inability to find lymphatics in the thyroid gland as indicating the relation between the vascular systems. I find, however, that it is not possible for fluids injected into the thyroid sinus or its plexus of lymphatics to pass in any quantity into the vessels within the thyroid gland; this is presumably because of the presence of valves.

The alternate expansion and contraction of the mouth and pharynx, forcing the stream of water through the branchial clefts, alternately fills and empties the thyroid sinus, so that by means of these respiratory movements the sinus acts somewhat after the manner of a venous heart. In this connection it is interesting to consider the observation of Favaro ('06), as quoted by Sabin ('09), that the relation of the veins and lymphatics in fishes is much more primitive than in mammals and that both lymph-hearts and vein-hearts may be present in these animals. The emptying and filling of the veins can readily be seen in the *Selachii* or *Raia* on removing the skin, or even through the integument in the living skate, the colored blood showing readily through the vascular walls. The relation of the lymphatics to the blood-sinus is so intimate that they must also be emptied and filled in the same way, though since they contain a colorless fluid they can not be so readily observed. I have, however, demonstrated that a colored fluid injected into these lymphatic vessels will overspread the thyroid region in ten to fifteen minutes and will almost entirely disappear within the next fifteen minutes; the lymphatic circulation must therefore proceed with considerable rapidity.

The thyroid artery approaches the organ from either side; in *Mustelus* and *Squalus* it enters at the extreme lateral angle of the triangular gland (fig. 19). In *Raia*, where the organ is of a more rounded form it enters near the middle of the lateral border. The branches of the artery ramify upon the surface of the organ send-

Fig. 2. Showing the form and relations of the thyroid sinus when injected with ink. *A*, in *Raia*. *B*, in *Carcharias*.

ing finer twigs into the interior. In this particular they offer an interesting analogy to the condition found by Major ('09) in man. Major says (page 484), "in the human the branching of the large arteries takes place mostly upon the surface of the gland, and having by their branching obtained their approximate distribution, the smaller branches are sent in." In the Elasmobranchs,

Fig. 3. Outline of the arterial supply of the thyroid gland of *Mustelus canis* as usually found. The vertical shading indicates the area supplied by the right thyroid artery, the horizontal shading that supplied by the left.

Fig. 4. Showing the less usual distribution of the thyroid arteries; the shading as in fig. 3.

branches the condition might well be described in the same words; this is the more remarkable inasmuch as Major states that this is not the condition in other mammals, *e.g.*, the dog and cat. The Elasmobranchs, therefore, seem to harmonize with the human rather than the lower mammalian condition as regards the distribution of the main branches of the thyroid arteries.

The left thyroid artery usually supplies a greater portion of the gland than the right (fig. 3), though the relative area is subject to extreme variation and in occasional instances the ratio may be reversed (fig. 4). The area of distribution in the great majority of individuals is approximately as indicated in fig. 3.

THE HYPOBRANCHIAL CIRCULATION AND THE ORIGIN OF THE THYROID VESSELS

In attempting to trace the circulation of the thyroid gland by means of injection experiments I was at once struck with the difficulty of reaching the gland by means of injections into the gill arteries, the ventral aorta or the heart. It was obvious that the thyroid artery has no direct connection with the ventral aorta or the afferent branchial vessels, a fact which seems to have been first observed by Simon ('44) who stated, without further explanation or any outline of his reasons therefor, that the thyroid gland in *Raia* "never receives the smallest share of supply from the branchial artery with which it is in contact." This fact seems to have been seldom recognized and never sufficiently emphasized by later writers.

The thyroid artery arises either from the mandibular artery, or by a common trunk with this artery, from an arterial sinus at the ventral extremity of the first branchial cleft (figs. 5 and 6); this sinus forms the ventral portion or connecting vessel of the efferent vascular loop contained in the hyoidean hemibranch and first holo-branch. From the dorsal extremity of this same loop the first efferent branchial artery passes to the dorsal aorta. It is therefore necessary for fluid injected into the ventral aorta or its immediate branches to pass through the gill capillaries before it can enter the thyroid arteries, and few injection fluids readily pass through capillary vessels. On the other hand, fluid injected into the thyroid artery or, as I later found, into any portion of the hypobranchial system passes readily into the vessels of the thyroid gland; such injections I repeatedly made, into the sinus at the ventral end of the first efferent branchial loop, into the thyroid artery, the median hypobranchial artery, and even into the coronary artery

taking care to prevent the escape of the fluid through the coronary vessels into the sinus venosus.

The hypobranchial system of vessels is so important for the thyroid gland as to deserve more than passing mention. T. J. Parker ('86) has described this system in connection with his much quoted work on the circulation in *Mustelus antarcticus*, but he makes no mention of its relation to the thyroid gland, in fact, he mentions neither the gland nor the thyroid artery; the gland was apparently not observed.

The main trunk of the hypobranchial system, in the species which I have examined is the median hypobranchial artery; it is formed by the commissural arteries coming from the ventral ends of the loops formed by the efferent branchial vessels which receive blood from the gill capillaries. These loops surround each branchial cleft, and within the gill they lie parallel to the afferent branchial arteries; they are just antero-internal to the afferent vessels. The ventral efferent vessels are very much smaller than either the dorsal efferent or the afferent (fig. 5). Opposite the ventral end of the second and third branchial clefts (sometimes only the second or the third) each loop gives off a commissural branch which passes inward and somewhat backward to the ventral surface of the ventral aorta where these vessels, with considerable variations, unite with their fellows of the opposite side to form a median hypobranchial artery which is frequently double so as to form a sort of elongated arterial circle. Sometimes the vessels fail to unite in front so that instead of a median hypobranchial there is a right and left hypobranchial artery, one on either side of the ventral aorta (fig. 5). Frequently the vessels so unite as to form an annular anastomosis which encircles the aorta, but portions of the ring may be absent. Some of these variations are indicated in figs. 5 and 6. The varied arrangements of these vessels are all indications of a more or less complete fusion of the commissural vessels to form a median hypobranchial artery. This vessel terminates posteriorly in a small sinus-like dilatation, single or double as the case may be. From this sinus the coronary vessels arise either as a median vessel which promptly divides, or as two or three independent vessels. From this same sinus a small paired

Fig. 5. Diagram of the hypobranchial arterial circulation in *Mustelus canis*; the insertions of the ventral divisions of the coraco-branchialis muscle are also shown. Ventral view. Anastomosis with the subclavian artery was wanting in this specimen.

artery passes backward on either side of the median line beneath the dorsal portion of the pericardium at the lateral margin of the cartilaginous floor of the pharynx formed by the basi-branchial cartilage; after anastomosing with its fellow of the opposite side beneath the apex of the cardiac ventricle it distributes its terminal branches to the wall of the esophagus and stomach near the cardia (figs. 5 and 6).

