CONTRIBUTIONS FROM THE BIOLOGICAL LABORATORY OF THE BUREAU OF FISHERIES AT WOODS HOLE, MASS.

THE PHYSIOLOGY OF THE DIGESTIVE TRACT OF ELASMOBRANCHS.

By MICHAEL X. SULLIVAN, Ph. D.

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INTRODUCTION.

The digestive tract in fishes has been studied quite extensively both from the histological and from the physiological standpoint. Most of this work has been done on European species, however, and has given rise to contradictory conclusions, especially from the viewpoint of physiology. Hence it seemed advisable to devote some attention to fishes found also in American waters, and I have accordingly undertaken the study of the digestive tract of elasmobranchs.[a]

This group of fishes was chosen for investigation, first, because of the relatively simple structure of their digestive tract, and second, because they may, from their position in the scale of evolution, form the groundwork for an extensive comparative study of fish in general. Thus we may arrive at a unified view of the changes, structural and physiological, which have taken place in the alimentary canal of fishes from the lowest to the highest. The treatment of the subject is partly histological, but mainly physiological.

HISTORICAL DISCUSSION.

THE DIGESTIVE TRACT IN FISHES.

MORPHOLOGY.

As in higher animals, the digestive tract in fishes may be divided into the following portions: Mouth, esophagus, stomach, small and large intestine. In fishes, however, two or more of these divisions may coalesce and become indistinguishable. As a rule there is a complicated dentition, but no salivary glands. The buccal cavity opens directly into the esophagus, and this in turn into a large stomach,

[a] The work embodied in this paper was done at the laboratory of the U. S. Bureau of Fisheries, Woods Hole, Mass. during the summers of 1905 and 1906, when the writer was a salaried assistant of the Bureau. I am indebted to Dr. W. L. Chapman for making the photomicrographs of the rectal gland (plate I).

which is usually furnished with a valve at its posterior end. The digestive tract may be straight, U-shaped, or Y-shaped—straight in *Branchiostoma* and the cyclostomes; syphonal or U-shaped in the elasmobranchs and in some teleosts, where the stomach presents the form of a bent tube, of which one half is the cardiac and the other the pyloric portion; cæcal or Y-shaped in most teleosts, where the cardiac division is a long, descending blind sac, with the cardiac and pyloric openings of the stomach lying close together. In most cases the pyloric tube is long and slender.

In many fishes, especially among ganoids and teleosts, a variable number of blind tubes open into the intestine immediately posterior to the pylorus. These tubes are termed the pyloric cæca and are often filled with the same material as is the intestine. When present in large numbers, the appendages often coalesce into a common duct. In the cyclostomes and dipnoans no pyloric appendages exist, and in the elasmobranchs pyloric cæca have been found by Turner (1873) only in the Greenland shark, *Læmargus borealis*, and by Gegenbauer (1892) in certain skates.

The duodenum receives the hepatic and the pancreatic secretions, and also the secretion of the pyloric appendages.

The intestine varies much in length, and in many fishes the absorbing surface is increased by folds of the mucous membrane, which wind spirally or are arranged in parallel lamellæ. These spiral valves are found in cyclostomes, selachians, ganoids, and dipnoans. In short, the digestive tract in fishes varies greatly, from a simple condition to a complex one, with valves, folds, and appendages.

The pancreas is the most constant of all digestive glands in vertebrates. In the lower fishes it occurs as a compact mass, while in the teleosts it is, as a rule, diffused and distributed about the pyloric cæca, hepatic duct, and in the liver. The presence of the pancreas in bony fishes in a widely diffused state was demonstrated by Legoius (1873). In the elasmobranchs the pancreas is comparatively large, and pinkish in color. It empties by a single duct into the duodenum.

Cæcal appendages at the end of the intestinal canal are of exceedingly rare occurrence in fishes. In the elasmobranchs, however, an appendage, the so-called rectal gland, exists near the end of the intestine. This gland varies from half an inch in length in the skate to three or four inches in the big sharks. According to Wiedersheim (1905, p. 422), cloacal appendages exist in the dipnoans and traces of a blind intestine may be found in certain teleosts, while in the Holocephali the place of the rectal gland is taken by glandular tissue within the walls of the rectum.

For a more detailed account of the gross anatomy of fishes the reader is referred to Home (1814), who described the intestines of thirty species, and to Rathke (1824), who described fifty-six species. General works on the intestinal canal of fishes are Siebold and Stannius (1854), Milne-Edwards (1860), Günther (1880), Oppel (1896, 1897, 1900, 1904), and Wiedersheim (1905).

HISTOLOGY.

Though Nehemiah Grew (Gamgee, 1893) made mention, in 1676, of a glandular secretion in the stomach of the horse, it was not until 1836 that the gastric glands were actually discovered. In this year Boyd (1836) discovered gastric glands in mammals and noticed their presence in some fishes. Following Boyd, Bischoff (1838), one of the earliest workers on the histology of the alimentary canal in fishes, studied the mucous lining of the stomach of a great many species. In Cyprinidæ he was unable to find gastric glands. In other species, however, he found them abundant. As we shall see later, the lack of gastric glands is not peculiar to the Cyprinidæ, since many other fishes have no functional stomachs.

Rathke (1841) found that the alimentary canal of *Amphioxus* (*Branchiostoma*) is composed of ciliated epithelium without glands, and Johannes Müller (1843), in his work on the myxinoids, arrived at the same results. By these investigators the simplicity and uniformity of the mucous lining of the alimentary canal in the lower fishes was fully established.

Vogt (1845) proved the existence of two kinds of cells in the stomach of the common trout, *Salmo fario*, i. e., cells of cylindrical epithelium, covering the surface, and round cells in the crypts. Vogt, however, did not recognize these crypts as gastric glands. Leydig (1852), on the other hand, clearly recognized the gastric glands in *Squatina angelus* and in *Torpedo galvani*, since he writes of the small round cells, containing highly granular protoplasm, as granular cells. In 1853 Leydig found such glands in the sturgeon, *Acipenser nasus*, but could not find them in the stomach of the loach, *Cobitis fossilis*. In a still later paper Leydig (1857) referred to these glands as "labdrüsen," thus signifying that they were like the gastric glands of higher vertebrates.

Since 1857 much work has been done on the histology of the alimentary canal of fishes, and especially on the histology of the stomach. Perhaps the best reviews of previous work are presented by Edinger (1877), Richet (1878), and Yung (1899). To the works of these authors I am greatly indebted.

One of the earliest workers on the glands of the stomach of fishes was Valatour (1861), who noticed the presence of gastric glands in various species and confirmed Bischoff's work in that he could find no functional stomach in Cyprinidæ. From 1861 to 1870 much advance was made in histological technic, and in 1870 Heidenhain discovered two kinds of granular cells in the mammalian stomach, and Rollet the same year confirmed his discovery. These cells are now known as chief and parietal cells. In fishes, on the other hand, Edinger (1877) could not find the two kinds of gastric cells distinguished by Heidenhain and Rollet in the mammalian stomachs. Of Edinger's work we may speak in detail.

Edinger (1877) made a detailed histological study of the entire digestive tract of fishes. According to his investigations, the stomachic crypts are only partly lined with gastric glands, for in the pylorus the crypts are functional merely as mucous glands. The pyloric appendages are simply evaginations of the intestinal wall and present the same structure as the part from which they arise. Properly speaking, no glands exist in the middle intestine, and the mucous cells are the only secretory part. The other epithelial cells are merely absorptive in function. Finally Edinger paid much attention to the question as to whether or not chief and parietal cells exist in fishes. He concluded that there is only one kind of cell in the gastric glands—a cell which is homologous to neither of these cells.

