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THE EMBRYOLOGY AND LARVAL DEVELOPMENT OF BAIRDIELLA CHRYSURA AND ANCHOVIA MITCHILLI

By Albert Kuntz, Ph. D.

School of Medicine, St. Louis University

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THE EMBRYOLOGY AND LARVAL DEVELOPMENT OF BAIRDIELLA CHRYSURA AND ANCHOVIA MITCHILLI.

By ALBERT KUNTZ, Ph. D.,
School of Medicine, St. Louis University.

INTRODUCTION.

The present paper embodies the results of observations made on the eggs and larvæ of two species of teleosts, *Bairdiella chrysura* and *Anchovia mitchilli*. The work was carried on at the United States Fisheries Laboratory at Beaufort, N. C., during the summer of 1913.

It is not the purpose of this paper to discuss at length any of the merely technically interesting points in the development of pelagic fish eggs. Nor does it contribute anything essentially new to our knowledge of the embryology of teleosts. The work was undertaken for the purpose of securing a record as complete as possible of the time of spawning and of the embryological and larval development of fishes with pelagic eggs breeding in these waters during the summer, one of the primary objects being to afford a ready means of identifying either eggs or larval fishes at any time during embryological and larval life.

Observations were made as far as possible on living material. The eggs were collected in the tow net. The larval fishes were taken primarily in the stow net, the bunt of which was provided with a hood of cheesecloth terminating at its apex in a large collecting bottle. A small per cent of the larval fishes taken in this manner were brought into the laboratory alive. The large majority of them, however, were dead before being taken from the net.

Eggs collected at the same hour on successive days were found to be in approximately the same phase of development. Obviously, spawning occurs regularly each day at approximately the same hour. Observations show that both species under consideration spawn regularly in the early evening, probably before 8 o'clock.

The eggs of these species are relatively small and contain but little yolk material. Embryological development, therefore, proceeds very regularly and requires a relatively short time. The eggs of *Anchovia mitchilli* require approximately 24 hours for hatching. Those of *Bairdiella chrysura* hatch in approximately 18 hours. The time required for hatching, doubtless, varies somewhat with the temperature of the water. The height of the spawning season of *Bairdiella chrysura* occurs during the last week of June and the first week of July. *Anchovia mitchilli* spawns freely during June, July, and August. The height of the spawning season of this species, doubtless, occurs in July. The average temperature of the water in the vicinity of the laboratory for the latter half of June was 27.15° C. The average temperature for the entire month of July was 27.77° C. These averages are based on daily readings taken at 5 o'clock p. m.

The young of *Bairdiella chrysura* were taken in small numbers at intervals throughout the latter half of June and the greater part of July. After the spawning season began to wane very few young of this species were taken. The young of *Anchovia mitchilli* were taken in considerable numbers throughout June, July, and August.

BAIRDIELLA CHRYSURA.

Spawning.—The eggs of *Bairdiella chrysura* were present in the plankton when work was begun on June 9, and were taken in the tow net nearly every day after that date until July 18, when they became relatively rare. Individual eggs were taken occasionally as late as August 15. Eggs of this species were at no time abundant. They were sufficiently numerous, however, to be readily obtained for study. They occurred in greatest abundance during the last week in June and the first week in July. These two weeks, doubtless, witnessed the height of the spawning season.

Adult specimens of *Bairdiella chrysura* were frequently taken in small numbers in the pound net and in the seine. Nearly all the adult fishes taken during June and July had already spawned. On June 20 and again on June 27 a single female ripe for stripping was brought into the laboratory. On the former occasion a few eggs were successfully fertilized. All of these eggs, however, died during early cleavage.

Eggs.—The eggs of this species are spherical in form and 0.7 to 0.8 mm. in diameter. The mature unfertilized egg is slightly yellowish in color. The yolk contains a relatively large oil globule. After fertilization has taken place and the blastodisc has become differentiated, the egg is almost perfectly transparent. The egg membrane is thin and horny. Between the egg membrane and the delicate vitelline membrane inclosing the yolk sphere there is a perceptible perivitelline space. The oil globule normally rests near the upper pole while the blastodisc hangs at the lower pole of the yolk sphere. The spherical form of the egg is maintained until the time of hatching.

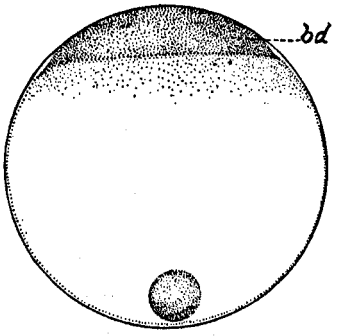
Segmentation.—In the mature unfertilized egg the yolk sphere is covered by a thin layer of protoplasm. After fertilization has taken place the protoplasm of this layer becomes concentrated at the pole opposite the oil globule, where it forms a lenticular cap on the surface of the yolk. This lenticular mass of protoplasm is the blastodisc. The "streaming" movements which occur in the protoplasm as it becomes concentrated to form the blastodisc have been well described and figured by Ryder (1882) for the cod [a] and more recently by other investigators for other species of teleosts.

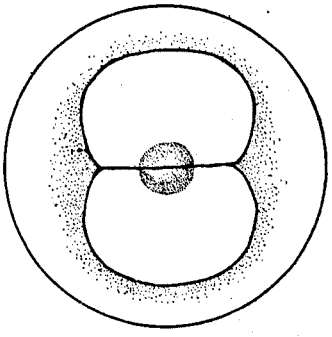
The fully developed blastodisc (fig. 1, *bd*) is circular in outline. Its periphery fades away almost imperceptibly into the very thin layer of protoplasm which remains at the surface of the yolk sphere. No protoplasm is noticeable within the yolk except in the vicinity of the oil globule. Here there is a small amount of protoplasm which can hardly be detected in the newly fertilized egg, but which, as development advances, becomes concentrated to form a protoplasmic cap covering about one-third of the surface of the oil globule.

Just before the first act of cleavage occurs one axis of the blastodisc becomes slightly longer than the other. The first plane of cleavage cuts the blastodisc at right angles to the longer axis (fig. 2). The second cleavage plane cuts the first at right angles (fig. 3). The first two cleavage furrows are meridional and cut deeply into the

[a] Ryder, John A.: Embryography of osseous fishes. Report United States Fish Commission 1882, p. 455–605.

blastodisc. In surface view the early blastomeres appear distinctly outlined peripherally (fig. 3). Viewing the early blastoderm in optical section from the side, however, it is apparent that the blastomeres are not entirely cut off peripherally, but are continuous with the thin layer of protoplasm at the surface of the yolk. This condition is illustrated in figures 25 and 27, in eggs of *Anchovia mitchilli*. The first four blastomeres are usually quite symmetrical and approximately equal in size. They





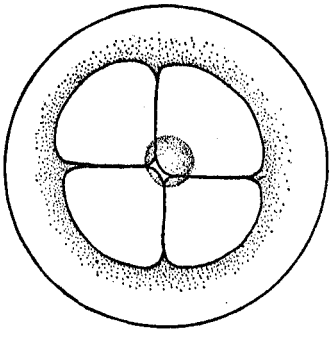
BAIRDIELLA CHRYSURA.

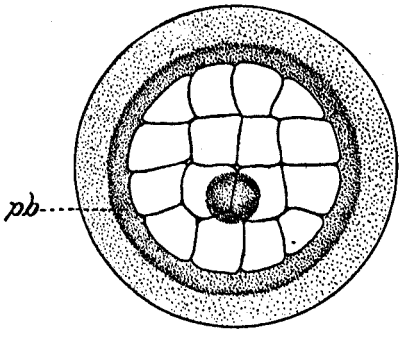
FIG. 1.—Egg with fully developed blastodisc (*bd*). × 55.

FIG. 2.—Egg with blastoderm of 2 cells. × 55.

also show a decided tendency to assume a spherical form, as is indicated by the deep indentations between the cells at the periphery of the blastoderm and the open area at the center (fig. 3). In the 4-cell stage the two axes of the blastoderm are approximately equal.

The third cleavage furrows cut the blastoderm approximately parallel with the first. When the third act of cleavage is completed and the blastoderm is composed of 8 cells,





BAIRDIELLA CHRYSURA.

FIG. 3.—Egg with blastoderm of 4 cells. × 55.

FIG. 4.—Egg with blastoderm of 16 cells; *pb*, periblast. × 55.