From the loop at the ventral end of the fourth branchial arch a very small anastomotic branch (less frequently arising as in fig. 6, *hypobr'*) passes backward along the lateral wall of the pericardium and penetrating between the precaval sinus and the coracoid arch anastomoses with the subclavian artery just prior to its division into the brachial (axillary) and the lateral (or hypogastric, a large artery lying parallel to the lateral vein. This anastomotic branch is undoubtedly that which T. J. Parker ('86) describes as the hypobranchial, which, according to his description receives blood from the subclavian and supplies the coronary arteries and whole hypobranchial system. Such is not the case, however, in any of the species I have studied and Hyrtl ('72) in his careful study of various species of the Selachii did not so find it, nor did Parker and Davis ('99). The hypobranchial is a very small artery, so small that its connection with the median hypobranchial is scarcely traceable, and in some individuals is entirely wanting (fig. 5), there being in these cases a small branch from the subclavian and a similar vessel from the median hypobranchial which follow the usual course but never unite, the subclavian branch distributing its blood to the muscles while the anterior division supplies the lateral pericardial wall and the adjacent muscles in front of the coracoid arch. Certainly where the vessel is wanting the flow of blood can not be in the direction indicated by T. J. Parker. Parker and Davis ('99), as already quoted, found the hypobranchial artery insignificant, though they did not record its absence.

If the median hypobranchial artery of *Mustelus* be injected the major portion of the fluid passes into the coronary arteries and thence through the coronary veins to the sinus venosus and auricle, while at the same time very little passes through the hypobran-

chial artery; the fluid pours into the sinus venosus and auricle very freely before it has even reached the subclavian by way of the hypobranchial artery. This is the case whether the fish has been previously bled or not. This experiment would certainly show

Fig. 6. Diagram of the hypobranchial arterial circulation in *Squalus acanthias*. Lateral view.

that the hypobranchial is of too small a caliber to supply the blood necessary to fill the coronary arteries, and if it can not supply this much it certainly is still less competent to supply blood for the whole hypobranchial system, which T. J. Parker's description

would seem to indicate was the case. The whole system may be readily injected from any one of the gill-loops with which it is connected. The true direction of flow is therefore from the efferent gill-loops through the commissural arteries to the median hypobranchial and from it to the muscular, pericardial, gastric, esophageal, and coronary branches, with only a relatively insignificant and inconstant anastomotic supply from the subclavian artery.

Anastomoses in both the arterial and venous systems, forming "circles" about the body wall and the viscera are of very frequent occurrence in this class of fishes as was pointed out for the venous system by T. J. Parker in 1880 (*vide supra*). The subclavian artery forms such a circle beneath the coracoid arch and several similar "circles" are formed by anastomosis between the two sides in the hypobranchial system as well as in other parts of the body with which we are not now specially concerned. The arrangement in the arterial system is therefore very similar to that which Parker found in the venous.

At the ventral extremity of each efferent gill-loop, at the point where the hypobranchial commissural arteries arise, is a small sinus-like dilatation (figs. 5 and 6, *s. a. v.*) which obviously serves as a reservoir where the blood coming from the two sides of the loop, which are in adjacent branchial arches, will intermingle, and from this sinus blood is distributed through the commissural arteries ("lateral hypobranchial" of Parker and Davis) anteriorly, posteriorly, or to the median hypobranchial in such proportion as the caliber of the several vessels and the course of the circulation dictate. The arterial sinus at the ventral end of the first gill-loop (first ventral sinus) is usually a trifle larger than the others. The thyroid artery arises from the anterior end of this sinus or from the adjacent portion of its anterior limb in the hyoid-dean hemibranch. It arises either as a separate and independent vessel or as a conjoined trunk with the mandibular artery; more frequently, in the specimens I have dissected, it was independent. The artery passes directly forward and inward to the extreme lateral border or angle of the thyroid gland. It continues its path along the surface of the thyroid gland (figs. 3, 4 and 19) near

its anterior border, distributing its main branches to the substance of the gland and small collateral branches to the floor of the pharynx in front of the hyoid arch and to the anterior third of the coraco-hyoideus and coraco-mandibularis muscles. A small median unpaired vessel arising from the left thyroid artery (less frequently from the right), penetrates the thyroid gland, divides, and enters the coraco-hyoideus to supply the antero-median portion of this muscle. This is very probably homologous with the anterior portion of the *arteria thyroidea impar*, derived from the median hypobranchial as described by Hyrtl ('72).

The left thyroid artery is usually larger, longer, and more extensive as to its area of distribution than the right. Lombard ('09) dissected a number of specimens of *Mustelus* and *Raia* and found that the left thyroid artery more frequently entered the dorsal surface, and the right the ventral surface, of the thyroid gland. I have found a somewhat similar condition, though I very frequently find both vessels coursing upon the ventral surface and sending their branches dorsally into the substance of the gland. Occasionally the right thyroid artery enters the dorsal surface of the gland and the left the ventral (fig. 3 and 19). The position and distribution of the thyroid arteries is, however, subject to considerable variation and, as I have already pointed out, the right may even supply a greater portion of the gland than the left thyroid artery (figs. 3 and 4).

The thyroid artery as described very probably in part corresponds to the vessel which was recognized by T. J. Parker ('86) as the coraco-mandibular artery. This latter is an obviously inaccurate designation, for the coraco-mandibular branches are insignificant as compared with the other ramifications of the artery. Hyrtl ('72) recognized and more accurately described the thyroid artery as arising from the "veins of the first gill-arch" in conjunction with the submental artery; his description appears to be accurate with the exception that the two arteries in my dissections appear more frequently to arise independently.

The observations which I have recorded concerning the origin

Fig. 7. The position and relations of the thyroid gland in *Raia*.

Fig. 8. Injected lymphatics in the tunica adventitia of the right commissural artery at its junction with the median hypobranchial. The specimen is from *Carcharias*, the fish having been injected with ink at a point just ventral to the right hyoidean hemibranch. The injection followed the lymphatics and was traced as far as the median hypobranchial artery in one direction and into the thyroid gland in the other.

and course of the thyroid artery and the hypobranchial system were carefully worked out with specimens of *Mustelus* and *Squalus* and verified in all their important particulars in *Carcharias* and *Raia*.

VEINS AND LYMPHATICS OF THE THYROID REGION

The numerous small veins of the thyroid gland discharge into the "thyroid sinus," which connects together the hyoid sinuses of the two sides. The conformation of the hyoid sinuses and their tributaries and connections have been well described by T. J. Parker ('86). In addition to the transverse anastomosis formed by the thyroid sinus, the hyoid sinus receives a submental vein from the region of the mandible and numerous small muscular branches from the neighboring muscles. This sinus and its connecting vessels can be most readily observed in *Raia*. The submental vein is seen to begin as a double transverse anastomosis; the larger, anterior, tributary lies close behind the cartilage of the inferior mandible; the smaller, posterior vessel arches across the floor of the mouth just in front of the hyoid arch and the anterior border of the thyroid gland. At the angle of the jaw these vessels unite in a small sinus which also receives a transverse anastomosis from in front of the maxilla, so that the mouth is thus encircled by an annular venous sinus. The thyroid sinus similarly forms a double transverse anastomosis, rather more deeply placed, behind the hyoid arch at the posterior border of the gland. These vessels convey the blood from the ventral cervical region to the hyoid sinus.