Edinger's conclusion has been generally accepted, although several authors have noted differences in the cells of the gastric crypts. Thus Cajetan (1883) called attention in the case of *Cobitis barbatula* to the fact that the cells of the stomach differ with respect to the dimensions of their granules and their staining reaction to osmic acid. Pilliet (1894) also mentioned some differences between the gastric cells of *Pleuronectes* according as they are situated at the superficial or the deeper portions of the gland.

Pilliet studied principally selachians and *Pleuronectes*. According to him, the glands of the stomach of the selachians are long. In *Pleuronectes* he noted a difference in the extent of the distribution of the glands, in that they are fewer in young or undeveloped fish. He likewise claimed that the cardiac portion of the stomach of *Pleuronectes* is essentially peptic, while the pyloric portion is essentially mucous.

According to Cattaneo (1866), who studied numerous fishes, the fishes highest in the scale of evolution repeat in their development the structure of the digestive tract as found successively in adult acraniates, cyclostomes, selachians, and ganoids. The least differentiated part of the intestine of the higher forms has a structure like the most differentiated part of the lower forms. Like Edinger, Cattaneo found in all species of fishes that the stomach and middle intestine are the most differentiated parts, while the esophagus and terminal intestine preserve a primitive character. With Edinger, he concluded that only one kind of cell is present in the gastric glands of fishes.

In addition to the writers already mentioned, Macallum (1886) described the intestines of some ganoids, Decker (1887) studied fresh-water fish, while W. N. Parker (1889), Hopkins (1890, 1895), Mazza (1891), Mazza and Perugia (1894), Claypole (1894), and Haus (1897) have added to our detailed knowledge of the digestive tract in fishes. More recently Yung (1899) made a detailed study of the digestive tract of *Scyllium canicula*, while Oppel (1896, 1897, 1900, 1904), in his "Lehrbuch der vergleichenden microskopischen Anatomie der Wirbelthiere," has made a comprehensive review of the previous work on the microscopical anatomy of the digestive tract.

PHYSIOLOGY.

The first experiments on the digestion of fishes were made by Spallanzani (1783), who worked on eels, pikes, carps, and barbels. Previously, Réaumur (1752) and Stevens (1777) had worked respectively on birds and man. Réaumur, indeed, made the first decisive step in the physiology of digestion. He introduced into the stomach of a kite small metallic tubes with the ends covered by a grating of threads or fine wire. He found that the gastric juice is acid and that it would digest meats and bones, but not vegetable grains or flour. Stevens proved the same thing for man, and in addition proved that the gastric juice would digest in vitro. Spallanzani in like manner passed into the stomach of his fish tubes filled with flesh, and, having left them in the stomach forty-two hours, found them covered with mucus, but with little or no flesh within them. From this work Spallanzani concluded that digestion is carried on best in the fundus of the stomach. He believed, however, that the stomach is not the only part capable of digesting food, but that the esophagus, in a more feeble way, also has digestive power. He likewise believed that digestion is accomplished without trituration, for the thin tubes which he used did not show any trace of deformation.

Spallanzani also showed that digestion goes on in vitro as in the stomach; consequently, hypotheses regarding vital force, coction, and fermentation have no reason to exist. Digestion is, on the contrary, a chemical phenomenon, not a process of putrefaction. Furthermore, this investigator saw that the acid is secreted by the stomach and that it might be seen exuding from the walls. He did not make mention, however, of digestion in the intestines or of the action of the bile. In his eyes, indeed, the stomach in all animals was the principal digestive organ.

Tiedemann and Gmelin (1827) made observations upon the contents of the intestinal tract of the trout, barbel (*Cyprinus barbus*), etc., and proved that in a fasting fish the mucus does not redden litmus, but that a stomach full of food contains free acid and coagulates milk. Tiedemann and Gmelin believed that the acidity is due to a mixture of acetic and hydrochloric acids. These workers also paid some attention to the liquid of the pyloric appendages. This liquid, they found, reddens litmus but slightly, and they believed that it mixes with the food dissolved by the stomach and accelerates assimilation.

In 1873 Fick and Murisier called attention to the fact that the ferment in the stomach of the trout and the pike differs from that of higher animals in that it digests food at a low temperature as well as at 40° C., while the higher organisms digest better at the higher temperature.

In the same year Rabuteau and Papillon (1873) recognized that the gastric juice of the skate is acid, and the former writer secured, by distillation, a colorless liquid which he considered hydrochloric acid.

A little later Homburger (1877) concluded from his researches upon *Cyprinus tinca*, *Chrondrostoma nasus*, *Scardmius erythrophthalmus*, and *Abramis brama* that the bile and extracts of the liver of these animals, as well as extracts of the intestinal mucous membrane, digest fibrin, emulsify fats, and convert starch to sugar.

In 1877 Krukenberg carried on investigations upon the intestines, and then upon the glands connected therewith, of widely different species belonging to all classes of fishes except dipnoans. From this work he concluded as follows:

No fish possesses salivary glands, although some have a diastase in the mucous membrane of the mouth, as, for example, *Cyprinus carpio* and *Lophius piscatorius*.

The action of the stomach is variable. With some selachians, ganoids, and teleosts this organ secretes pepsin similar to that of mammals in that it acts only in an acid medium, but different in that it can act at a lower temperature. In some cases, as in certain teleosts (*Zeus faber*, *Scomber scomber*), the stomach produces pepsin only in its anterior part, while the fundus secretes a mixture of pepsin and trypsin or a juice capable of digesting fibrin in an acid or in an alkaline medium. With other teleosts (*Gobius, Cyprinus*) the stomach, or the organ, considered as such does not furnish any enzyme at all. Digestion in these instances is carried on exclusively in the middle intestines.

In the selachians and the ganoids pepsin is produced not only in the stomach, but also in the anterior end of the middle intestines, in the selachians to the place where the pancreatic duct empties, and in the ganoids to the pyloric appendages.

In the selachians the massive pancreas secretes trypsin, while in the ganoids and teleosts, which have a diffused pancreas mixed with hepatic tissue, a ferment similar to pepsin can be extracted from the liver. This ferment is absent from the liver of the selachians.

In the case of the Cyprinidæ trypsin is found both in the liver and in the mucous membrane of the middle intestine. The middle intestine, indeed, should be regarded as the principal seat of digestion in these fishes.

As regards the function of the pyloric appendages in most fishes, they inclose only mucus and chyle and are absorbing organs, while in other cases they secrete either a trypsin-like ferment, as in the *Thymnus vulgaris*, a mixture of pepsin and trypsin, or sometimes a mixture of pepsin, trypsin, and diastase.

Finally, in many species the liver, or hepato-pancreas, and the middle intestine secrete a diastatic ferment, as does even the buccal mucous membrane.

The general tendency of Krukenberg's studies, therefore, is to establish the existence of an evolution of the digestive function from the invertebrates (molluscs, crustacea, etc.) to the higher vertebrates. By the great variation in the distribution of the ferments, fish, according to him, show the principal stages of this evolution.