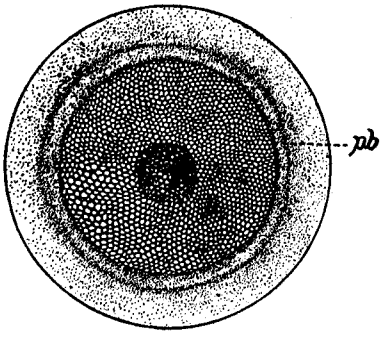
one axis is again distinctly longer than the other. In the 16-cell stage (fig. 4) the blastoderm is usually more or less nearly circular in outline.

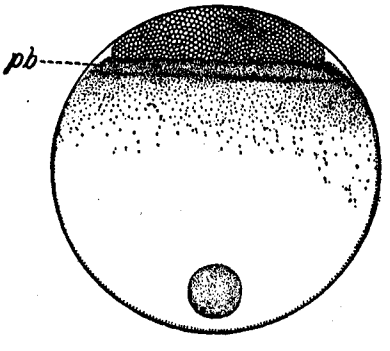
While blastoderms in the early cleavage stages show considerable variation, cleavage in these eggs may in general be said to proceed very regularly. The majority of the blastoderms observed in the 4-cell stage were almost ideally symmetrical. The same may be said of many of the blastoderms of 8 cells. At this stage irregularities are not uncommon, however. A marked tendency toward regularity is apparent also in blasto-

derms of 16 and 32 cells. This tendency may still be recognized in blastodorms of 64 cells.

The successive acts of cleavage follow each other in rapid succession. Eggs showing blastoderms in advanced stages of cleavage may be observed within three or four hours after the time of spawning. Such eggs were usually observed between 9 and 11 o'clock p. m.

Formation of the periblast.—During the early cleavage stages the marginal cells of the blastoderm are not definitely limited peripherally, but are continuous with the thin layer of protoplasm which remains at the surface of the yolk sphere. At the periphery of the blastoderm this protoplasmic layer is concentrated to form a low ridge. This ridge of protoplasm gives rise to the periblast (fig. 4, *pb*). As segmentation advances nuclei become apparent in the periblast. These nuclei, as observed by Agassiz and Whitman[a] (1884), are, doubtless, derived from the marginal cells of the blastoderm. The cells at the margin of the blastoderm gradually become more definitely limited peripherally until in the advanced stages of cleavage they are completely cut off from





BAIRDIELLA CHRYSURA.

FIG. 5.—Egg with blastoderm of many cells, late cleavage stage, surface view; *pb*, periblast. × 55.

FIG. 6.—Egg with blastoderm of many cells, late cleavage stage, lateral view; *pb*, periblast. × 55.

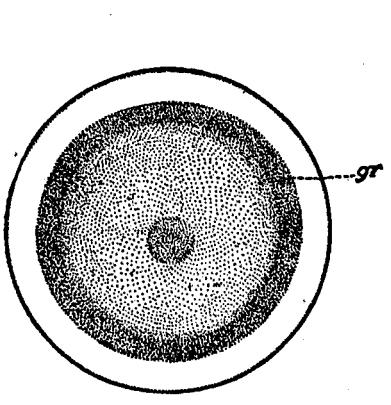
the periblast (fig. 6). The blastoderm is now more or less dome-shaped and beneath its central area may be observed a perceptible cleavage cavity. During the later cleavage stages the periblast becomes somewhat more definitely outlined, increases somewhat in width, and also sends a thin sheet of protoplasm centripetally beneath the cleavage cavity.

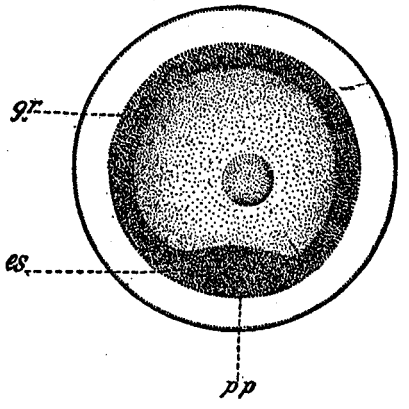
Formation of the germ ring and differentiation of the embryo.—While the marginal cells of the blastoderm are becoming cut off from the periblast there appears a slight thickening at the periphery of the blastoderm. This thickening represents an early stage in the differentiation of the germ ring. It is caused primarily by the thinning of the central area of the blastoderm and secondarily by the ingrowth (invagination) of the marginal cells. The part played by invagination in the formation of the germ ring and the embryonic shield is discussed at some length by Wilson (1889) in his paper on the embryology of the sea bass.[b] Evidence of invagination first appears at the

[a] Agassiz and Whitman: On the development of some pelagic fish eggs. Proceedings of the American Academy of Arts and Sciences, vol. 20, 1884.

[b] Wilson, H. V.: The embryology of the sea bass (*Serranus atrarius*). Bulletin of the United States Fish Commission, vol. IX, 1889, p. 209–277, pl. LXXXVIII–CVII.

posterior, i. e., the embryonic pole of the blastoderm. At this pole a broad tongue of cells, several layers in depth, may be observed before any evidence of invagination is apparent around the rest of the periphery of the blastoderm. Figure 7, plate II, illustrates an early stage in the differentiation of the germ ring. In this blastoderm invagination was not yet apparent. The following figure (fig. 8) illustrates a blastoderm in which the broad tongue of cells is already growing forward from the embryonic pole, and the entire germ ring is well differentiated. At this stage the central area of the blastoderm has become materially thinner than the peripheral area. Viewed from the under side the blastoderm is now distinctly concave. Between its concave surface and the periblast there is a perceptible subgerminal cavity closed in on all sides by the germ ring. The blastoderm gradually increases in size by centrifugal growth. The germ ring, therefore, which in its earlier stages is comparatively narrow, increases in width both by the invagination of the marginal cells and by the centrifugal growth of the blastoderm.





BAIRDIELLA CHRYSURA.

FIG. 7.—Egg with blastoderm showing early germ ring (*gr*). × 55.

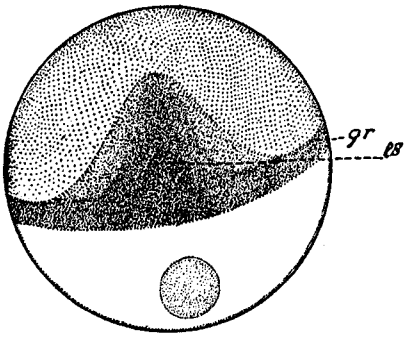
FIG. 8.—Egg with blastoderm showing fully developed germ ring (*gr*) and beginning of embryonic shield (*es*); *pp*, posterior pole of blastoderm. × 55.

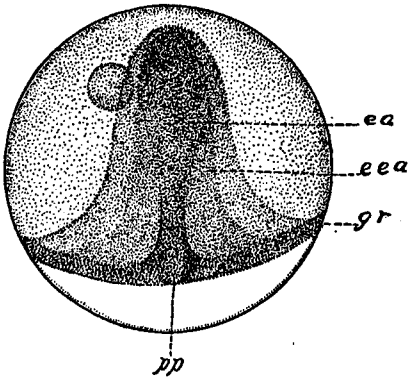
While the germ ring is becoming differentiated the cells forming the surface layer of the blastoderm become thin and flattened. This flattening of the surface cells is less apparent in the region of the germ ring, especially in the neighborhood of the embryonic pole, than in the central area of the blastoderm. In the neighborhood of the embryonic pole the surface cells remain relatively thick and more or less polygonal in form.

After the germ ring is completely differentiated the blastoderm increases in size more rapidly than in the earlier stages and advances around the surface of the yolk sphere. The broad tongue of cells which grows into the subgerminal cavity from the embryonic pole of the germ ring also increases in size, and the area of the blastoderm immediately over this ingrowing tongue of cells becomes differentiated. This differentiated area represents an early stage in the formation of the embryonic shield (fig. 9).

Soon after the embryonic shield has become distinctly outlined there occurs a thickening along its antero-posterior axis. This relatively opaque linear area repre-

sents the axis of the future embryo. We may now distinguish an embryonic and an extra-embryonic area within the embryonic shield. The differentiation of the embryonic axis begins in the head region and gradually advances posteriorly until it reaches the posterior pole of the blastoderm. When the embryonic area becomes distinctly





BAIRDIELLA CHRYSURA.

FIG. 9.—Egg showing later stage in differentiation of embryonic shield; *gr*, germ ring; *es*, embryonic shield.

FIG. 10.—Egg showing embryonic shield (*es*) with embryonic area (*ea*) outlined; *eea*, extra-embryonic area; *gr*, germ ring; *pp*, posterior pole of blastoderm.

outlined it is somewhat broader in the anterior or head region than in the posterior region. Observed in surface view (fig. 10) the embryonic area now has a more or less regular spatulate form. While the embryonic shield is growing forward into the subgerminal cavity and the embryonic axis is becoming differentiated, the germ ring is continually advancing around the yolk sphere. By the time the embryonic axis becomes well differentiated the blastoderm covers more than three-fourths of the surface of the yolk (fig. 11).