The thyroid veins open into the thyroid sinus as several small branches, the largest of which are a median vein, leaving the organ near the middle of its dorsal surface, and two anterior veins which leave the same surface near the anterior margin of the organ, but a little to either side of the median line. Other smaller veins leave the lateral margins of the organ passing either to the thyroid or the hyoid sinus. The thyroid veins must contain valves, for although the vessels can be readily traced with the dissecting microscope and even with the naked eye, it is with difficulty that

fluid injected into the thyroid or hyoid sinus can be forced back into the venous channels of the thyroid gland; an extreme pressure will accomplish this result to a limited extent only. I have been able to find some traces of valves in microscopical sections.

The hyoid sinus passes around the base of the hyoidean hemibranch to connect with the jugular vein, through which a portion of its blood is transmitted to the precaval sinus beneath the coracoid arch, and thence to the sinus venosus and auricle. The flow through the hyoid sinus in this direction is quite intermittent, and, as already indicated, it is chiefly dependent upon the muscular force of the pharynx as it alternately relaxes and contracts to force water through the gill-openings.

Blood is also transmitted from the hyoid sinus to the heart by the more ventral and direct path through the inferior jugular (anterior cardinal) vein. This vessel maintains a more constant flow, receiving blood from the ventral cervical region and the branchial arches, along the ventral ends of which it courses to terminate in the precaval sinus.

In *Raia* the thyroid sinus is thin, and its investment of connective tissue containing the lymphatic plexus is less pronounced than in the other species studied, so that, except when the sinus is fully distended with blood, the gland in *Raia* is not much obscured. In *Mustelus* the sinus is larger and the fascia about it is more voluminous so that the gland is usually more or less obscured, though there is much individual variation: the same is true of *Squalus*. In *Carcharias* the vascular walls in the sinus are so thick, and the connective tissue about it so abundant that in most of the animals examined the outline of the thyroid gland contained within this mass could only be discerned on holding up the stretched membranous mass between the eye and the bright sun so that the intense transmitted light showed the yellowish orange gland contained within the connective tissue mass.

The thyroid lymphatic plexus forms an extensive group of vascular channels surrounding the gland and the vessels of the blood sinus. It is contained in a fold of the deep cervical fascia which stretches across from side to side between the ventral ends of the first branchial clefts: it is broad in the mid-portion,

but tapers from the postero-lateral angle of the thyroid gland outward to the tissue surrounding the hyoid sinus. The vessels form perivascular lymphatics about the venous sinuses. Ink or a colored fluid injected into the connective tissue about the hyoid and thyroid sinuses readily fills the anastomosing vessels forming a sheetlike mass of peculiar form (fig. 2, *thyr. sn.*). Ink thus injected can also be traced into the perivascular lymphatics of the hypobranchial arterial vessels (fig. 8) as far backward as the walls of the coronary arteries; it can likewise be found in small perivascular lymphatics in the walls of the thyroid arteries and to some extent in the broad venous spaces between the vesicles of the thyroid gland, indicating that the lymphatic vessels to some extent may open into the veins of the thyroid. The vessels of the lymphatic plexus in the cervical fascia are apparently connected with the blood-vessels of the thyroid sinus, for excessive pharyngeal contraction in the living fish forces blood into areas which otherwise appear to be occupied only by lymphatic vessels. The blood-vessels may without doubt be classed as "venae lymphaticae" and the lymphatics as vasa lymphatica" after the terminology of Favaro ('06), who says that the same vessel may in fishes carry either blood or lymph at the same or different times so that these vessels may in this sense be either vasa or venae lymphaticae. Fluid injected into the lymphatics spreads so rapidly over so great an area that it seems almost impossible to trace a connection with the blood sinus by means of injections; the fluid enters the blood-vessels so readily that one is unable to exclude the possibility of an intra-venous injection.

The statement by Baber ('81) that he was able to demonstrate no lymphatics in the thyroid gland of Elasmobranchs led me to pay special attention to the study of these vessels by injection methods. As I have already pointed out, Baber states that "in both the skate and the Conger-eel an extensive system of vessels lined with epithelium becomes injected by the method of puncture." He then injected the blood vessels of a Conger-eel with Berlin blue through the "efferent branchial vein" and "dorsal aorta and thereupon states that "in the Conger-eel at least, there is no evidence of any system of lymphatic vessels," emphasizing

his statement by the use of italics. I can confirm that portion of Baber's statement which says that an extensive system of vessels within the thyroid gland can be readily injected by the method of puncture, but I would maintain that neither that procedure, nor the injection of the dorsal aorta or efferent branchial vessels with Berlin blue, would demonstrate the absence of lymphatics; that they may still be present, I have demonstrated both in microscopical sections and by injection (figs. 9, 10 and 11).

Fig. 9. Lymphatics, "vasa lymphatica," and veins, "venae lymphaticae," of the thyroid gland. The "vasa lymphatica" have been injected with ink and the thyroid gland cleared and mounted *in toto*; the lumen of the follicle and the follicular epithelium are only indistinctly seen. At X in the specimen the two sets of vessels anastomose.

Injection by puncture does not always fill the extensive system of vessels observed by Baber. If one takes care to use only a very gentle pressure, this system, which completely surrounds each vesicle, is only filled near the point of injection, while at the margins of the injected area the fluid spreads through more minute vessels which lie in closer contact with the vesicular epithelium (fig. 9, L). I believe these last are true vasa lymphatica in the sense

of Favaro and I find the contents of the vesicles apparently secreted into them as in the mammalian thyroid (fig. 10). The larger vascular channels then are *venae lymphaticae*, readily injected by puncture if the pressure is excessive, the veins being easily entered because of their large caliber and extremely thin walls; they transmit only lymph when the intravenous blood-pressure is low within the gland, but fill with blood when from any cause the pressure is raised. I have invariably found some blood cells in the *venae lymphaticae*; I have never found them filled with blood in all the three score animals I have examined except in one case in which as a result of injury the thyroid gland was greatly congested. In this case they were filled to distension. In microscopical sections I have been able to trace the connection of the *vasa* with the *venae lymphaticae* (fig. 10). I have been unable to demonstrate positively the presence of any valves at the orifices of these vessels, but the extreme obliquity of the anastomosis considered in conjunction with the very thin vascular walls might well serve a valvular function when the blood pressure is low, though with increased pressure and venous distention some blood would be forced back into the *vasa lymphaticae* and even into the vesicles. The frequent occurrence of red blood corpuscles within the vesicles of all animals is well known and in the Elasmobranchs it is thus accounted for. The intimate relation between the venous and lymphatic systems pointed out by Sabin ('09) would possibly suggest that an homologous vascular relation may account for the presence of red blood corpuscles within the vesicles of the mammalian thyroid gland.