Luchhau (1878), by means of glycerin extracts of the mucous membrane of the stomach of the salmon, pike, and sandre, secured juices that would peptonize fibrin. Contrary to Fick and Murisier (1873), he observed that the peptonizing action is more rapid at 40° C. than at 15° C. Luchhau also examined the digestive activity of the juice of certain Cyprinidæ (*Cyprinus carpio*, *C. blicca*, *C. carassius*, *C. tinca*, *C. erythrophthalmus*, and *Abramis brama*), which do not have a functional stomach. In no case did he find an enzyme digesting in an acid medium—that is, pepsin; but he did find that fibrin is digested by the neutral or alkaline extract of the intestinal mucous membrane and that the digestive power is greater at 40° C. than at lower temperatures. Luchhau compared the ferment of the intestines of Cyprinidæ to the trypsin of mammals, and in addition to the trypsin-like ferment he found the diastatic ferment also. The trypsin-like ferment, he asserted, is secreted in the middle region, while the diastatic ferment is secreted along the whole length of the intestine. He did not find a fat-splitting ferment, nor, unlike Krukenberg, did he find any ferment which would digest albumen.

In researches upon the composition of the gastric juice, Richet (1878) analyzed the gastric juice of different fishes. He proved conclusively the presence of hydrochloric acid, free or combined with organic substances, such as tyrosin and leucin. He found the acidity to be high, in the case of *Scyllium canicula* even as high as 1.5 per cent hydrochloric acid. The digestive power of *Scyllium canicula* he found to be greater than that of *Lophius piscatorius*. In a later paper Mourrut and Richet (1880) found that the liquid in the stomach lost its digestive power by filtration. An acidity of 2.5 per cent was found by them to prevent peptonization, while moderate heat favored the action of the ferment. Mourrut and Richet did not observe that either *Lophius* or *Scyllium* produced a diastatic ferment in the stomach.

In a still later study of digestion in fish, Richet (1882) confirmed the facts previously given by him and declared that the gastric juice of sharks digests the chitin of crustacea. Further, the pancreas of *Scyllium* and of *Galeus* has no action on proteids but is limited to the transformation of starch to sugar and to the emulsification of oil.

Raphael Blanchard (1882) investigated the rectal gland of elasmobranchs and the pyloric appendages of teleosts. He found that the former organ produces both a diastatic and a fat-splitting ferment. The pyloric appendages, according to this investigator (1883), represent, in a certain sense, the pancreas, since they secrete a diastatic enzyme and a trypsin-like enzyme.

The presence of the trypsin-like ferment in the pyloric appendages has been proved also by W. Stirling (1884, 1885), who worked on the herring, cod, and hake, and made glycerin extracts of the stomach and pyloric appendages. In the stomach he found a ferment acting in acid and in the pyloric appendages one acting in an alkaline medium, from which observations he concluded that the stomach secretes pepsin, the pyloric appendages trypsin.

Decker (1887) found the stomach of fish to be sometimes neutral, sometimes alkaline, and he likewise found that the esophagus of the hake digested fibrin much more rapidly than the stomach did. He found in these species, moreover, that the esophagus, the intestine along its whole length, the cloaca, and the pyloric appendages all produced a ferment comparable to pepsin.

According to the researches of Knauthe (1898) all the intestinal mucous membrane of the carp, and especially the anterior portion of the intestine, produces a strong tryptic ferment, as does also the liver, or hepato-pancreas. The intestinal mucous membrane, except that of the mouth and the hepato-pancreas, produces amylolytic and fat-splitting ferments. The bile, he concluded, has of itself no digestive action on proteids or fats mixed with extracts of the intestinal mucous membrane or of the hepato-pancreas; it augments their action. The bile has, however, a diastatic action which is at the maximum at 23° C.

Bondouy (1899) investigated the function of the pyloric tubes in teleosts and came to the conclusion that they played an active part in digestion. They secrete trypsin and amylopsin, but no lipase. On the other hand, Bondouy believed the pyloric tubes have but little function as absorptive organs.

Yung (1899) in a very comprehensive and detailed work on elasmobranchs, including *Scyllium canicula*, *Acanthias vulgaris*, *Lamna cornubica*, *Galeus canis*, and *Carcharias glaucus* found that—

(1) The buccal and esophageal membranes have no digestive action.
(2) The stomach digests proteids.
(3) The acidity of the stomach may be as high as 1 per cent.
(4) The stomach may or may not convert the food into anti-peptone.
(5) The gastric juice of *Scyllium canicula* acts better at 38° C. than at 20° C.
(6) The formation of pepsin is limited to the stomachic sac.

Very little study has been given to the physiology of the pancreas of fishes. Bernard (1856) proved that the pancreas of the skate converts starch to sugar and acidifies fats. Krukenberg (1877), in his work on selachians, found that the pancreas of these fishes was secreting trypsin, the proteolytic ferment, but no amylopsin, the starch-splitting ferment, nor lipase, the fat-splitting ferment. Richet (1878), however, was unable to find trypsin in the pancreas of selachians, but did find the starch-splitting and fat-splitting ferments. Yung (1899), working on *Squalus acanthias*, found amylopsin and lipase, but only occasionally trypsin. Yung attempted to get the juice by a fistula, but had little success. His water glycerin extracts were only occasionally active. He found that extracts of the spleen aided the activation of the pancreas. More recently, Sellier (1902) found that the pancreas of several selachians studied by him does not of itself digest proteid, but must be activated by the juice of the spiral valve.

From this synopsis of the literature, it may be seen that there is by no means unanimity of opinion regarding the physiology of the digestive tract of fishes.

OBSERVATIONS AND EXPERIMENTS.

HISTOLOGY OF THE ALIMENTARY CANAL OF THE SMOOTH DOGFISH AND THE SAND SHARK.

The intestine of both *Mustelus canis* and *Carcharias littoralis* is bent twice upon itself; the first of these bends is between the stomachic sac and the pyloric tube, the second between the pyloric tube and the middle intestine. From an anatomical standpoint these two bends divide the alimentary tube into three portions, of which the first two constitute the anterior intestine, the third the middle and terminal intestine. As a rule the middle intestine, or what we might call the duodenum, is short. In *Mustelus canis*, indeed, there is almost no duodenum or valve-free portion between the pyloric tube and the spiral valve. On the other hand, in *Carcharias littoralis*, *Carcharhinus obscurus*, *Dasyatis centrura*, *Lamna cornubica*, and *Tetronarce occidentalis*, the middle intestine, or duodenum, is well marked off from the spiral valve and pyloric tube. From a histological standpoint the entire intestine may be divided into buccal, esophageal, stomachic, pyloric, duodenal, valvular, rectal, and cloacal mucous membrane.

Upon the digestive tract of European selachians, as Yung shows in his paper, "Recherches sur la digestion des Poissons" (1899), considerable histological work has been done. Yung himself made a thorough study of the alimentary canal of *Scyllium canicula*. The histology of *Mustelus canis* and *Carcharias littoralis* is practically the same and agrees in most respects with that of the European form *Scyllium canicula*. Histological study of the digestive tract of the American species shows the following facts:[a]

BUCCAL MUCOUS MEMBRANE.

The mucous membrane of the buccal cavity is smooth, often covered with fine papillæ and moistened with mucus. Sections of the mucous membrane of the buccal cavity showed epithelium and connective tissue, but no glands. The epithelium is of the stratified pavement type. The epithelial cells next to the connective tissue are cylindrical, finely granular, and possess oval nuclei. Above this layer of cylindrical cells are several layers of large mucous cells, which are oval and contain a substance that stains with the ordinary mucus stains. The nucleus is very small, elongated, and pressed against the cell wall. Finally, the superficial epithelium consists of one or two layers of flat or oval cells which form a fine membrane.

MUCOUS LINING OF THE ESOPHAGUS.