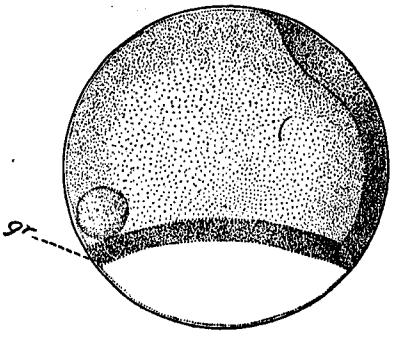
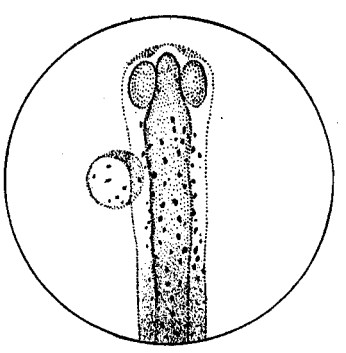


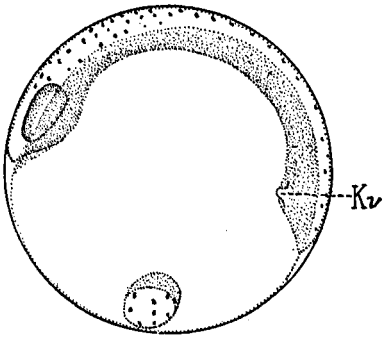
FIG. 11.—Same as figure 10, lateral view. × 55.

The further differentiation of the embryo advances very rapidly and the germ ring continues to advance round the yolk until the blastoderm covers the entire surface of the yolk sphere and the blastopore is completely closed. In the eggs observed while the germ ring was advancing round the yolk sphere the posterior pole of the blastoderm maintained approximately the same position with respect to the oil globule. Inasmuch as the oil globule maintains a more or less constant position with respect to the early blastoderm, it is obvious that the posterior pole of the blastoderm remains at a relatively fixed point. This Wilson (1889) observed to be the case also in the eggs of *Serranus atrarius*. In the eggs under observation the closure of the blastopore occurred before 1 o'clock a. m. This is probably not more than six hours after fertilization.

At the time of the closure of the blastopore the embryo extends about halfway round the circumference of the yolk sphere. There is as yet no evidence of pigmentation in either the egg or the growing embryo. Within one and one-half or two hours after the closure of the blastopore, yellow chromatophores become sparsely distributed over the

dorsal and dorso-lateral aspects of the embryo. A few yellow chromatophores are apparent also on the surface of the oil globule. The distribution of chromatophores at this stage is illustrated in figures 12 and 13. Kupffer's vesicle (fig. 13, *Kv*) now appears as a small bubblelike body on the ventral surface near the posterior end of the embryo.





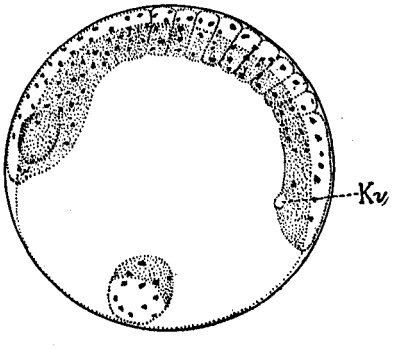
BAIRDIELLA CHRYSURA.

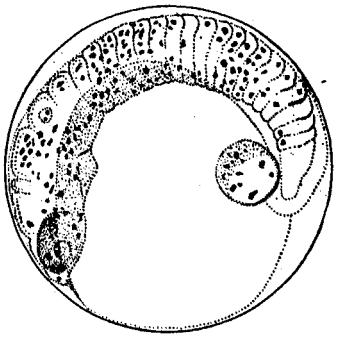
FIG. 12.—Early embryo showing distribution of chromatophores, dorsal view. × 55.

FIG. 13.—Early embryo showing distribution of chromatophores; *Kv*, Kupffer's vesicle. × 55. Lateral view.

An hour later (fig. 14) the chromatophores have become more numerous and are distributed more or less uniformly over the entire dorsal and lateral surfaces of the embryo. Kupffer's vesicle has now reached its maximum development. After this it gradually decreases in size until it disappears. The length of the embryo now exceeds half the circumference of the yolk sphere and shows 10 to 12 somites.

As development advances and the time of hatching approaches, the distribution of the chromatophores undergoes a material change. A few hours before hatching the





BAIRDIELLA CHRYSURA.

FIG. 14.—Egg with embryo showing 10 somites; *Kv*, Kupffer's vesicle. × 55.

FIG. 15.—Egg with advanced embryo. × 55.

embryo becomes quite active within the egg membrane. The posterior portion of the body is now free from the yolk sphere and narrow fin folds are apparent both dorsally and ventrally (fig. 15).

Larval development.—At the time of hatching the larval fishes are 1.5 to 1.8 mm. in length. The head is slightly deflected at the anterior end of the large oval yolk sac.

The oil globule appears as a yellowish opaque body on the surface of which are scattered a few yellow chromatophores. It is located in the posterior region of the yolk sac. The fin folds are continuous. The dorsal fold arises just posterior to the head; the ventral fold is continuous with the yolk sac. The depth of each fin fold is less than the depth of the body. The body is brownish yellow, marked by five vertical yellow bands. These vertical bands are composed of more or less closely aggregated chromatophores. A few scattered chromatophores occur also between the vertical bands. The fin folds and the posterior tip of the body are transparent. Figure 16 illustrates a larval fish about two hours after hatching.

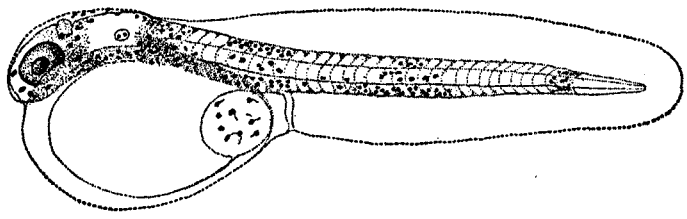


FIG. 16.—*Bairdiella chrysura.* Newly hatched fish, actual length 1.8 mm.

For some time after hatching the general color of the body remains unchanged. The distribution of the yellow chromatophores, however, undergoes marked changes. Five hours after hatching (fig. 17) the vertical bands have become broken up. A distinct vertical yellow band remains located approximately two-thirds the distance from the vent to the posterior end of the body. Another less distinct vertical band occurs just posterior to the head. Groups of scattered chromatophores occur in the head region and above the vent. A few more or less isolated chromatophores occur also on the posterior half of the body.

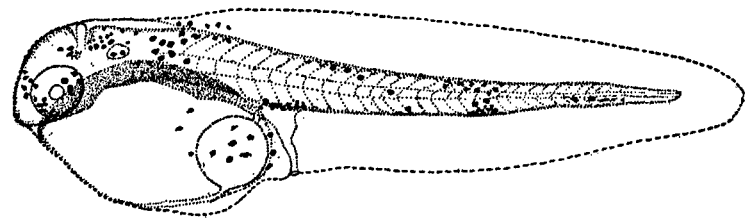


FIG. 17.—*Bairdiella chrysura.* Larval fish 4 to 5 hours after hatching, actual length 2 mm.

At one day after hatching (fig. 18) the young fish has grown to a length of 2.4 to 2.6 mm. A small mass of yolk remains unabsorbed. The head is no longer deflected, but slightly elevated. The body is distinctly flattened. The greatest depth of the body occurs posterior to the head. From this point the body tapers gradually toward the posterior end. The depth of each fin fold is greater than the depth of the posterior half of the body. The general color of the body remains brownish yellow. The fin folds and the posterior one-fifth of the body remain transparent. The yellow chromatophores have become fewer. The posterior vertical band now consists of a dorsal and a ventral group of chromatophores. There is no distinct vertical band in the anterior region at this stage, but a few yellow chromatophores remain scattered over the head and the anterior region of the trunk.

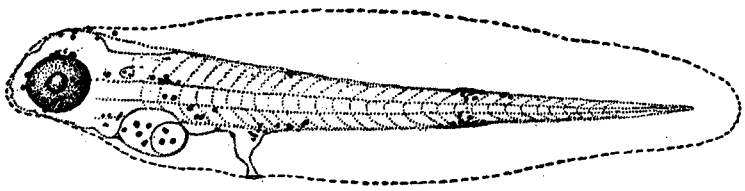


FIG. 18.—*Bairdiella chrysura.* Larval fish 1 day after hatching, actual length 2.5 mm.