THE HISTOLOGY OF THE ELASMOBRANCH THYROID GLAND

The thyroid gland in Elasmobranchs consists of a mass of vesicular follicles (figs. 10, 11 and 13 to 18) which very closely resemble those of the mammalian gland. The vesicles are lined by epithelium of a low columnar type, contain more or less colloid material, and are loosely bound together by a connective tissue framework which is very richly supplied with blood-vessels.

The shape of the gland in *Mustelus canis* (fig 1, C) is sufficiently

Fig. 10. Section of the thyroid gland of *Mustelus canis* showing the anastomosis at the point X of the vasa and venae lymphaticae.

Fig. 11. Typical section of the thyroid gland of *Mustelus canis*; the intimate relation of the epithelium of the thyroid follicles to the lymphatics and blood vessels is accurately shown.

peculiar to deserve passing mention. It may be described as consisting of two triangles whose bases are fused in the median line, the apices directed outward, the anterior borders convex and conforming to the anterior margin of the basi-hyal cartilage, the posterior borders concave and free, except for their attachment to the deep cervical fascia. In the median line the conjoined bases are prolonged backward to form a short median projection; anteriorly a shallow notch separates the two lateral triangular halves. The gland is approximately bilaterally symmetrical (figs. 1, 3 and 4).

The thin, almost membranous character of the gland in *Mustelus canis* offers an excellent opportunity for the recognition of a lobar or lobular structure if such exists, for the whole gland is frequently no more than four or five follicles in thickness and may be stained, cleared and mounted *in toto*, giving very excellent microscopical pictures of the entire organ. I have not been able to find any indication of definite lobes or lobules. Portions of the thyroid substance are here and there wanting, as observed by Lombard ('09), and these deficient areas occur more frequently in the posterior than in the anterior half of the gland. In one of the thirty-two fishes of this species the deficiencies were so great that the gland was only represented by a few specks which were positively identified as portions of the thyroid only after microscopical examination. A similar case was found in *Squalus*, and one gland from *Carcharias* consisted of three small pieces.

Occasionally the posterior border presents a notched deficiency in or near the median line. There may be one, two or three such notches, either symmetrically or asymmetrically disposed. Deficiencies of the thyroid tissue also occur within the gland and may, or may not, be connected with the notches in the posterior border. These deficiencies are all of inconstant occurrence, irregular location, and could scarcely be taken to indicate any suggestion of definite lobes. They seem rather to be due to the extreme thinness of the gland and in many of the thicker specimens they are in no way indicated. When present they are occupied by connective tissue continuous with the glandular capsule. Frequently they transmit the larger thyroid vessels.

Bits of thyroid tissue of inconstant form or location are occasionally separated from the body of the gland by narrow partitions of connective tissue; they are most frequently found near the border of the gland or adjoining an area in which the thyroid substance is deficient. Since they possess no constant relation to the vascular supply, the detached masses can not correspond in any sense to true anatomical lobules. The arteries branch irregularly, for the most part after a somewhat dicotymous fashion (fig. 12), the arterial twigs passing off at acute angles. Partially injected specimens in which the injection fluid has passed through the arteries but has not penetrated in quantity into the veins

Fig. 12. Terminal divisions of the thyroid artery. The area occupied by injected capillaries, "venae lymphaticae," directly connected with each terminal arteriole is roughly indicated by the dotted lines.

show areas of injected capillaries surrounding the terminal arterioles (fig. 12), but the extent of these injected areas and their relation to the artery seems to be dependent rather on the pressure of the injection than on any constant or characteristic relation to the vascular system. I can not recognize any probable vascular or anatomical unit which might in any sense serve as an anatomical lobule or structural unit, as described for various other glands by Born, Mall and others.

In *Raia* the occurrence of partially detached groups of thyroid follicles is more frequent than in the other species, but the number of such groups present in a gland varies from two or three to a score or more. The groups are outlined by connective tissue in

which broad venous spaces to a certain extent encircle the quasi-lobule. The veins thus lie at the periphery while the artery on reaching the group promptly breaks up into a plexus of broad capillary spaces—*venae lymphaticae*—which surround the follicles within the quasi-lobule. The number of follicles in the group varies from four or five to several score.

In *Carcharias* the condition is similar to that in *Mustelus*, there being no indication of lobular groups except about the occasional irregular deficiencies in the thyroid mass. Except for the anatomical disintegration of the gland in one fish there was similarly no indication of lobulation in *Squalus*, but as none of my specimens from this species were prepared as total mounts I can not speak with the same certainty as in the other species.

The form of the thyroid follicles is subject to considerable variation, but, in general, they may be said to be of ovoid shape, and, as pointed out for the mammalian thyroid by Streiff ('97), they present frequent diverticula. The Elasmobranch thyroid differs from those described by Streiff in that they show very little tendency to branch and no indication of a tubular character when the whole follicles are examined in total mounts of the gland (fig. 13). In cut sections diverticula are of frequent occurrence and are apparently the result of pronounced infoldings of the follicular wall rather than of any protuberance, or of any tendency of the follicle to branch. Fig. 13 shows characteristic follicles from all four species; the figures are of whole follicles and differ from the cut sections in that only the largest infoldings of their wall are visible. As already indicated, diverticula are more apparent in sections than in the preparations (total mounts) from which the drawings have been made. The particular follicles drawn from *Carcharias* present rather greater infoldings than those from the other species. I have not, however, observed that this is characteristic of *Carcharias*. In the figure the magnification is the same for the several Selachian species but less by one half for *Raia*; the follicles of *Raia* are, therefore, relatively about twice as large as shown.

The size of the follicles is subject to considerable variation as regards the individual follicles, the different thyroids, and the

Fig. 13. Outline of the follicles of thyroid glands as seen in total mounts. *A*, from *Mustelus canis*, $\times 152$. *B*, from *Squalus acanthias*, $\times 152$. *C*, from *Carcharias litoralis*, $\times 152$. *D*, from *Raia erinacea*, $\times 80$.

various species. I have tabulated the results of some of the measurements.

SPECIES	MAXIMUM DIAMETER OF FOLLICLE	MINIMUM DIAMETER OF FOLLICLE	AVERAGE DIAMETER OF FOLLICLE	AVERAGE LENGTH OF FISH	RATIO FOLLICLE TO FISH	AVERAGE DIAMETER THYROID GLAND
<i>Mustelus</i>	.160mm.	.017mm.	.067mm.	67.8cm.	101.7	13.6mm.
<i>Squalus</i>	.079	.013	.047	53.7	115.	no data
<i>Carcharias</i>	.273	.023	.100	117.2	117.2	13.7
<i>Raia</i>	.340	.053	.167	46.2	27.7	6.5

The very large relative size of the follicles of *Raia* is at once apparent. They are approximately four times as large, relatively to the length of the fish, as in the case of any other species. When compared with the diameter of the gland the ratio is again increased, but this difference is in part compensated for by the increased thickness of the gland in *Raia* as compared with the other species. The thyroid gland of *Raia* is 1.5 times as thick as that of *Carcharias*, and 2 to 2.5 times the thickness of the gland in *Mustelus*.