Numerous papillæ and longitudinal folds occur in the inner lining of the esophagus. The folds are fine at their beginning, but thicken toward the cardiac end of the stomach. They vary in number and frequently anastomose. Transverse folds form a boundary more or less marked between the esophagus and the stomach. The mucous membrane of the esophagus is whitish, in strong contrast to that of the

[a] As fixing agent I used corrosive acetic, and as stain hematoxylin and eosin.

stomach, which is always reddish in color, especially noticeable when the stomach is full of food. In the beginning of the esophagus the epithelium is similar to that of the buccal cavity, but it is gradually replaced by an epithelium consisting of ciliated cylindrical cells and goblet cells.

MUCOUS MEMBRANE OF THE STOMACHIC SAC.

The mucous membrane of the stomach has a reticulated appearance, due to numerous folds. Some of these folds are continuations of the longitudinal folds of the esophagus, while others are transverse and oblique. In the pyloric tube the folds are extremely fine. Histologically, the mucous membrane of the stomachic sac differs from that of the esophagus by the absence of cilia and of goblet cells, and by the presence of true peptic glands.

The epithelium of the stomachic sac is of two kinds, superficial and glandular. The first is composed of a single layer of prismatic or pyramidal cells with oval nuclei. In these, two portions may be distinguished—one, finely granular, which incloses the nucleus and occupies four-fifths of the length of the cells; and another, the superficial part or the part nearest the cavity of the stomach, composed of a highly refractive, nonstaining, transparent substance. These two portions, as Yung (1899) has pointed out in his work on *Scyllium canicula*, corresponded to what Oppel (1897) called the protoplasmic portion and the upper portion. The refractive superficial portion of these cells has been called "Pfroph" or "plug" by Biedermann (1875), and is considered by Oppel as a substance comparable to mucus. The superficial epithelium, which is rather uniform in character, covers all the folds of the mucous membrane and the superficial portions of the glandular tubes.

The glandular tubes begin in the cardiac end of the stomach and extend to the pylorus, being most plentiful in the middle of the stomachic sac. Each gland is a cylindrical tube, with the canal narrow in the upper part but wider toward the bottom. The tubes in the middle of the stomach are longer than those of the cardiac end or the pyloric end of the sac. In every case they are separated from each other by a fine layer of connective tissue. The epithelium of the neck of the peptic crypts consists of cylindrical cells, like those of the superficial epithelium, which become little by little shorter and thicker. They are distinguishable from the superficial layer, however, by the absence of the mucous plug, and by the presence of a large round nucleus. They differ from the neighboring peptic cells by their clearer contour, smaller size, and the smaller amount of granulation. The body of the gland is occupied by cells which are irregularly polygonal in shape, highly granular, and closely packed together. These cells are all of one kind, and can not be differentiated into chief and parietal cells, such as Heidenhain and Rollet have found in the mammalian stomach.

PYLORIC TUBE.

In the long narrow pyloric tube we find crypts and the same superficial epithelium as in the stomachic sac. The crypts, however, are short and the polygonal peptic cells are absent.

INTESTINE.

The intestine of elasmobranchs may be divided into two portions—a small intestine or duodenum, and a large intestine. The former is short, varying from one-half an inch to 2 inches in length. The latter is longer and very wide; it is divided into two portions—the colon, containing the spiral valve, and the rectum, which is short.

From the end of the pyloric tube to the cloaca the histology of the intestine is practically the same and consists essentially of cylindrical and goblet cells. No glands are present, but the villi project into the lumen of the intestine both in the duodenum and in the spiral valve. The epithelium which covers these villi is the same throughout and consists of cylindrical and goblet cells. Since the villi are more prominent in the spiral valve, it would be well to consider this part of the intestine in detail.

SPIRAL VALVE.

A spiral valve is present in the colon of cyclostomes, selachians, ganoids, and dipnoans. Its histological structure in *Mustelus canis* and *Carcharias littoralis* is like that of the duodenum. The villi stop abruptly at the point where the rectal gland opens into the intestines. The folds of the spiral valve are formed from the mucosa of the walls of the intestines. Through the middle of each fold passes the muscularis mucosa. From the center connective tissue extends into the villi. A cross section of a fold shows: (1) Epithelium of upper surface, (2) connective tissue, (3) connective tissue and muscular tissue, (4) connective tissue, (5) epithelium of undersurface.

RECTAL GLAND.

The rectal gland, glandula or processus digitiformis, is a compound tubular gland varying from one-half inch in the skate to four inches in the mackerel shark. It opens into the rectum by a duct, which, beginning at the central canal of the gland, runs forward along the edge of the mesentery to enter the dorsal wall of the lower end of the spiral valve or the top of the rectum. The gland consists of three layers: (1) an outer fibro-muscular layer, (2) a middle glandular layer, and (3) a central region consisting of ducts and blood vessels arranged round a central lumen.

The middle layer is composed of a number of branched tubules radially arranged and separated by capillaries which are usually gorged with blood. The high power shows mono-nucleated cubical cells not clearly defined from each other and of a glandular appearance.

The central layer begins at a varying distance from the periphery by the sudden transition of the gland cells into the epithelium of ducts which open into the central lumen. In many cases the more superficial cells have undergone a mucoid change and a band of clear cells is visible lining the duct. The microscopical appearance of the gland is shown in plate I.

PHYSIOLOGY OF THE DIGESTIVE TRACT OF ELASMOBRANCHS.

While studying the food of the dogfish, *Mustelus canis*, at the laboratory of the Bureau of Fisheries, Woods Hole, Mass., during the summer of 1904, Irving A. Field found that 16 per cent of the dogfish contained lobsters, 34.17 per cent rock crabs, and 20.1 per cent spider crabs. The carapace of these organisms consists of salts and chitin, the latter highly resistant to reagents. As the carapace was found in varying degrees of decomposition, and, further, since the carapace of crabs and lobsters fed to the fish could not be found in the stomach after four days of digestion, the question arose as to whether the dogfish actually does digest chitin. I therefore began, during this summer, a physiological study of the alimentary canal of *Mustelus canis*. During the summers of 1905 and 1906 the investigation was extended to include *Carcharias littoralis*, *Squalus acanthias*, *Tetronarce occidentalis*, *Carcharhinus obscurus*, *Raja erinacea*, *Lamna cornubica*, and *Dasyatis centrura*. The work consisted of—

(a) The preparation, for artificial digestion, of extracts of buccal, esophageal, and gastric mucous membranes.

(b) The study of the normal content of the stomach.

(c) The study of the acidity of the stomach.

(d) Determining whether or not *Mustelus canis* digests chitin.

(e) The preparation of extracts of the intestinal mucous membrane and of the pancreas.

(f) The study of the activation of the pancreas.

(g) The study of the rectal gland.

BUCCAL CAVITY.

The elasmobranchs as a rule swallow their food whole, without mastication. Naturally we should suppose that little digestion goes on in the buccal cavity. This probability is increased by the absence of glands. Since Krukenberg (1877) claimed, however, that the buccal mucus of some fish, especially of *Cyprinus carpio* and *Lophius piscatorius*, possesses a diastatic action, it seemed proper to test the action of various kinds of infusions of the buccal mucous membranes of selachians.

The buccal cavity of a number of these fish, freshly killed, was scraped. The mucus thus collected was white in color, neutral in reaction, and gave a good test for mucin, but showed no diastatic activity. Scrapings of the buccal cavity of all the elasmobranchs obtainable gave the same results.

The buccal cavity of elasmobranchs, then, as Yung has already shown for *Scyllium canicula*, secretes mucin comparable to that of the saliva of man, but with no diastase.

Water extracts of the buccal mucous membrane have no permanent emulsifying action on olive oil.