During the following day the larval fishes do not increase in size materially. They undergo material changes in form and color, however. At two days after hatching (fig. 19) they remain 2.5 to 2.8 mm. in length. The yolk is completely absorbed. The depth of the head is now greater than the depth of the body. The fin folds remain continuous and the depth of each fold remains greater than the depth of the body posterior to the vent. The general color of the body is light brownish yellow, marked by two distinct vertical bands. The anterior vertical band is located just posterior to the head. It is composed of yellow chromatophores on a blackish background. The general macroscopic effect of this band is blackish. The posterior vertical band is located approximately two-thirds the distance from the vent to the posterior end of the body. It is composed of a dorsal and a ventral group of yellow chromatophores on a diffuse blackish background. The macroscopic effect of this band is yellowish. Yellow chromatophores no longer appear on other parts of the body. The fin folds and the posterior end of the body remain transparent.

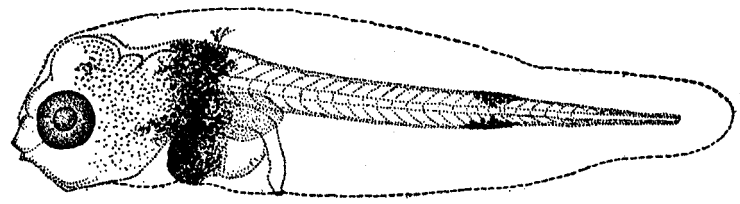


FIG. 19.—*Bairdiella chrysura.* Larval fish 2 days after hatching, actual length 2.6 mm.

The critical period for these larvæ begins during the third day after hatching. When kept in dishes of sea water they began at this time to die rapidly. Few survived until the fourth day. Means of keeping the larvæ alive for a longer period was not available. Observations on the later larval development, therefore, were made on larval fishes taken alive in the stow net.

After the critical period is passed the little fishes feed actively and probably grow comparatively rapidly. Figure 20 illustrates a young fish 3.5 mm. in length. The relative depth of the body in fishes of this size is materially greater and the trunk tapers more rapidly toward the posterior end than in larvæ which have not yet passed the critical period. The posterior end of the notochord is slightly elevated. The posterior end of the body is asymmetrical and betrays an ancestral heterocercal condition of the tail. The fin folds remain continuous. The depth of each fold is now less than the depth of the body posterior to the vent. The general color of the body is somewhat lighter than in the earlier larvæ. Both vertical bands are distinctly blackish. Yellow pigment is still present in the vertical bands, but is obscured by the denser blackish ground color. From the anterior vertical band two blackish bands extend antero-ventrally. One of these blackish bands terminates in proximity with the eye, the other extends diagonally over the preopercle and cheek. The posterior vertical

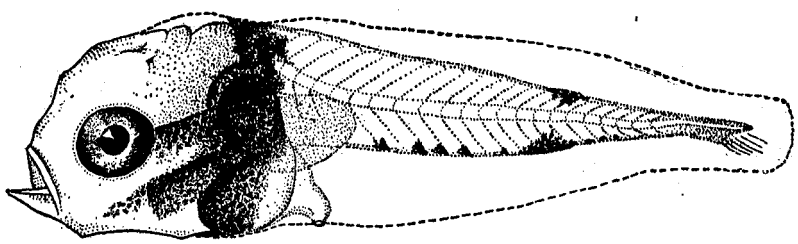


FIG. 20.—*Bairdiella chrysura.* Larval fish 3.5 mm. in length.

band is composed of a dorsal and a ventral pigmented area. These two areas are now so widely separated that in lateral view the band no longer appears continuous. Several blackish pigment spots occur also along the ventral margin of the body between the vent and the posterior vertical band.

Larval fishes 5 mm. in length (fig. 21) retain the same general form as the one 3.5 mm. in length above described. The posterior end of the notochord is curved upward more strongly and the heterocercal character of the tail is more apparent. The general color of the body has changed to silvery gray. The anterior vertical band and

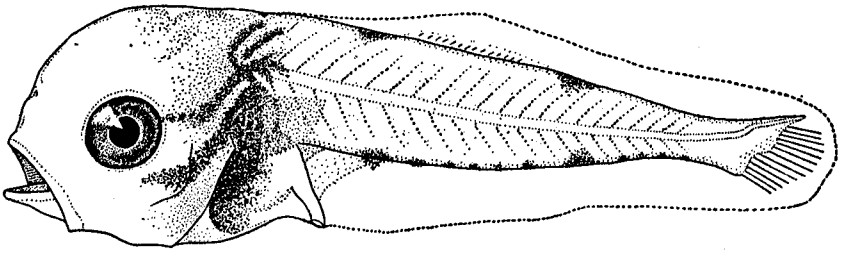


FIG. 21.—*Bairdiella chrysura*. Larval fish 5 mm. in length.

the dorsal and ventral pigmented areas in the region in which in the earlier larvæ the posterior vertical band is located are distinctly blackish. A small dark area occurs dorsally opposite the vent. Several small darkly pigmented areas occur also along the ventral margin of the body posterior to the vent.

As the little fishes grow larger the trunk posterior to the vent becomes relatively deeper until there is no longer an abrupt break in the ventral contour of the body. The caudal end of the body gradually becomes symmetrical dorso-ventrally and the tail assumes its true homocercal character. The general color of the body remains silvery

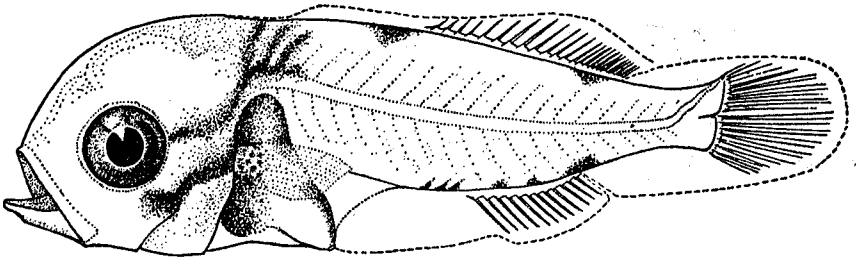


FIG. 22.—*Bairdiella chrysura*. Larval fish 7.5 mm. in length.

gray, distinctly darker dorsally than ventrally. The anterior vertical band and the other darkly pigmented areas are retained until the little fishes have grown to a length of 8 to 9 mm. After this they gradually disappear. In fishes 11 to 12 mm. in length (fig. 23) there remain only traces of these pigmented areas.

After the little fishes have attained a length of 7 to 8 mm. (fig. 22) they rapidly assume the general form and appearance of the adult individuals of the species. In fishes 10 to 12 mm. in length (fig. 23) the fins are well differentiated and the full numbers of fin rays are already present. Fishes of this size have the general

appearance of adult individuals. However, the depth of the body in the thoraxic region is relatively great and the head is relatively large and blunt. They are also somewhat lighter in color.

Figure 24 illustrates a young fish 30 mm. in length. The fins are now fully differentiated and the entire surface of the body is covered with scales. However, the scales are still small and deeply embedded in the skin. They are, therefore, not

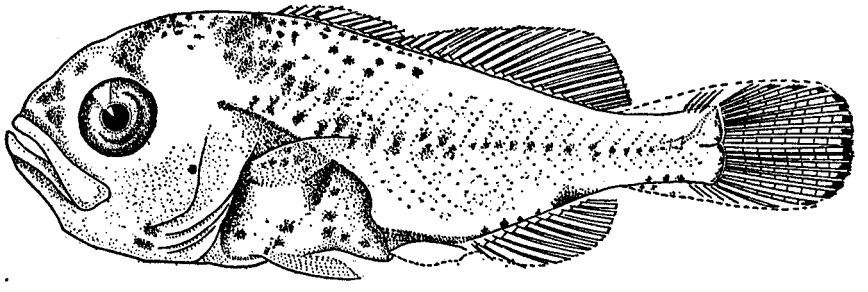


FIG. 23.—*Bairdiella chrysura*. Larval fish 11 mm. in length.