The column of ratios in the above table would indicate that in the Selachians the size of the follicle is in approximate proportion to the size of the fish, but that in *Raia* the relative size of the follicle is many times as great; the actual number of follicles in the thyroid gland of *Raia* is only a small fraction of those in the gland of any of the other species. It is readily susceptible of mathematical proof that the combined circumference of large follicles contained in a given area is less than the combined circumference of smaller follicles in the same area; hence the gland of *Raia* with its larger follicles will contain proportionately less epithelium than the glands of the other species. I estimate that the difference is just about sufficient to render the volume of secretory epithelium in the gland of *Raia* relative to the size of the fish equal to the volume of secretory epithelium in each of the other species.

But it is equally susceptible of mathematical proof that the cubical contents of the combined follicles is greater in the gland having the larger follicles; hence there is in *Raia* a greater volume of intrafollicular space than in the other species. It is scarcely susceptible of proof but entirely reasonable to suppose that the epithelium of different individuals of the same or different species so closely allied and the Batoidei and the Selachii is approximately equally active as regards its secretory function. There is ample evidence that the fluid secreted by the thyroid epithelium into the cavity of the follicle finds its way through the wall of the follicle to the neighboring vascular spaces so that the direction of flow must, in part at least, be from the epithelium into the follicle and thence through the follicular wall to the vessels. In view of these facts the rate of this secretory flow in *Raia*, with its relative y

large intrafollicular space must be slower than in the other species with their relatively small intrafollicular cavities, or, to express it differently, there is relative stagnation in intrafollicular secretory flow in the case of the thyroid follicles of *Raia*. It is well known that an albuminous secretion which is rendered relatively stagnant within the epithelial cavities of the body tends to produce colloid masses whose microchemical reactions more or less closely resemble those of the colloid material of the thyroid gland; this occurs, *e.g.*, in the ducts and tubules of the resting mammary gland and in dilated cystic tubules in the kidney. We would therefore expect that in the thyroid follicles of *Raia* with their relatively stagnant secretory flow we should find an increased amount of colloid material. This I find to be the case, the proportionate volume of colloid present in the follicles of *Raia* being decidedly greater than in the other species. Similarly I find it the rule that the larger follicles contain relatively more colloid than the small follicles in the same gland. The volume of colloid contained in the thyroid follicles, therefore, can not be regarded as an index of the activity of the secretory epithelium; it would rather appear as a sort of by-product whose volume was dependent upon the rate of flow in the fluid from which it was formed. This view harmonizes the appearance of colloid material in the thyroid gland with the occurrence of similar material in the other glandular portions of the body, and with those theories of thyroid secretion which regard the colloid as a by-product rather than as the secretion. Moreover the great variations in the amount of colloid in the thyroid follicles are then explicable upon the basis of variations in the rate of secretory flow which, in turn, is dependent upon the physiological factors of blood and nerve supply as well as upon the anatomical factors.

It is also interesting to observe that the volume of secretory epithelium in the several species examined remains in each case approximately proportionate to the size of the thyroid gland and to the size of the fish. The relation of the epithelium to the follicular content and to the blood vessels and lymphatics seems to me to indicate most clearly that the secretion is poured out from the epithelial cells so as to find its way, on the one hand directly into

the vessels, and on the other hand into the follicular cavity, whence it eventually passes through the follicular wall to reach the vessel. It is in the course of the latter flow that the colloid appears and its volume is dependent upon the rate of flow.

The question arises as to whether or not the colloid may serve for the storage of secreted materials, somewhat after the manner in which the hepatic glycogen may be considered as stored carbohydrate to be delivered as the needs of the economy necessitates; if so the colloid material should show further evidences of change, at least, under certain conditions. That the colloid does undergo changes is evidenced by the appearance within its otherwise homogeneous mass of such structural alterations as vacuolation, basophile degeneration, and disintegration into granules of greater or less size, changes which are frequently observed (figs. 14, 15 and 18). As to the physiological nature of these changes in colloid, and their possible connection with a storage function I can offer no conclusive proof, but it seems to me quite possible that such a relation exists.

The thyroid follicles are lined by a simple columnar type of epithelium (fig. 11) whose cells show considerable variation in height. In the same gland the epithelium lining certain vesicles measured as much as .010 mm., others only .006 mm. The epithelium of occasional vesicles was even lower, but was possibly open to the criticism of mechanical distortion since the colloid was often crowded against one side instead of lying in the middle of the follicular lumen, even though the tissues had been prepared with the greatest care. Being anxious to avoid any possible distortion of the tissue, I removed nearly all of the glands studied without allowing them to be touched by either instrument or fingers, the knife or scissors being passed through the muscle beneath, and the gland, supported on a thin layer of muscle, dropped bodily into the killing fluid.

As a rule those follicles which were well filled with colloid possessed low epithelium, in those with taller epithelium the reverse was the case. In making this comparison the surface area of the sections of colloid mass was compared with that of the containing follicle. The average height of the epithelium of a number of fol-

licles in *Mustelus* which were well filled with colloid was .077 mm., while in a similar number of follicles which were either devoid of colloid, or nearly so, the height of the epithelium averaged .087 mm.

Each epithelial cell possesses a fairly distinct cell-wall. Many cells appear to have a well marked cuticular border which appears to be more highly refractive than the endoplasm, but wherever the colloid lies in contact with the surface of the cell the cuticular border is obscured. I have also noticed that it is less pronounced in the thinner sections so that I am inclined to regard it as an optical diffraction line rather than a true cuticular membrane. The conformation of the free ends of the epithelial cells tends to confirm this opinion. These cells project slightly into the lumen of the follicle by means of a somewhat convex free border so that the height of a cell is greater in its axis than at its margin. Thus the lower portions of a cell will, in the thicker sections (.010 mm. or more), show through the taller central or axial portions and so account, at least, for a portion of the cuticular appearance.

The exoplasmic membrane is specially distinct in the epithelium of the Elasmobranch thyroid gland. Baber ('81) called attention to this fact, and described it as an intercellular network enveloping the cells and connecting the lumen of the follicle with the surrounding tissue spaces. If the lining epithelium of the follicle be cut parallel to the surface the resulting sections will show the membrane as a distinct mosaic within whose meshes the cells are apparently contained. It appears to me that this mosaic, which is distinct from the intercellular colloid observed by Langendorf (see page 200), is rather to be regarded as a cell membrane than as an intercellular substance, for there are many portions where in thin sections a narrow intercellular space is distinctly apparent and is bounded on either side by the exoplasmic membrane of adjacent cells. Occasionally the cells are separated by wider intervals through which the follicular lumen is placed in direct communication with the surrounding tissue spaces.

There appears to be no distinct basement membrane upon which the follicular epithelium may rest. At intervals the cells are invested with a very small amount of loose connective tissue (fig. 11), but in large part the epithelium rests directly upon the

walls of the venous channels and lymphatic vessels (fig. 14). Thus the relation of the epithelium to the vascular lumen is a very intimate one.