Five-tenths per cent hydrochloric acid extracts of the buccal mucous membrane filtered free of mucin have no peptonizing action on white of egg or pig fibrin. The buccal mucous membrane then contains no pepsin-like enzyme.

Conclusions: The buccal mucus of all the elasmobranchs examined contains mucin but no digestive ferment.

ESOPHAGUS.

The reaction of the esophagus of a fasting fish is neutral. Tested when the fish is in full digestion, on the other hand, the reaction of the esophagus is acid, due, undoubtedly, to regurgitation from the stomach.

Mucus was scraped from the esophagus of ten fasting smooth dogfish. This mass was divided into two equal portions and one portion was made slightly alkaline with sodium carbonate, while the other was acidified to the extent of five parts of hydrochloric acid in one liter. To each portion thymol was added to prevent the action of microbes. Into test tubes containing the alkaline and acid solutions, respectively, fibrin was placed. In no case was the fibrin digested, whether the tubes were kept at 18° C. or at 37° C.

Therefore the esophagus produces neither trypsin nor pepsin. If the mucous membrane is scraped from the esophagus of a fish in full digestion, extracts of this mucus may have a slight digestive action on fibrin in acid solution. This digestive action, however, is due to some pepsin which has come from the stomach, for if the esophagus is well washed before scraping the esophageal mucus is found to have no action on fibrin.

In like manner neither water extracts nor weakly alkaline extracts of the mucous membrane of the esophagus have any diastatic action on starch paste even after a lapse of ten hours. Yung (1899) in two cases found a diastatic ferment in the mucus of the esophagus. These cases were one *Scyllium canicula* in full digestion and one *Acanthias vulgaris*. He concludes from his experiments, however, that as a rule the epithelial elements do not produce a diastatic ferment.

Water extracts of the esophageal mucus have no action on olive oil.

Conclusion: The esophagus has of itself no digestive action.

MUCOUS MEMBRANE OF THE STOMACHIC SAC.

The mucous membrane of the stomachic sac was scraped and triturated in equal parts of glycerin and 0.5 per cent hydrochloric acid. Neutralized extracts did not coagulate milk. Therefore the rennet enzyme and its zymogen are absent from the mucous membrane of elasmobranchs.[a] The acid extracts digested uncooked white of egg and fibrin rapidly, but acted very slowly on cooked egg. As a rule the products of digestion by the acid extracts were peptones. Alkaline extracts of the stomachic sac showed no digestive activity.

The only proteolytic ferment in the stomachic sac of elasmobranchs is, accordingly, pepsin similar to that of higher vertebrates. There is one great difference, however, between the pepsin of mammals and that of fish: The pepsin of fish acts at a low temperature far better than does that of mammals. Moreover, Fick and Murisier (1873) and Hoppe-Seyler (1877, cited by Yung, 1899) claimed that the fish pepsin acts better at 10° C. or 15° C. than at 37° C. Luchhau (1878) and Yung (1899), on the other hand, observed that the peptonizing action of the stomach of fishes is greater at 40° C. than at 15° C.

While I should admit that the pepsin of fish acts rapidly on fibrin at 15° C., I must conclude from my experiments that the pepsin of *Mustelus canis*, *Carcharias littoralis*, and *Galeocerdo tigrinus* digested fibrin better at 37° C. than at 20° C. In this conclusion I am in exact agreement with Yung, who found in *Scyllium canicula* that the pseudopepsin of fish acted more rapidly at the higher temperatures. Before leaving the question as to the action of pepsin it must be said that the artificial digestion in no way approximates the natural digestion as carried on in the stomach,

[a] Certain experiments have led me to believe that the rennin zymogen (pexinogen) may exist in the mucous of the stomachic sac of at least some of the elasmobranchs and may be extracted by appropriate methods as the active enzyme rennin (pexin). To this question I hope to return at another time.

because in natural digestion the products are rapidly carried off, the stomach is in constant movement, and the pepsin and hydrochloric acid are constantly being renewed.

In no case, whether the solution was acid, neutral, or alkaline, did I find that glycerin extracts of the mucous membrane of the stomachic sac of the various elasmobranchs had the power of converting starch to sugar. In concluding that the mucous membrane of the stomachic sac of elasmobranchs does not produce a diastatic enzyme I should be in exact agreement with Richet (1882), who studied *Scyllium* and *Acanthias*, and with Yung (1899), who extended his studies further—to *Galeus* and *Lamna cornubica*. Upon ethyl butyrate, likewise, I found that the watery extracts had no effect whatever.

MUCOUS MEMBRANE OF THE PYLORIC TUBE.

Peptic glands are absent from the pyloric tube. To study the physiology of this tube, I took 10 smooth dogfish (*Mustelus canis*) and 10 sand sharks (*Carcharias littoralis*). After carefully washing the inner surface of the pyloric tube I scraped off the mucus and macerated it in glycerin and 0.5 per cent hydrochloric acid solution. After twenty-four hours the liquid was filtered. To the filtered liquid small pieces of fibrin were added. In twelve hours the digestion mixture was tested and showed syntonin, but no peptones. Contrary to Krukenberg's (1877) results from work on selachians, we must conclude with Yung that the formation of pepsin is limited to the cardiac end of the stomach or the stomachic sac.

The pyloric tube has likewise no action on starches or fats.

Conclusion: The only active ferment secreted by the stomach of elasmobranchs is pepsin.

CONTENT OF THE STOMACH.

The study of the content of the stomach is really the study of the content of the stomachic sac. The content of the stomach varies greatly. Sometimes it is strongly acid and viscid; sometimes it is liquid and holds in suspension alimentary débris, more or less recognizable, oil, fish in various stages of decomposition, chitin, etc.

An analysis was made of the contents of the stomachic sac of *Mustelus canis*, *Carcharhinus obscurus*, *Carcharias littoralis*, *Squalus acanthias*, *Tetronarce occidentalis*, *Raja erinacea*, *Lamna cornubica*, and *Galeocerdo tigrinus*. The acid content of the stomach was neutralized and an abundant precipitate of syntonin occurred. The filtrate was boiled, and if a precipitate occurred was again filtered. The solution was boiled and again treated with an excess of ammonium sulphate. The precipitate showed albumoses. The filtrate was then tested with the biuret reaction. As a rule syntonin, proteoses, and peptones were found in the stomach content. Occasionally, however, no peptone could be found in the stomach content of *Mustelus canis* and *Carcharias littoralis*.

Conclusions: The stomach of *Mustelus canis*, *Carcharias littoralis*, *Squalus acanthias*, *Tetronarce occidentalis*, *Carcharhinus obscurus*, *Raja erinacea*, *Lamna cornubica*, and *Galeocerdo tigrinus* secretes pepsin and converts proteids partly to antipeptone.

Acidity of the gastric juice.—Richet (1878) found the acidity of the gastric juice of fish to be much greater than that of mammals. Thus he found the acidity of fish he studied to be as follows:

Fish.	Parts in 1,000.
Skate (Raja clavata)	14.6
Lophius piscatorius	6.2
Squalus squatina	6.9
Scyllium catulus	6.9
Scyllium canicula	14.9
Pike	6.0

The acidity Richet found to be due to an acid not soluble in ether. He believed that the acid was hydrochloric acid combined with some organic substance, as leucin or tyrosin.

Yung (1899) in his study of the gastric juice of *Scyllium canicula* found the mean of four analyses to be 0.84 per cent.