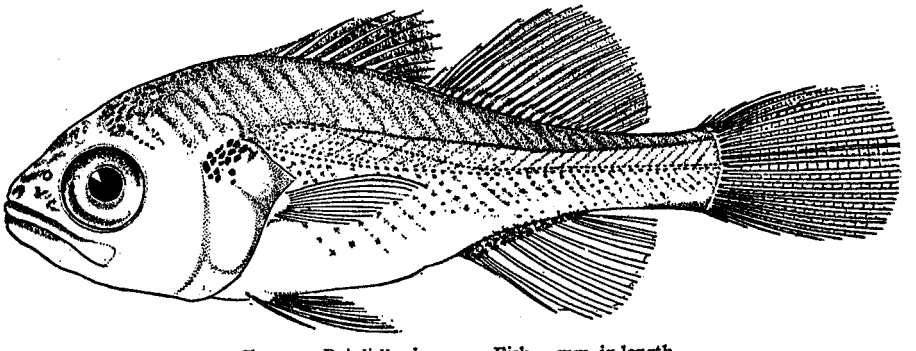


FIG. 24.—*Bairdiella chrysura*. Fish 30 mm. in length.

shown in the drawing. In form and color fishes of this size are practically identical with adult individuals. In short, they show all the diagnostic characters of the species.

ANCHOVIA MITCHILLI.

Spawning.—The eggs of *Anchovia mitchilli* were present in the plankton when work was begun on June 9, and were collected in the tow net nearly every day after that date until August 23, when the work was discontinued. During the second and third weeks in June the eggs of this species were not abundant, though they were sufficiently numerous to be readily obtained for study. Toward the close of June they became numerous, and they were much more abundant in the plankton during July and August than the eggs of any other fishes spawning during these months. The height of the spawning season is probably reached during July.

As already indicated, this species, like *Bairdiella chrysura*, spawns regularly in the early evening, probably before 8 o'clock p. m. On a few occasions newly spawned eggs were collected before 6 o'clock p. m. Usually, however, no newly spawned eggs were

taken before 8 o'clock p. m. Eggs were found occasionally in the early cleavage stages as late as 9.30 o'clock p. m. Newly spawned eggs were taken in the tow net alike on the flood and the ebb tides.

Eggs.—The eggs of this species are not spherical, but slightly elongated. The major axis, which is 0.65 to 0.75 mm. in length, is 0.1 to 0.3 mm. longer than the minor axis. These eggs are almost perfectly transparent and contain no oil globule. Furthermore, the yolk is composed of separate masses. It has the appearance under the microscope of being broken up into large cells. As observed by Wenckebach [a] in 1886 and later by other European naturalists, the elongated form of the egg and the segmented character of the yolk is characteristic also of the European anchovy (*Engraulis encrasicholus*). The eggs of this species, however, are somewhat larger than the eggs of *Anchovia mitchilli*. The difference in length of the major and the minor axes in the eggs of the former species also is considerably greater. According to Heincke and Ehrenbaum [b] (1900), the greater diameter of the eggs of the European species is 1.1 to 1.5 mm., and the lesser 0.7 to 0.9 mm. These measurements approximate very closely the dimensions of the eggs of the American species, *Anchovia brownii*.

Eggs in advanced stages of development and newly hatched larvæ were rarely taken in the tow net at the surface of the water. This fact suggests that before the time of hatching the specific gravity of the eggs is increased sufficiently to cause them to sink. This conclusion is verified by the results of experimental observations. Eggs placed in a dish of sea water 12 to 16 hours after fertilization float at the surface for several hours and then sink to the bottom of the dish. After hatching the larval fishes may be found at any level in the dish. The eggs of this species are very delicate. When placed in a dish of sea water many die before hatching. All the eggs alike, however, sink to the bottom before any are hatched.

Embryology.—The eggs of *Anchovia mitchilli*, like those of *Bairdiella chrysura*, develop in a manner typical for pelagic teleostean eggs, and the development differs from that of Bairdiella only in a few unimportant details. The embryological development of *Anchovia mitchilli* will therefore be discussed but briefly and with reference to the above discussion of the embryology of *Bairdiella chrysura*.

As indicated above, the eggs of *Anchovia mitchilli* are not spherical, but slightly elongated. As the thin protoplasmic layer investing the yolk becomes concentrated to form the blastodisc, the protoplasm "streams" toward one pole of the major axis. When fully differentiated the blastodisc appears as a lenticular cap of protoplasm lying on the somewhat flattened lower end of the yolk mass. The periphery of the blastodisc fades away almost imperceptibly into the very thin layer of protoplasm which remains at the surface of the yolk. Between the thin egg membrane and the delicate vitelline membrane there is now a perceptible perivitelline space.

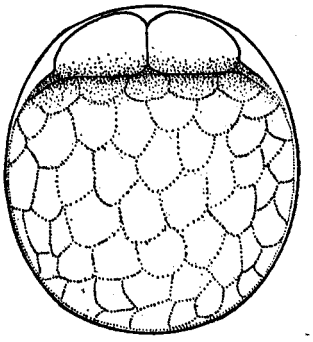
Cleavage in these eggs advances with great regularity. It conforms in all essential details to the process of cleavage, as above recorded, in the eggs of *Bairdiella chrysura*. In many instances the early blastoderms in these eggs are even more symmetrical than in the eggs of the latter species. Early blastoderms which are quite typical of the eggs

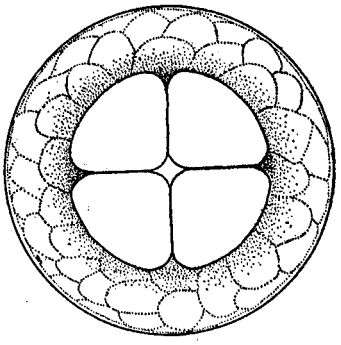
[a] Wenckebach, K. F.: De embryonale outwikkeling van de ansjovis (*Engraulis encrasicholus*). Verhandeling der Kaiserlichen Akademie van Wetenschappen. 1887.

[b] Heincke, Fr., und Ehrenbaum, E.: Eier und Larven von Fischen der Deutschen Bucht. II. Die Bestimmung der schwimmenden Fisheier und die Methodik der Eimessungen. Wissenschaftliche Meeresuntersuchungen, n. f., bd. III, Abteilung Helgoland, 1900, p. 127–332, taf. IX–X.

of *Anchovia mitchilli* are illustrated in figures 25, 26, and 27. Figure 28 illustrates an egg in an advanced stage of cleavage in which the marginal cells of the blastoderm are already cut off from the periblast. Eggs in this stage of development were usually observed between 11 and 12 o'clock p. m.

The germ ring (fig. 29, *gr.*) and the embryonic shield (fig. 30, *es*) are differentiated in the manner described above in the eggs of *Bairdiella chrysura*. Soon after the germ



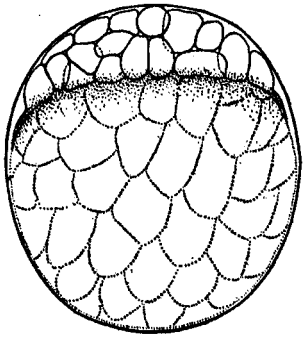


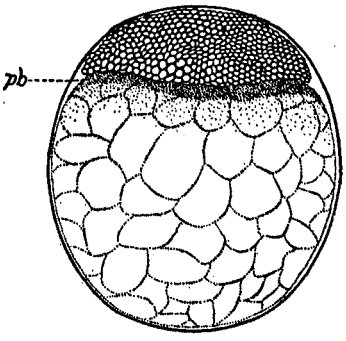
ANCHOVIA MITCHILLI.

FIG. 25.—Egg with blastoderm of 2 cells, lateral view. × 60.

FIG. 26.—Egg with blastoderm of 4 cells, surface view. × 60.

ring is fully differentiated the blastoderm begins to grow around the yolk more rapidly than in the earlier stages. The posterior pole of the blastoderm, however, does not remain at a relatively fixed point, as is the case in many teleostean eggs, but recedes as the anterior pole advances. As the blastoderm grows around the yolk, therefore, its center remains at one pole of the major axis of the egg. The blastopore finally closes at the opposite pole (fig. 34, *bl*). When the embryo is fully differentiated, therefore, it lies approximately parallel with the major axis of the egg (fig. 35).





ANCHOVIA MITCHILLI.

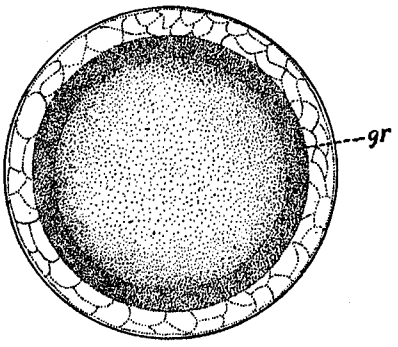
FIG. 27.—Egg with blastoderm of 32 cells, lateral view. × 60.

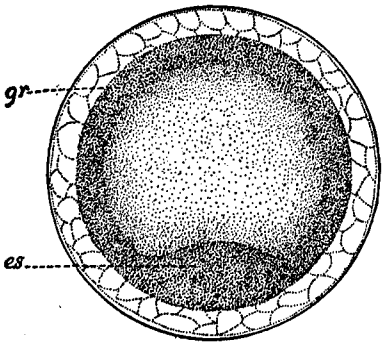
FIG. 28.—Egg with blastoderm in advanced stage of cleavage; *pb*, periblast. × 60.