The cytoplasm of the "chief" cells is relatively clear, but contains a coarsely granular eosinophile reticulum. Some cells appear much more granular than others. In such cells as are filled with colloid, "colloid cells," the granular reticulum is entirely obscured (fig. 14, A).

The nuclei of the chief cells are spheroidal, vesicular, and are placed near the base of the cell. From the apices of many of these cells threads of secretion extend to the central colloid mass. The apices of many of the chief cells appear ragged, frayed, and often shrunken, so that the height of the cell is decreased. Such cells present an appearance suggestive of an advanced stage of secretion. Other cells contain granules at the distal ends which are arranged in vertical rows, giving this portion of the cell a somewhat rodlike appearance; such cells are usually well filled with granules. Occasionally a similarly ragged and rodlike appearance is seen at the base of the cell and it suggests that secretion may also be discharged at that point. Such a possibility is rendered more probable by the absence of basement membrane and the intimate relation to the lymphatics and blood vessels, these cells often resting directly upon the vascular endothelium. Laterally the epithelial cells frequently are separated from one another, leaving considerable spaces or channels through which secretion may find its way from the follicular lumen to the neighboring vessels; such channels are often occupied in part by colloid and in a few cases I have traced the colloid in a continuous line from the intrafollicular mass to the interior of the vasa and venae lymphaticae (fig. 10).

The above observations suggest that secretion may either be discharged from the chief cells into the lumen of the follicle and thence find its way through the follicular wall to the blood and lymphatic vessels, or that it may be discharged from the cells directly into the vessels; this is in harmony with the conditions indicated in the thyroid gland of mammals.

Fig. 14. A, section of the thyroid gland of *Mustelus* showing a follicle completely surrounded by "colloid cells." At *y* a carmine granule, derived from the injection mass, lies in the vena lymphatica. B, section of the thyroid gland of *Mustelus* showing follicles lined entirely by "chief cells."

The colloid cells described by Langendorf ('89) are remarkably distinct in most of the sections of *Elasmobranch* thyroids and constitute one of the most characteristic features of the thyroid gland of these fishes. The colloid cells are distinctly acidophile and are easily recognized in specimens stained with hematoxylin and eosin if the eosin is used in dilute solution and allowed to act for one-half hour or more. They present a glistening, highly refractive colloid appearance, which is in marked contrast to the granular chief cells. The colloid cells occasionally occur singly, but are more frequently disposed in groups along one side of the follicle. One such group (fig. 14, A), more extensive than the others, was seen to include fully three-fourths of all the epithelium in its follicle. The groups are often in contact with the central colloid mass, and the colloid within the cell may then appear continuous with that within the follicle. Occasionally a group of such epithelium appears to have been completely engulfed by the colloid mass, the epithelial nuclei then appearing well within the colloid. Such appearances might suggest mechanical distortion, but as the surrounding follicles show no evidences of injury, and, as already stated, the tissues were very carefully prepared, I am more inclined to agree with Bozzi ('95) that these appearances are the result of vital phenomena.

The nuclei of the colloid cells are small and deeply stained, so deeply, in fact, that in the usual preparations they frequently show neither nuclear wall nor karyosomes. Unlike the nuclei of the chief cells they are usually situated near the inner extremity of the cell rather than at its base. The greater the cell is distended with colloid the farther its nucleus is pushed toward the cell's apex; in the most distended cells there was frequently some distortion and even fragmentation of the nucleus. A further continuation of this process would account for at least a portion of the extruded and disintegrating nuclei found within the intrafollicular colloid masses (figs. 11 and 18).

The intrafollicular colloid closely resembles that of the mammalian thyroid gland. It is strongly acidophile and is usually homogeneous or very finely granular in appearance. Frequently a minor portion of the mass, *e.g.*, one side, is finely granular while

the major portion is clearly homogeneous. The well-known filaments pass at frequent intervals from the colloid mass to the epithelial surface. Some of these filaments can be traced to the free surface of the epithelial cell while others quite clearly enter the intercellular spaces, where, in tangential sections of the follicle, they form intercellular masses simulating the net-work described by Baber ('81) and interpreted by Langendorf ('89) as the ramification of colloid cells.

Occasionally the colloid mass appears to have been disintegrated into small spherules .007 to .008 mm. in diameter (fig. 15). The size of these spheres is suggestive of the red blood cells of mammals, but the red cells of Elasmobranchs are ovoid and larger. Of the spherules some are distinctly acidophile but many are slightly basophile, none, or very few, are strongly basophile. All the spherules are homogeneous, and I have observed in the more basophile no tendency to chromatolysis such as one might expect to find if the spherules of this type were thought to represent degenerating nuclei of the red cells, nor have I been able to trace stages of transition from the nucleus to the basophile spherule. Since all the blood cells of the species studied are nucleated one could not well infer that the acidophile spherules could represent any stage in the disintegration of red blood cells, for none of these spherules contain even traces of chromatin. On the other hand, both red and white blood cells can occasionally be found within the colloid quite independently of the spherules I have described; in this particular the Elasmobranch thyroid is in accord with the well known structure in other vertebrate orders. The appearance, location, disposition and reactions of the spherules indicate their origin from the solid colloid masses, from which they would appear to be formed by disintegration with progressively increasing basic reaction. That the reverse process occurs, viz., that the spherules may represent intermediate stages in the formation of the colloid masses, is contraindicated by the fact that only very few follicles contain spherules, nor does there appear to be any indication of a tendency of the spherule to fuse. On the other hand, a tendency to further disintegration is quite apparent, and the possibility is suggested that the colloid in this way may be trans-

Fig. 15. Section of the thyroid gland of *Raia* showing the colloid within a follicle disintegrated into spheroidal masses of varying size and depth of stain.

Fig. 16. Section through the ventral margin of the thyroid gland of *Mustelus* with sections of the very broad venae lymphaticae of the thyroid sinus simulating an endothelial capsule about the gland.

formed to such a state that it may be secreted through the wall of the follicle, in which case the intrafollicular colloid would presumably assume the nature of stored secretion which is first poured out from the cells as a fluid, is then condensed through retention within the follicle, to form colloid, and is later disintegrated, passing out of the follicle with the secretory flow. The chemical analyses of the thyroid gland, showing the common relation of iodine to the colloid and to the active principle of thyroid secretion would harmonize with such an hypothesis.

Vacuolation of the colloid mass (fig. 14) is of frequent occurrence; it may result either from the inclusion within the intrafollicular colloid of portions of the peripheral cup-like impressions which Langendorf has surmised result from the secretion pouring out from the surface of the epithelial cells, or it may be further evidence of disintegration of the colloid with formation within its substance of fluid droplets, rather than of solid spherules. The vacuoles are filled with a clear fluid and occasionally contain basophile granules. A colloid mass may contain many small vacuoles mostly at or near the periphery of the mass and containing few, if any, basophile granules, or it may contain one or more vacuoles of relatively large size which occupy the interior of the mass and may be more or less completely filled with the granules. These granules stain deeply with hematoxylin and similar dyes, and they are either amorphous or somewhat crystalline in form. The origin of the chromatic material within the vacuoles may be from chromatolysis of the nuclei of either the disintegrating follicular epithelium or of blood cells included within the colloid. Evidences of disintegration of epithelium and extrusion of the nuclei as well as of the penetration of the nuclei together with blood cells (fig. 20) into the colloid mass are frequently seen. But the disintegration of such cells and nuclei can scarcely account for the much more numerous vacuoles in which no basophile chromatic substance is found.