The acidity of the stomach of elasmobranchs is greatest when the fish is in full digestion. Indeed, the fasting stomach is practically neutral. In order to study the acid, phenolphthalein, alizarin, and dimethyl-amido-azobenzol were used as indicators as recommended by Webster and Koch (1903) in their Laboratory Manual of Physiological Chemistry (p. 36), and experiments were made to determine: (a) The total acidity of the stomach content in terms of hydrochloric acid; (b) the physiologically active hydrochloric acid; (c) the free hydrochloric acid. The results are given in the following table:

Species.	Total acidity in percentage hydrochloric acid.	Physiologically active hydrochloric acid, average percentage.	Highest percentage free hydrochloric acid.
Mustelus canis	0.04–1.00. Average, 0.78. 50 individuals.	0.538. 6 individuals.	0.2.
Carcharias littoralis	0.1–1.2. Average, 0.87. 25 individuals.	0.614. 10 individuals.	0.31.
Squalus acanthias	Average, 0.67. 60 individuals.	No tests.	No tests.
Carcharhinus obscurus	Average, 0.55. 2 individuals.	0.493. 2 individuals.	0.254.
Lamna cornubica	0.275. 1 individual.	0.229. 1 individual.	0.172.
Galeocerdo tigrinus	0.93. 1 individual.	0.812. 1 individual.	None.
Tetronarce occidentalis	0.51. 1 individual.	No tests.	None.

In the case of *Carcharias littoralis* and *Carcharhinus obscurus* the physiologically active hydrochloric acid was determined as follows: (a) By neutralizing 10 c.c. of the stomach contents, evaporating, calcining, and finding the total chlorides by titrating with normal silver nitrate; (b) by evaporating, calcining, and finding, by titrating with the silver nitrate solution, the chlorides in a nonneutralized 10 c.c. of the stomach content; (c) subtracting (b) from (a). The results were as follows:

Total acidity in terms of hydrochloric acid (phenolphthalein indicator):

	Per cent.
Carcharias littoralis	1.1
Carcharhinus obscurus	.92

Physiologically active hydrochloric acid by $AgNO_3$=C:

Carcharias littoralis 0.660
Carcharhinus obscurus .... .525

Action of the gastric juice on chitin.—Lobsters and crabs form part of the food of several of the elasmobranchs. The shell of these crustaceous consists of chitin and salts. This chitin is very resistant to reagents. According to Hammarsten (1901), chitin, to which he gives the formula $C_{60}H_{100}N_8O_{38}+n(H_2O)$, is insoluble in boiling water, alcohol, ether, acetic acid, dilute mineral acids, and dilute alkalies. It is dissolved without decomposing in cold concentrated hydrochloric acid. Since chitin is so resistant it is interesting to know whether the chitin-eating fish digest chitin or whether it passes through the body unchanged.

An analysis of lobster shells given by Herrick (1895) from the work of Albert W. Smith is as follows:

TABLE SHOWING COMPOSITION OF THE CARAPACE OF THE LOBSTER (3 SPECIMENS).

Composition, air dried.	1.	2.	3.
Weight in grams	11.87	21.51	8.48
Calculated as calcium carbonate	43.68	32.83	32.93
Calcium phosphate	7.70	8.32	11.18
Calcium sulphate	.58	.53	.99
Magnesium carbonate	3.50	2.39	3.38
Sodium carbonate	1.51	1.80	2 31
Alumina	.68	2.04	1.04
Silica	.14	.29	.08
Organic matter and water	42.21	51.80	48.09

Richet (1878) believed that the chitin of the shell of crabs, lobsters, etc., is digested by dogfishes and sharks, although he recognized that it is extremely difficult to dissolve chitin by artificial digestion. Yung (1899), on the other hand, claimed that the selachians do not digest chitin, for he found pieces of chitin not only in the stomach but even in the spiral valve and rectum. More recently Zaitschek (1904) has proved quantitatively that the chitin in the wings of insects is absolutely undigested by hens.

To determine whether the elasmobranchs, and especially the smooth dogfish, digest chitin, the following experiments were made:

1. Several fishes were fed with crabs and lobsters and in the course of from one to five days were killed. In some cases the shells were found in the stomach in a macerated state. On the other hand, no compact chitin could be found after ninety hours of digestion, but in the spiral valve might be found a gritty dark-brown or reddish mud.

2. The gastric juice was drawn from several large dogfish. Into small quantities of this juice lobster shells were placed. The mixtures were kept at a constant temperature—some at 18° C., some at 38° C. After twelve hours the only change found in the shell was that the edges were softened a trifle.

3. The mucous membrane of the stomach of five dogfish was scraped, triturated in glycerin and 0.5 per cent hydrochloric acid. This juice, although it acted quickly on fibrin, did not digest the chitin in forty hours.

4. Experiment 2 was repeated with the difference that at frequent intervals the gastric juice was renewed and the chitin was subjected to frequent grinding. In this

way there was formed a pulverized mass of a dark brown or dark red color, which approximated the granular mass found in the intestines of the chitin-swallowing fish.

5. Pieces of chitin were placed in acid of strengths varying from 0.5 to 35 per cent hydrochloric acid. Carbon dioxide was set free in each case, but in greater quantities with the stronger acids. The chitin became softened, pliable, but did not dissolve.

6. Crabs were fed to dogfish confined in small aquaria. The excrement of the fish was carefully watched, and in this excrement, known by its shape and color, some pieces of the softened but otherwise unchanged chitin could be found. No evidence was gathered that the fish ever regurgitated any of the chitin, though Yung believes that regurgitation might take place.

Conclusion: The conclusion to be drawn from the experiments is that *Mustelus canis* and other chitin-swallowing fishes do not digest the chitin. The frequent change of the gastric juice, combined with the movements of the stomach, dissolves out the salts, softens the shell, and breaks it up into a fine mass, such as may be found in the spiral valve. The chitin is not regurgitated, but on the contrary is excreted in a finely divided mass.

MIDDLE INTESTINE.

Extracts of the middle intestine or duodenum of the various elasmobranchs show no digestive activity. Whether or not the cylindrical cells and goblet cells lining the mucous membrane of the duodenum play any part in activating the pancreatic juice will be discussed under pancreatic digestion.

SPIRAL VALVE.

Extracts of the mucous membrane of the spiral valve showed no digestive action on starches, fats, or proteids; nor indeed was it possible to demonstrate any inverting power, though we may presume that such power may exist in this mucus. The main function of the spiral valve is absorptive. Its further action in pancreatic digestion will be taken up in the discussion of the function of the pancreas.

PANCREAS.

As may be seen by reference to page 9, there has been but little experimentation on the pancreas of fishes, which fact is warrant for discussing the physiology of the pancreas of elasmobranchs. In order to get at the problem with the greatest clearness, it would perhaps be well for us to outline the growth of knowledge concerning pancreatic digestion, from the first experiments on the pancreas to the later researches leading to our present-day understanding of the action of this organ.

The pancreatic ducts, according to Haller (1764), were discovered by Wirsung in 1642. Although Wirsung appears to have observed the pancreatic juice, he did not pursue the subject further, and little was made of his discovery until Regner De Graaf took up the matter. In 1664, De Graaf, according to Foster (1901), made the first successful pancreatic fistula and collected the juice from a dog. But De Graaf did not obtain a definite grasp of the function of the pancreas. Eberle (1834), however, announced that a watery infusion of the pancreas when shaken with oil emulsifies it. In 1836, according to Corvisart (1857), Purkinje and Pappenheim discovered the proteolytic power of the pancreas, and nine years later Bouchardat and Sandras (1845) discovered and established with precision, by means of observation carried on with the aid of pancreatic infusions as well as of small quantities of pancreatic juice obtained from hens and geese, that this secretion possesses powerful diastatic properties.