In the majority of the eggs observed the blastopore closed between 4 and 5 o'clock a. m.—i. e., approximately 10 hours after spawning. At this time the length of the embryo is somewhat greater than half the greater circumference of the egg. Soon after the closure of the blastopore, Kupffer's vesicle arises as a bubble-like body on the ventral aspect of the embryo near its posterior extremity (fig. 35, *Kv*). The vesicle soon reaches its maximum development and then gradually decreases in size until it disappears.

After the closure of the blastopore the embryo increases in length until it extends more than two-thirds around the greater circumference of the yolk (fig. 36). In some instances, before the time of hatching, the embryo extends entirely around the circumference of the yolk.

Larval development.—The time required for hatching, as already indicated, is approximately 24 hours. Hatching usually occurs between 6 and 9 o'clock p. m. The



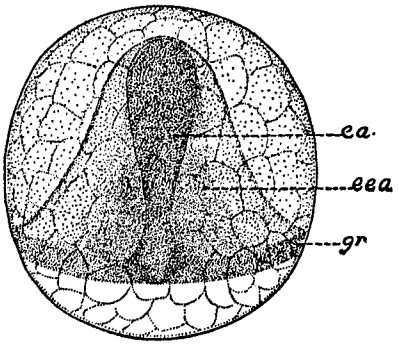


ANCHOVIA MITCHILLI.

FIG. 29.—Egg with blastoderm, showing early germ ring (*gr*). × 60.

FIG. 30.—Egg with blastoderm, showing fully developed germ ring (*gr*) and beginning of embryonic shield (*es*). × 60.

newly hatched larvæ (fig. 37) are 1.8 to 2 mm. in length. The yolk sac, which remains comparatively large, is greatly elongated and tapers to a point posteriorly. The segmented character of the yolk, already noted in the egg, is still apparent. The head of the young fish is deflected at the anterior end of the yolk sac. The body is appreciably flattened and comparatively slender. The fin folds are continuous. The depth of



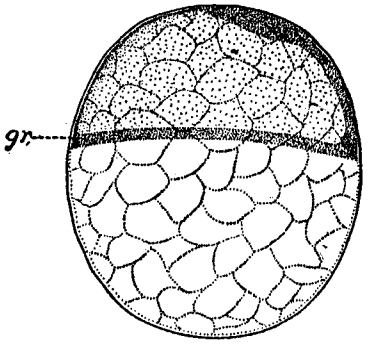


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FIG. 31.—Egg showing advanced stage in development of embryonic shield (*es*), embryonic area (*ea*) outlined. × 60.

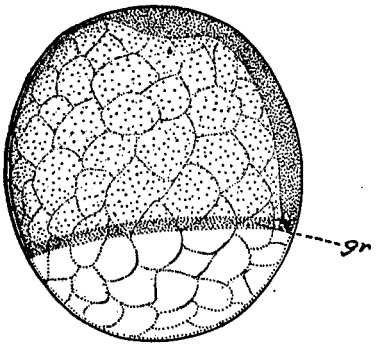
FIG. 32.—Same as figure 7, lateral view; *gr*, germ ring. × 60.

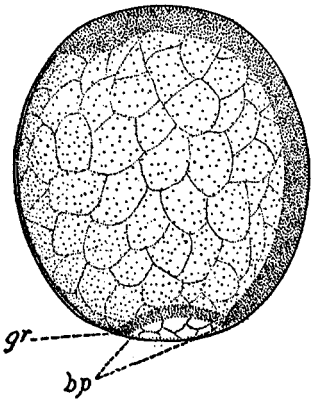
each fin fold is less than the depth of the body. The larval fish is almost perfectly transparent and shows no evidence of pigmentation.

At 12 hours after hatching (fig. 38) the larval fish has grown to a length of 2.6 to 2.8 mm. The remaining yolk mass retains its elongated form and its segmented character. The head of the young fish is no longer deflected.

The yolk sac decreases in size until at 15 to 18 hours after hatching it is completely absorbed. For some time after the yolk is absorbed the larval fishes increase in size

very slowly. Nor do they undergo any material changes in form or appearance. They are relatively long and slender and highly transparent. At 36 hours after hatching (fig. 39) the mouth is apparently functional and soon begins to show the form character-



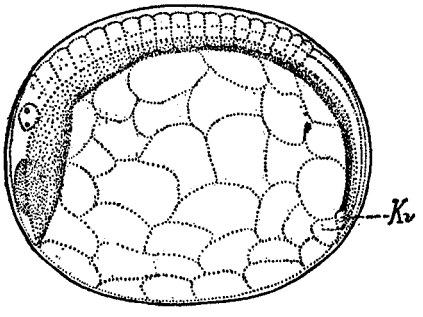


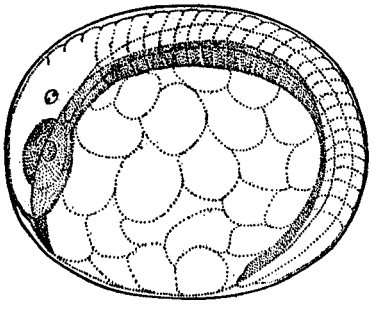
ANCHOVIA MITCHILLI.

FIG. 33.—Egg showing blastoderm spreading over yolk; *gr*, germ ring. × 60.

FIG. 34.—Egg showing blastopore nearly closed; *bp*, blastopore; *gr*, germ ring. × 60.

istic of anchovies. The maxillaries are comparatively long. The lower jaw is long and narrow. The tip of the head, however, does not as yet extend forward beyond the mouth.





ANCHOVIA MITCHILLI.

FIG. 35.—Egg with embryo showing 18 to 20 somites; *Kv*, Kupffer's vesicle. × 60.

FIG. 36.—Egg with advanced embryo. × 60.

The critical period for the larvæ of this species begins before the close of the second day after hatching. When kept in dishes of sea water many of them died before reaching the third day. Observations on the later larval development were made on larval fishes collected in the stow net.

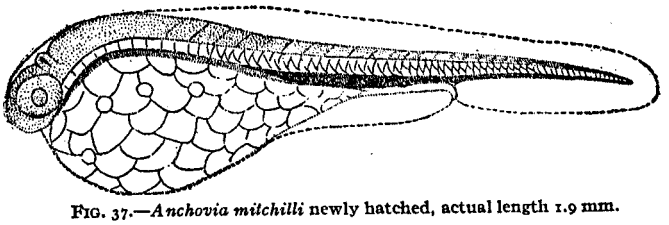


FIG. 37.—*Anchovia mitchilli* newly hatched, actual length 1.9 mm.

Larval fishes 3 to 4 mm. in length (fig. 41) do not differ markedly in appearance from larvæ in which the yolk sac is just absorbed. They retain the same general form and remain almost perfectly transparent. The fin folds remain continuous. Their relative depth, however, has materially decreased.

Fishes 5 mm. in length (fig. 42) illustrate an early stage in the differentiation of the dorsal and the anal fins. In larvæ of this size the posterior region of the intestine is already convoluted. In lateral view these convolutions have the appearance of vertical folds. This character is apparent externally until the little fishes have attained a length of 15 to 20 mm.

In fishes 7 to 8 mm. in length (fig. 43) the dorsal and anal fins are becoming definitely outlined. In some instances the full number of fin rays is already present.

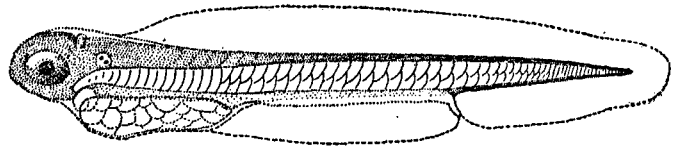


FIG. 38.—Larval fish 12 hours after hatching, actual length 2.7 mm.

FIG. 39.—Larval fish 36 hours after hatching, actual length 2.9 mm.

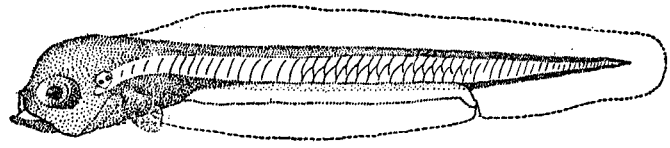


FIG. 40.—Larval fish 3 days after hatching, actual length 2.7 mm.