The follicles of the thyroid are supported within the meshes of a connective tissue stroma in which the blood-vessels lie. The volume of connective tissue is never great, much less than in the mammalian gland. There is more connective tissue in the gland

of *Raia* than in the other species examined; in *Mustelus* and *Squalus* there is so little that one wonders at the relative compactness of the organ. In these *Selachii* the epithelium rests directly upon the walls of the blood-vessels, and they in turn consist of little else than endothelium and form broad sinuses rather than capillaries or venules.

The gland is inclosed by a very thin connective tissue capsule and its tissue is thus always sharply defined from the surrounding structures. In *Mustelus* and *Squalus*, and to some extent in the other species, the broad vascular channels of the thyroid sinus are in direct contact with the capsule, so that in sections the ventral surface of the gland often appears clothed with an endothelial coat derived from these vessels (figs. 16 and 17); a similar disposition of the vascular endothelium of collapsed blood-vessels is also occasionally seen on the margins and dorsal surface of the gland.

The blood vessels have been in each case carefully studied by dissection, injection, sections, and transparent total mounts of the gland. Both blood vessels and lymphatics were demonstrated beyond doubt, though lymphatics have not hitherto been observed in these fishes and their existence was denied by Baber ('81). Fig. 9 shows the lymphatics filled with injection mass, lying between the blood channels and the follicular epithelium; they appear as perivascular lymphatics in the wall of the *venae lymphaticae*. Similar vessels, perivascular lymphatics, are found in the walls of the arteries and veins of the thyroid, the thyroid sinus, and the arteries of the hypo-branchial system (fig. 8).

The course of the larger blood-vessels was readily followed in injected specimens in which the whole gland was examined under the microscope. The arteries course upon the surface of the gland, the major portion of them being always on the ventral surface. Fig. 19 shows the distribution of the arteries in the thyroid of *Mustelus*, and figs. 3 and 4 indicate the relative area of the gland supplied by the arteries of the right and left side, the left thyroid artery, as in figs. 3 and 19 usually supplying the greater part of the organ, though occasionally the major part, as in fig. 4, is supplied from the right side. Twigs from the superficial branches here and there penetrate the gland, break into arterioles, and

Fig. 17. Section through the ventral margin of the thyroid gland of *Squalus* showing peripheral venae and vasa lymphatica.

Fig. 18. Section of the thyroid gland of *Carcharias* showing the disintegrating nuclei of leucocytes or red blood cells within the intrafollicular colloid.

promptly empty into groups of broad interfollicular, blood capillaries, the *venae lymphaticae* (figs. 9, 11, 12), which envelop the follicles on all sides. From the *venae lymphaticae* the veins pass out of the gland at its posterior and lateral borders and dorsal surface to enter the thyroid sinus; a few veins from the lateral border of the gland pass directly to the hyoid sinus.

The course of the lymphatics was much less easily determined than that of the blood vessels. "Stick injections," as ordinarily made, spread so rapidly through the loose connective tissue of the gland and so easily entered and filled the *venae lymphaticae* that they entirely obscured the smaller *vasa lymphatica*. The *venae lymphaticae* thus injected form a dense almost opaque mass, showing that the thyroid may well occupy the place in these fishes assigned to it by Tscheuwsky ('03) as the most vascular of mammalian glands. After several futile attempts to inject the lymphatics in the ordinary way the method was so altered as to inject only minute areas under a very low pressure. In this way it was found that at the margins of the injected area the fluid which had entered the vessels traveled farther than that in the connective tissue spaces, and in many cases the *vasa lymphatica* were filled beyond the limits of the injected *venae lymphaticae*, so that in the outermost zone of the injected area the true lymphatics could be readily studied, the *venae lymphaticae* in this zone being either empty or only partially filled.

The *vasa lymphatica* are, for the most part, perivascular channels (fig. 9), but they are also in direct contact with the epithelial walls of the follicles (fig. 10). The *vasa lymphatica* could not be followed for any great distance through the injected zone, for, on the one hand, they entered the area of opaque injection mass, and in the other direction they ended abruptly, often with a small knob-like dilatation. By means of serial sections I was able to determine in uninjected specimens that the *vasa lymphatica* opened at the points of terminal dilatation directly into the *venae lymphaticae* (fig. 10, X). Having demonstrated the connection between the two sets of vessels in uninjected specimens, showing the true relation of *vasa* and *venae lymphaticae*, many points in the injected specimens could be readily found at which it seemed quite certain that the injection mass was

Fig. 19. Drawn from a total mount of the thyroid gland of *Mustelus canis*, showing the course and distribution of the thyroid arteries and the origin of some of the veins of exit. The vessels on or near the ventral surface are indicated by the solid black lines, those on or near the dorsal surface of the gland by dotted lines.

Fig. 20. A section from the thyroid gland of *Carcharias*, showing invasion of epithelium and colloid by leucocytes.

passing from the vasa lymphatica directly into the venae. The relatively intimate relation between the veins and lymphatics in fishes is well known; Wiedersheim ('07) and Favaro ('06) have recently emphasized the fact so far as the tail vessels of fishes were concerned. This intimate relationship seems to be quite as obvious in the thyroid vessels and in those of the region occupied by the thyroid sinus (*vide supra*).

That the vasa lymphatica are true lymphatics and not blood-vessels is shown by the fact that in many cases they are of altogether too small caliber to transmit the large red blood-cells of the Elasmobranch fishes. Moreover, the quasi valvular nature of their anastomosis with the veins, as already described, renders highly improbable the regurgitation of blood-cells into the vasa lymphatica even in the larger vessels.

In mammalian thyroid glands, especially in dogs, one now and then observes instances where the colloid has accumulated beyond the bounds of the follicles, giving to sections of the organ the appearance of a tissue completely infiltrated by the waxy colloid substance. No such appearance was found in the Elasmobranch thyroids which were studied, unless the follicles lined chiefly by colloid cells could be so interpreted.

SUMMARY

1. The Elasmobranch thyroid gland closely simulates the human, both in the form and structure of its follicles and the distribution of its blood-vessels.

2. The gland rests upon the basi-hyal cartilage whose anterior margin forms an excellent guide to its location.

3. The pyramidal lobe of mammals is often represented in Elasmobranchs by a process passing forward and reaching the floor of the pharynx through a notch in the anterior margin of the basi-hyal cartilage; this notch is sometimes converted into a foramen.

4. Baber's opinion that lymphatics are not present in the thyroid of Elasmobranch fishes was founded on insufficient evidence and is incorrect.