As pointed out by Gamgee (1893, p. 203), this discovery of the sugar-forming enzyme of the pancreas has been erroneously attributed to Valentin (1844). Valentin recognized that starch was changed, but did not say that the change was a conversion to sugar. His own words are: "Wie man sieht erlauben diese Erfahrungen noch keine irgend bestimmenden Schlüsse. Höchstens deuten sie darauf hin, dass vielleicht die Pancreasflüssigkeit die Fähigkeit habe die Stärke löslich zu machen und beweilen eine Umsetzung derselben einzuleiten." No further study of the pancreas seems to have been made until Bernard took up the work.

Bernard (1856), by means of a pancreatic fistula, proved that the secretion is an alkaline fluid with a threefold action on starches, fats, and proteids. He concluded, however, that the pancreatic juice alone has no action upon proteids, but that it is able to dissolve them either when they have been first of all subjected to the action of bile or when it acts in conjunction with bile. To this proteolytic function of the pancreatic juice, indeed, Bernard gave little weight.

In 1857 Corvisart called attention to the proteid-digesting power of the pancreatic juice, and although his observations were more or less discredited by some, they were confirmed by Meissner (1859), Danilewsky (1862), and Kühne (1867), the latter particularly contributing greatly to our knowledge of tryptic digestion.

The great interest awakened in the proteolytic activity of the pancreas by the researches of Kühne (1867) was intensified by the publication of a remarkable memoir by Heidenhain (1875). In this paper the author described for the first time those changes in the secreting cells of the pancreas which correspond to the different states of activity, and announced that the fresh pancreas does not contain the proteolytic ferment, but an antecedent body which he called zymogen. This zymogen he found could be extracted from the gland, and under suitable treatment would yield the proteolytic ferment. Since Heidenhain's discovery the antecedent bodies of other enzymes have been discovered. To the antecedent of the proteolytic enzyme of the pancreas, trypsin, the name trypsinogen has been given.

As a result of the study of the pancreas by the various investigators, this organ has long been known to secrete an alkaline juice and three enzymes or their zymogens, namely, trypsin, acting on proteids; amylopsin, acting on starches; and steapsin or lipase, acting on fats. Notwithstanding all the study of the pancreatic juice, however, in many ways the knowledge of its action was somewhat uncertain. Sometimes the pancreatic extracts would show a little digestive power, while the juice collected by a fistula was, as a rule, inactive. To explain these variations, investigations were made on the correlation between the pancreas and other organs and juices. Heidenhain (1875) had observed that when an aqueous solution of dried pig's bile was added to a glycerin extract of the pancreas, the proteolytic power of the latter was increased. Chittenden (1885) noticed that bile in a pancreatic extract containing salicylic acid increased tryptic action. Martin and Williams (1890) and Rachford and Southgate (1895) also noticed the stimulating action of bile on tryptic digestion. Chittenden and Albro (1898), however, found that normal bile exerts very little influence on pancreatic proteolysis and may retard as well as aid. Bruno

(1899), on the contrary, found that the bile even doubled the action of the pancreas, and that this action was not lost by boiling, and Delezenne (1902) verified, in the main, Bruno's work, but declared that bile does not activate inactive pancreatic juice. What part the bile plays in activating the pancreas is not yet fully decided.

The spleen has also been claimed to play a certain part in digestion. Schiff (1862), Gachet and Pachon (1898), Bellamy (1901), and Mendel and Rettger (1902) have shown that the spleen when congested during digestion increases the proteolytic power of the pancreas. On the other hand, Heidenhain (1883), Ewald (1878), and Hammarstein (1901) do not find that the spleen had any action on pancreatic digestion, while Noel Paton (1900) has shown that there is not necessarily any difference in the nitrogenous metabolism of dogs before and after splenectomy. Frouin (1902) has demonstrated that the removal of the spleen from dogs with an isolated stomach does not interfere with their nutrition even during a meat diet. Further, Camus and Gley (1902), Bayliss and Starling (1903), and Hekma (1904) have shown that extracts of the spleen have no activating action. The influence of the spleen on pancreatic digestion is still open to debate.

An activating principle more easy of demonstration is that discovered by Dr. N. P. Schepowalnikow (1898) in the succus entericus, or the juice of the small intestines. This juice, though possessing no proteolytic action itself, has the power of augmenting the activity of the pancreatic ferment, and especially of the proteolytic ferment—trypsin. Indeed, it was found that the succus entericus would convert an otherwise inactive pancreatic juice into an active juice. To the ferment, since such the activating principle was found to be, Pawlow gave the name of enterokinase. Others have corroborated Schepowalnikow's work, and Delezenne (1902) found enterokinase not only in the duodenum, jejunum, and slightly in the ileum, but also wherever leucocytes abound. In other words, he claims that the activating principle is generated by the white blood corpuscle.

From the size of the pancreas in the elasmobranchs we should expect this organ to play a large part in the work of digesting food. Krukenberg (1877), in his work on selachians, found that the pancreas of these fishes was secreting amylopsin, steapsin, and trypsin. Richet (1878), however, was unable to find trypsin. More recently Yung (1899), working on *Squalus acanthias*, found amylopsin and lipase, but only occasionally trypsin. Yung attempted to get the juice by a fistula, but had little success. His water glycerin extracts were only occasionally active. He found that extracts of the spleen aided the activation of the pancreas. Sellier (1902) found that the pancreas of several selachians studied by him does not of itself digest proteid, but must be activated by the juice of the spiral valve.

To determine just what part the pancreas of selachians plays in digestion and what enzymes it secretes, my work comprised experiments as follows:

1. Pancreatic fistulæ were made to obtain pure pancreatic juice.
2. Water glycerin extracts and sodium carbonate extracts were made to extract the zymogens or enzymes.
3. Extracts of the pancreas were combined with bile, and with extracts of the duodenum, spiral valve, spleen, stomach, and rectal gland.
4. The fresh pancreas was used to determine the presence of lipase.
5. The content of the spiral valve was studied.

Pancreatic fistulæ.—In order to collect the pancreatic juice a slit was made in the abdomen and in the wall of the duodenum of *Carcharias littoralis* in such a way as to cut the pancreatic duct. Into the central end of the duct was fastened a small glass cannula. To the outer end of the cannula was fastened a small sterilized rubber balloon. After the sand shark had been sewed up it was set free in a large aquarium. In a few days the balloon was taken off and the juice collected with a pipette. As a rule the quantity of juice thus collected was small and had no digestive activity. Owing to the difficulties of keeping the fish alive for a prolonged period and of feeding them, I made but six fistulæ and then abandoned this kind of work for the pancreatic extracts.

The proteolytic enzyme. Activation of the pancreas.—The pancreas was ground in a mortar with glass, and the comminuted mass was treated with water and glycerin. After twenty-four hours the mixture was filtered through cotton. To test the digestive activity of the extracts, at first I used Mett's tube and fibrin. Finding both of these media unsatisfactory, I employed Fermi's (1902) gelatin method, using 10 per cent gelatin and 0.6 per cent carbolic acid. The gelatin was placed in small test tubes and the upper layer of the gelatin marked on the tube by means of a blue pencil. The amount digested was measured in twenty-four hours if the experiment was carried on at the room temperature; in three hours if at 37° C. The pancreatic extracts had as a rule very little digestive activity. Accordingly, I added water glycerin extracts or water chloroform extracts of the mucous lining of the stomachic sac, pyloric tube, duodenum, spiral valve, rectal gland, and spleen to determine whether or not any of these extracts would activate the pancreas. Controls were made in each case. The results may be found in the table.