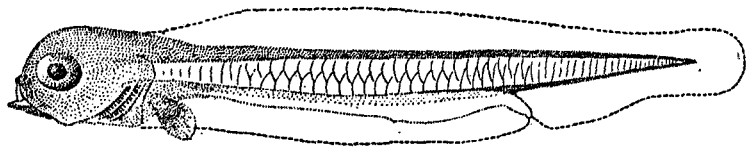


FIG. 41.—Larval fish 3.4 mm. in length.

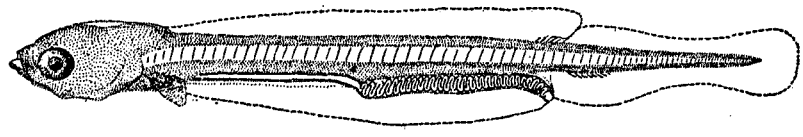


FIG. 42.—Larval fish 5 mm. in length.

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A few small darkly pigmented areas are now apparent along the ventral margin of the body in the thoracic region and at the base of the anal fin.

As the young fishes grow larger they become less transparent, but show very little pigment. They undergo no marked changes in form, but gradually assume the appearance of adult fishes, showing all the diagnostic characters of the species. The silvery, longitudinal band characteristic of adult anchovies, however, does not appear until the young fishes have attained a considerable size.

During the early summer larvæ of *Anchovia mitchilli* and *Anchovia brownii* were frequently taken together. In this stage the two species are very similar and might readily be confused, the larvæ of the latter, however, being somewhat the longer and comparatively more slender. The vent is also located correspondingly farther posteriorly in the latter than in the former. As soon as the dorsal and anal fins have become fully differentiated, the young of either species may be recognized by the character of the anal fin. The number of anal fin rays in *Anchovia brownii* usually does not exceed 20. In *Anchovia mitchilli* the anal fin rays number 25 to 28. In the latter species the anal fin also is longer and terminates less abruptly and nearer the base of the caudal fin than in the former.

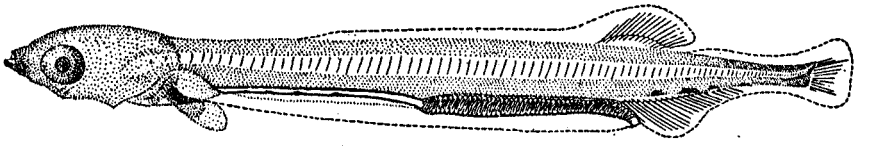

FIG. 43.—Larval fish 7.5 mm. in length.

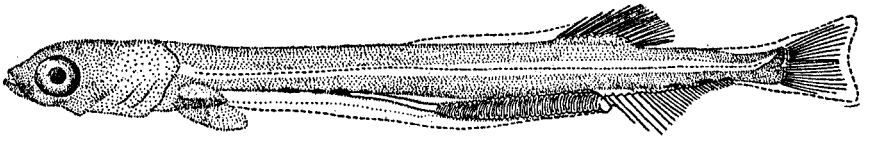

FIG. 44.—Larval fish 10 mm. in length.

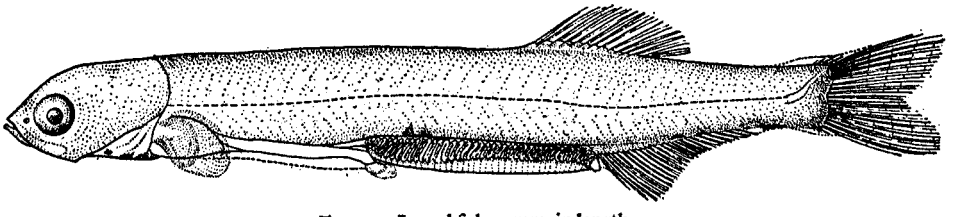

FIG. 45.—Larval fish 15 mm. in length.

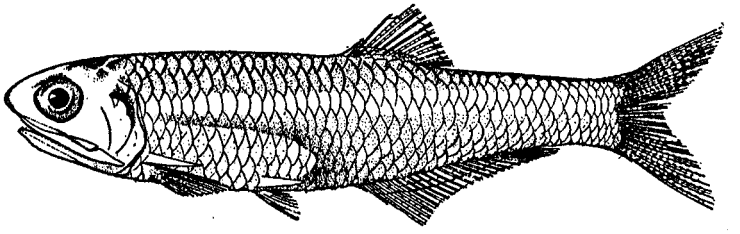

FIG. 46.—Adult fish 7 cm. in length.

ANCHOVIA MITCHILLI.

Figure 46 illustrates an adult fish. The adult of this species does not differ markedly in form and appearance from the adult of *Anchovia brownii*. The average length of the body is somewhat greater and its relative depth is somewhat less in the latter, while the silvery lateral band of *A. mitchilli* is narrower and less distinct than in *brownii*. More distinctive characters are the anal fin, as indicated above, and the position of the vent. In the larvæ of both species the vent is located opposite the middle of the dorsal fin or farther posteriorly. In the adult of *Anchovia mitchilli* the vent is located opposite the origin of the dorsal fin, while in the adult of *Anchovia brownii* the vent is located approximately opposite the middle of the dorsal fin.

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THE SKELETAL MUSCULATURE OF THE KING SALMON

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THE SKELETAL MUSCULATURE OF THE KING SALMON.

By CHARLES WILSON GREENE and CARL HARTLEY GREENE,
Department of Physiology and Pharmacology, Laboratory of Physiology, University of Missouri.

GENERAL ARRANGEMENT OF THE SKELETAL MUSCULATURE.

The general muscular structure of the king salmon has not previously been described. One must therefore be guided by the general descriptions and comparisons as between the musculature of the different parts of the body of the king salmon and that of other fishes of related groups.

The major amount of the muscle mass of the salmon is represented by the great lateral muscles. These extend from the head and pectoral arch on either half of the body straight along the sides to the base of the tail. Each great mass is grossly divided longitudinally into dorsal and ventral portions, vertically into the well-known segments or myomeres. Out of the extreme dorsal portion of the mass certain special longitudinally arranged muscles have been developed. Along the mid-ventral line similar longitudinal differentiations have occurred. In the head region the usual complex differentiations of muscles have taken place. These muscles are undoubtedly derived primarily from the great lateral muscle.

In like manner, the muscle region at the base of the tail, the caudal peduncle, has been differentiated into a number of definite and special muscles which produce the complex movements of the caudal fin.

The pectoral girdle and the pelvic girdle, together with the corresponding fins, have a number of highly specialized muscles. Also the great median fins, the dorsal fin, and the anal fin, each are supplied with a complex group of muscle filaments.

These great groups form the basis of the subdivisions which are followed in this paper in describing the muscles in anatomical detail. In gross outline the groups are as follows:

Muscles of the trunk, the great longitudinal muscles
Muscles of the head region.
Muscles of the caudal fin.
Muscles of the pectoral girdle.
Muscles of the pelvic girdle.
Muscles of the dorsal fin.
Muscles of the anal fin.

MUSCLES OF THE TRUNK, THE LONGITUDINAL MUSCLES.

The longitudinal muscles have been developed out of the great lateral muscle and form the major mass of muscle substance on each side of the body of the king salmon as in other fishes. The extreme anterior portion of the embryonic lateral muscle is differentiated into numerous highly specialized small muscles in the head region, and it is similarly, though less complexly, differentiated in the caudal region also.

The great lateral muscle is subdivided both longitudinally and transversel . Longitudinally the subdivisions are indicated superficially by more or less distinct longitudinal connective tissue areas. The most developed and largest septum however, is indicated by the connective tissue band lying immediately under the lateral line, where a thick septum extends from the under surface of the skin directly down to the lateral-ventral surfaces of the centra of the vertebral column. This septum completely divides the great lateral muscle into dorsal and ventral portions, the division extending from the base of the skull to the middle of the base of the caudal fin. Kingsley, in his Comparative Anatomy of Vertebrates, speaks of these great divisions as the epaxial and hypaxial muscles.

The extreme dorsal portion of the epaxial muscle on each side has become further differentiated by the separation of a definite cylindrical bundle extending from the occiput to the base of the tail, but interrupted at the dorsal fin, and modified at the soft dorsal fin. This muscle is the homologue of Owen's supracarinalis.

That portion of the lateral muscle lying below the lateral line, the hypaxial, has its extreme ventral portion cut off into definite masses which for the most part are cylindrical in form. This portion is the homologue of what McMurrich [a] calls in the catfish "the great ventral muscle," the infracarinalis of Owen. It extends from the gular plate to the base of the caudal fin, but is interrupted at the pelvic girdle and at the anal fin respectively.