5. Lymphatics are present in considerable numbers both in and

about the thyroid gland and can be demonstrated by injection and in sections; they are true "vasa lymphatica."

6. The blood-vessels within the thyroid gland terminate in a network of "venae lymphaticae" which invest the follicles, receive the vasa lymphatica, and transmit either or both blood and lymph, under varying conditions of blood-pressure.

7. The thyroid artery arises from the ventral end of the efferent hypobranchial arterial loop contained in the hyoidean hemibranch and the adjacent half of the first holobranch by an independent origin or by a common stem with the mandibular or submental artery.

8. The thyroid veins in these fishes for the most part enter the "thyroid sinus," a mass of veins and lymphatic vessels which pour their blood into the hyoid sinuses.

9. The rhythmic respiratory movements of the pharyngeal wall cause the thyroid sinus to act somewhat after the manner of a "vein-heart" or "lymph-heart."

10. The hypobranchial arterial system is formed as described by Hyrtl in 1858 and 1872, and the direction of the flow of its blood is from the gill vessels toward the coronary and other terminal arteries, as indicated by Hyrtl and again by Parker and Davis in 1899, and not from the subelavian artery toward the coronaries, as described by T. J. Parker in 1886. The hypobranchial artery of T. J. Parker, forming an anastomosis between the subelavian and median hypobranchial arteries, is of insignificant importance and is frequently wanting.

11. The relative volume and distribution of the "colloid" in the glands of different species indicates that this substance is a retention product, formed from the albuminous secretion of the follicular epithelium.

12. The further changes occurring in the "colloid" indicate the possibility of its usefulness as a sort of stored-up secretion.

13. The follicular epithelium contains both "chief" and "colloid" cells, the latter being even more numerous and characteristic than in the mammalian thyroid.

14. The parenchymal epithelium is in intimate relation with the vasa and venae lymphaticae; it rests directly upon or in close proximity to the endothelial wall of these vessels.

EXPLANATION OF FIGURES

Abbreviations

<i>I—V</i> , first to fifth branchial clefts	<i>L.</i> , vasa lymphatica
<i>A. lat.</i> , lateral artery	<i>l. c.</i> , lateral commissural arteries
<i>A. thyr.</i> , thyroid artery	<i>Lat. hypobr.</i> , lateral hypobranchial artery
<i>A. thyr. imp.</i> , arteria thyreoidea impar	<i>M. c. br.</i> , coraco-branchial muscle
<i>Art.</i> , artery	<i>M. c. hy.</i> , coraco-hyoideus muscle
<i>b. hy.</i> , basi-hyal cartilage	<i>M. c. mdb.</i> , coraco-mandibularis muscle
<i>cap.</i> , fibrous capsule of the thyroid gland	<i>Med. hypobr.</i> , median hypobranchial artery
<i>ch. ep.</i> , chief cells of the follicular epithelium	<i>p. c.</i> , pericardial arteries
<i>col.</i> , colloid	<i>pharyn.</i> , pharyngeal arteries
<i>com.</i> , commissural artery	<i>R B C</i> , red blood cells.
<i>cor.</i> , coronary arteries	<i>s. a. v.</i> , ventral arterial sinus of the first hypobranchial loop.
<i>coraco-mdb.</i> , coraco-mandibular artery	<i>sbcl.</i> , subclavian artery
<i>coracoid</i> , coracoid artery	<i>thyr.</i> , thyroid gland
<i>D A.</i> , dorsal aorta	<i>thyr. sn.</i> , thyroid sinus
<i>en.</i> , vascular endothelium	<i>V.</i> vein
<i>ep.</i> , epithelium of the thyroid follicles	<i>V A.</i> , ventral aorta
<i>epibr.</i> , epibranchial artery	<i>V L.</i> , venae lymphaticae
<i>fol. thyr.</i> , follicles of the thyroid gland	<i>W B C</i> , white blood cells
<i>gastric</i> , gastric arteries	<i>x.</i> , point of anastomosis of vasa and venae lymphaticae
<i>hy. sn.</i> , hyoid sinus	<i>y.</i> , injected carmine granules in the venae lymphaticae
<i>hypobr.</i> , hypobranchial artery	
<i>hypobr.'</i> , its anastomosis with the commissural artery.	

BIBLIOGRAPHY

- BABER, E. C. 1881 *Phil. Trans., Roy. Soc. London*, 172, 577.
- BALFOUR, F. M. 1881 *Treatise on Comp. Anat.*, London 2, 626.
- BRIDGE, T. W. 1904 *Cambridge Nat. Hist.*, London, 7.
- COLE, F. J. 1905 *Anat. Anz.*, 27, 323.
- DE MEURON, P. 1886 *Rec. zool. suisse*, 3, 517.
- DOHRN, A. 1884 *Mitth. a. d. zool. Station z. Neapel*, 5, 102.
- ECKER U. WIEDERSHEIM. 1904 *Anat. des Frosches*, Braunschweig, 205.
- FAVARO, 1906 Quoted by Eisler, *Schwalbe's Jahresb.*, 12, 323.
- GUIARD, J. 1896 *Thèse Paris*.
- HYRTL, J. 1858 *Denks. d. k. Akad. Wissen., Wien, Math. Nat. Cl.*, 15, 1.
1872 *Denks. d. k. Akad. Wissen., Wien, Math. Nat. Cl.*, 32, 263.
- LAGENDORF, O. 1889 *Arch. f. Physiol., Suppl. Bd.*, 219.
- LOMBARD, G. D. 1909 *Biol. Bull.*, 18, 39.
- MAJOR, R. H. 1909 *Am. J. Anat.*, 9, 475.
- MAURER, F. 1886 *Morph. Jahrb.*, 11, 129.
1888 *Morph. Jahrb.*, 13, 296.
- MÜLLER, W. 1871 *Jena. Zeitschr.*, 6, 428.
- PARKER, G. H. AND DAVIS, F. K. 1899 *Proc. Bost. Soc. Nat. Hist.*, 29, 163.
- PARKER, T. J. 1880 *Trans. and Proc. New Zealand Institute*, 13, 413.
1886 *Phil. Trans., Roy. Soc. London*, 177, 685.
- PEREMISCHKO 1867 *Zeitschr. f. wis. Zool.*, 17, 279.
- SABIN, F. R. 1909 *Am. J. Anat.*, 9, 43.
- SCHAFFER 1906 *Anat. Anz.*, 28, 65.
- SIMON 1844 *Phil. Trans., Roy. Soc. London*, pt. 1, 295.
- STOCKARD, C. R. 1906 *Anat. Anz.*, 29, 91.
- TSCHEUWSKY 1903 *Arch. f. d. ges. Physiol.*, 97, 210.
- TURNER 1874 *J. Anat. and Physiol.*, 8, 285.
- VAN BEMMELEN, J. F. 1887 *Zool. Anz.*, 10, 88.
- WAGNER, R. 1853 *Handwörterbuch d. Physiol.*, Braunschweig, 4, 111.
- WIEDERSHEIM, R. 1907 *Comp. Anat. of the Vertebrates*, p. 432.