TABLE SHOWING THE ACTIVATING ACTION OF THE VARIOUS EXTRACTS UPON TRYPTIC DIGESTION.

Species.	Pancreas.	Pancreas duodenum.	Pancreas spiral valve.	Pancreas rectal gland.	Pancreas spleen.	Pancreas bile.
Carcharias littoralis	0 or +	0 + or ++	+++	0 or +[a]	+	+; — ++[b]
Carcharhinus obscurus	0 or +	++	+++	0 or +	0 or +	0 or +
Lamna cornubica[c]	0 or +	?	++	?	?	?
Mustelus canis	0 or +	0 + or ++	+++	0 or +	0 or +; ++[a]	—; ++[b]
Squalus acanthias	0 or +	0 or +	++	0 or +	0 or +	0 or +
Raja erinacea	+	+	++	0 or +	0 or +	0 or +
Dasyatis centrura	0		++	0	0	0

Species.	Bile.	Duodenum.	Spiral valve.	Rectal gland.	Spleen.	Pancreas and stomachic sac.	Pancreas and pyloric tube.
Carcharias littoralis	0	0	0	0	0	+	+
Carcharhinus obscurus	0	0	0	0	0	+	+
Lamna cornubica[c]	0	0	0	0	0	0	0
Mustelus canis	0	0	0	0	0	0	0
Squalus acanthias	0	0	0	0	0	0	0
Raja erinacea	0	0	0	0	0	0	0
Dasyatis centrura	0	0	0	0	0	0	0

+, quantity digested by pancreas.
++, greater quantity.
+++, still greater quantity.
0, no digestion.
—, less than pancreatic extract alone.
[a] Only a few tests were made to determine the activating action of the rectal gland.
[b] The bile often increased the proteolytic activity of the pancreas, but occasionally diminished this activity.
[c] Only two sets of experiments were made.
[d] The spleen occasionally showed activating action.

The conclusions to be drawn from the table are:

(1) The pancreatic extracts may or may not show proteolytic activity.

(2) The duodenum as a rule causes the inactive pancreatic extract to digest the gelatin and increases the digestive activity of an active pancreatic extract.

(3) The greatest activation is produced by the spiral valve.

(4) The spleen occasionally activates the pancreatic extract.

(5) The bile sometimes activates, sometimes has no effect whatever, and sometimes slightly diminishes the digestive activity of the pancreatic extracts.

(6) Extracts of the duodenum, spiral valve, and rectal gland have no digestive activity.

(7) The bile alone has no digestive power.

(8) Extracts of the rectal gland have no activating influence.

(9) Extracts of the mucous membrane of the stomachic sac and pyloric tube have no activating action.

Amylopsin and lipase.—Krukenberg (1877), Richet (1878), and Yung (1899) found amylopsin and lipase in the pancreas of European elasmobranchs. To determine the presence of these enzymes in the pancreas of the American elasmobranchs experiments were made (1) on the amylolytic activity and (2) on the lipolytic activity.

1. Water-glycerin extracts of the pancreas, slightly acidified with acetic acid, were made at different times from different lots of fishes. To 5 c. c. of a starch paste, free from sugar, was added 5 c. c. of the extract. After a short period the mixture was examined for sugar by Fehling's test. If negative, the tests were repeated at hourly intervals for six hours. In every case control tests were made. The results of the experiments are as follows:

Species.	Diastatic enzyme (amylopsin).	
	Number of tests.	Positive.
Carcharias littoralis	6	4
Carcharhinus obscurus	2	2
Lamna cornubica	2	0
Mustelus canis	10	6
Squalus acanthias	2	1
Raja erinacea	10	0
Tetronarce occidentalis	1	1
Dasyatis centrura	1	1

The table shows that the pancreas of elasmobranchs may secrete the diastatic enzyme or its zymogen. Even in the case where the tests were negative I should expect on further investigation to find that the pancreatic extracts have the power to convert starch to sugars.

2. To determine the presence of the lipolytic ferment of the pancreas, the fresh pancreas of *Mustelus canis*, *Carcharias littoralis*, and *Raja erinacea*, the only elasmobranchs available at the time, was used. The methods employed and the results obtained are as follows:

(a) The fresh pancreas of the three fish mentioned was cut finely and mixed separately with a little water to make a thin paste. To a small quantity an equal volume of olive oil and litmus solution was added, and this mixture was kept at 30° C. for twenty-four hours. When a small quantity of a 1 per cent sodium carbonate solution was added to the oil mixture a permanent emulsion was formed in every case. When olive oil alone was treated with water and sodium carbonate the emulsion was not permanent.

(b) A neutral ethereal solution of butter was made and litmus added to a distinctly blue tint. This was placed in contact with a teased bit of fresh pancreas, as recommended by Gamgee (1893, p. 213). In a short time the liquid bathing the pancreas became faintly pinkish in spots, showing that a slightly acid reaction had developed.

(c) Aqueous extracts of the pancreas separated a small amount of butyric acid from a dilute solution of ethyl butyrate.

From these experiments it is to be concluded that lipase is secreted by the pancreas of *Mustelus canis*, *Carcharias littoralis*, and *Raja erinacea*, although its activity as demonstrated in vitro is not very great.

RECTAL GLAND.

Blanchard (1882) studied the function of the rectal gland, or digitiform gland, in *Acanthias vulgaris*, *Mustelus canis*, *Scyllium catulus*, *Scyllium canicula*, *Raja punctata*, and *Raja maculata*. In every case he found that extracts of the gland emulsified oil and converted starch to sugar, but had no action on white of egg or cane sugar. Extracts made by me of the rectal gland, or processus digitiformis, of *Carcharias littoralis*, *Carcharhinus obscurus*, *Lamna cornubica*, *Mustelus canis*, and *Raja erinacea* had no digestive action on fibrin or starch, nor did they hydrolyze ethyl butyrate. In the secretion of the gland I found considerable mucin. To the structure and physiology of the rectal gland I shall return in a later paper. At present, however, I should decide that the rectal gland has no digestive activity.

SUMMARY.

The results of the histological work in the present investigation may be summarized as follows:

1. The mucous membrane of the bucal cavity of *Mustelus canis* and *Carcharias littoralis* consists of stratified epithelium, with goblet and cylindrical cells, but no glands.

2. The mucous membrane of the esophagus possesses ciliated cylindrical cells and goblet cells, but no glands.

3. Gastric crypts exist in the stomachic sac of the elasmobranchs. There is no differentiation into chief and parietal cells. The epithelium of the crypts consists of cylindrical cells and polygonal cells.

4. The pyloric tube has the same kind of superficial epithelium as the stomachic sac and similar crypts. The polygonal cells, however, are absent.

5. The epithelium of the intestines from pyloric tube to cloaca consists of cylindrical cells and goblet cells.

6. The rectal gland is a compound tubular gland.

The physiological study and experiments produce the following conclusions:

7. In the elasmobranchs examined, neither the buccal mucous membrane nor the mucous membrane of the esophagus has any digestive activity.

8. The stomachic sac secretes pepsin and hydrochloric acid.

9. The total acidity of the stomach contents, in terms of hydrochloric acid, may reach as high as 1 per cent.

10. The physiologically active hydrochloric acid may be as strong as 0.6 per cent.

11. The gastric juice does not digest chitin.

12. The middle intestines and spiral valve have no digestive activity, but activate the pancreas.

13. The spiral valve possesses the greatest activating power.

14. The pancreas secretes trypsinogen as a rule, but may secrete trypsin.

15. The pancreas secretes amylopsin, the starch-splitting ferment, and lipase, the fat-splitting ferment.

16. The rectal gland has no digestive activity.

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