The lateral muscles proper are further differentiated into a superficial and a deep portion. These subdivisions are rather intimately bound together at their surfaces of approximation. But in gross anatomical features, in minute histological structures, and in physiological properties, they are so characteristically different that they were considered as distinct muscles. They have been described and given distinctive names by the senior author.[b]

The entire list of longitudinal muscles, including the divisions of the lateral muscles and special differentiations at the mid-dorsal and mid-ventral regions, is as follows:

I. Divisions of the great lateral muscle proper.
  1. Musculus lateralis superficialis.
    a. The epaxial division.
    b. The hypaxial division.
  2. Musculus lateralis profundus.
    a. The epaxial division.
    b. The hypaxial division.

[a] McMurrich, J. P.: The myology of Amiurus. Proceedings of the Canadian Institute, vol. II, p. 330.

[b] Greene, Chas. W.: An undescribed longitudinal differentiation of the great lateral muscle of the king salmon. Anatomical Record, 1913, vol. 7, p. 99–101.

II. Supracarinales, the dorsal longitudinal muscles.
    3. Protractor dorsalis.
    4. Retractor dorsalis.
III. Infracarinales, the ventral longitudinal muscles.
    5. Protractor ischii.
    6. Retractor ischii (protractor analis).
    7. Retractor analis.

DIVISIONS OF THE GREAT LATERAL MUSCLE.

The great lateral muscle, as the term is applied to the adult fish, does not include the dorsal and ventral differentiations given in II and III of the above list. It does, however, include all the muscle mass extending from the base of the skull and the pectoral girdle to the base of the caudal fin except the supracarinales and the infracarinales, respectively. This mass, as just described, is divided longitudinally into four actual divisions. An epaxial and a hypaxial portion is formed by the lateral line septum. Each of these great divisions is differentiated longitudinally into a thin superficial portion and a thick deeper portion as previously indicated. Each of these may now be described in fuller detail.

MUSCULUS LATERALIS SUPERFICIALIS.

This muscle extends over the surface of the deeper division of the great lateral muscle, the profundus, for its full extent from the head to the base of the tail. It is thickest in the mid-lateral line. There are two separate and distinct portions, the epaxial and hypaxial divisions. Each of these divisions forms a thin sheet, becoming thinner as it extends out from the lateral line, dorsally in the epaxial and ventrally in the hypaxial divisions, respectively. The muscle is several millimeters thick in the king salmon in the lateral line region, while its extreme dorsal and ventral borders are represented in thickness by only a few fibers. The dorsal limit of the superficialis is along the line about two-thirds the distance from the lateral line to the mid-dorsal line of the salmon body. The ventral division varies somewhat in its extent. In the anterior portion of the body the superficialis extends only about one-half the distance from the lateral line to the mid-ventral line. In the posterior part of the body the margin of the superficialis extends two-thirds to three-fourths this distance. These epaxial and hypaxial divisions of the superficialis muscle are sharply separated from each other by the lateral line septum.

The muscle as a whole is characterized by a darker appearance than the profundus. The latter is the usual salmon pink color in the well-conditioned fish, though lighter in color in the fish of poorer quality. The superficialis is separated from the profundus by a rather weakly marked sheet of connective tissue. In macerated examples the superficialis can readily be separated from the profundus. On the whole, however, the two muscles are very intimately connected. Histologically the demarcation line is sharp and distinct, but by methods of gross anatomy this line is not so readily determined.

The superficialis has been observed by the senior author in a number of other fishes. In some of these, for example the California sardine, *Clupanodon cæruleus*, this muscle is relatively more highly developed than in the king salmon. In the

literature, however, thus far no previous reference to or description of this differentiation of the great lateral muscle in other fishes has been found other than the sentence of Miescher's quoted below.[a] Miescher speaks of "a thin muscle plate lying along the side of the body just beneath the skin which degenerates strikingly (cutaneous muscle)." I interpret this statement as referring to the lateralis superficialis, though there is nothing else in the context that suggests that Miescher recognized this portion of the lateral muscle as a differentiation out of the total mass. The differentiation is described in part by the papers of the senior author dealing with subjects in salmon anatomy and physiology.[b]

Histologically the superficialis is distinguished from the profundus by its strikingly different type of muscle fibers. The fibers of the superficialis are more compact, more uniform in diameter, and relatively smaller in size than the fibers of the profundus. The fat-storing property of this muscle has been specifically described in a previous paper.[c] Analyses made of this muscle showing the percentage of fat in the fish from the mouth of the Columbia River gave the total of the fat in the fresh wet muscle as high as 30 per cent. In no other muscle of the salmon is such an enormous quantity of fat stored, and especially nowhere else are such quantities stored within the fibers.

MUSCULUS LATERALIS PROFUNDUS.

This muscle forms the major portion of the great lateral muscle as defined above. It extends from the occiput and pectoral girdle to the base of the caudal fin. The muscle is characterized in the fish of first quality especially by its rich pink color. The body of the profundus fills the entire space between the superficialis and skin on the one hand, and the skeletal complex on the other. The two divisions, the epaxial and the hypaxial, are very sharp and distinct for the entire extent of the muscle. The attachments of the muscle are better understood after a discussion of the arrangement of its segments.

The profundus is distinguished from the superficialis always by its characteristic difference in color, as previously referred to. The king salmon in the Columbia River shows an especially rich color in this muscle, though the color fades as the period of starvation progresses during the spawning migration. The form and size of the fibers vary within wide limits while the length of the individual fibers remains more constant. In contradistinction to the superficialis the fibers of the profundus vary in diameter from 25 or 30 to as much as 200 or 250 micra. No such variation in size occurs in the fibers of the superficialis. This characteristic alone is sufficient to diagnose the two muscles.

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[a] Miescher, Friedrich: Statistische und biologische Beiträge zur Kenntniss vom Leben des Rheinlachses im Süsswasser. Schweizerischer Fischerei-Ausstellung zu Berlin, 1880, p. 186. Also reprinted in Histochemische u. Physiologische Arbeiten von Friederich Miescher, 1897, p. 145. Miescher's exact words are: "Am starksten degenerirt eine gesonderte dünne Muskelplatte, die an der Seite des Körpers direct unter der Haut liegt (Hautmuskel)."

[b] Greene, Chas. W.: The storage of fat in the muscular tissue of the king salmon and its resorption during the fast of the spawning migration. Bulletin U. S. Bureau of Fisheries, vol. XXXIII, 1913.

[c] Greene, Chas. W.: A new type of fat storing muscle in the salmon, Oncorhynchus tschawytscha. American Journal of Anatomy, vol. 13, 1912, p. 175–178.

MYOMERES OF THE GREAT LATERAL MUSCLES.

The entire lateral muscle mass, including the superficialis and profundus of both the epaxial and the hypaxial divisions, is subdivided into vertically marked segments, the myomeres (Wiedersheim). The myomeres are separated by connective tissue septa, the myocommata. The septa, and hence the myomeres, are not simple vertical sheaths but are very complexly folded "so as usually to form semiconical masses" (Owen).[a] The surface markings of the septa, forming the borders between the myomeres, present zig-zag lines across the sides of the fish. These septa are not so simple as the surface lines would indicate, as shown in the figure presented (pl. II). From this figure of three myomeres taken from about the middle portion of the body it is obvious that each myocomma as a whole forms a rather complex membrane. Owen has described the form of the myocomma in *Perca fluviatilis* and illustrated the same with a fair figure. Allis [b] figures the surface markings of the anterior portion of the body of *Amia* in his figure 33, the deep folds of the myomeres in figure 34, and the septa after dissecting away the muscles in figure 35, all of the same region. Allis' figures are splendid artistic reproductions of the anatomical facts. The region figured by him is near the pectoral girdle where the myomeres and septal folds are relatively simple.

The form of the myomere and of the septum varies somewhat in different regions of the body but is always complex and intricate. The variations are from one and the same type. In that part of the body from which the figure is taken, in fact also the myomeres of the entire side of the salmon, the surface markings have the general outline of the letter "W" with the bottom of the letter turned toward the tail. The middle limb of the curve coincides with the lateral line. (See pl. I.) For the entire anterior half of the body the myocommata at the mid-line form sweeping curves. At about the anterior border of the anal fin this curve gives way to a point of gradually increasing sharpness. On the caudal peduncle at the lateral line each myocomma makes a sharp pointed union as between the dorsal and ventral halves.

The dorsal or epaxial half of the musculature has the bend in the myocommata directed posteriorly. That portion of the myocomma on the surface between the lateral line and the mid-dorsal bend runs in a sweeping curve, almost vertical at the anterior portion of the body, set at an angle of about 60° under the dorsal fin, about 45° over the middle portion of the anal fin, and about 30° on the caudal peduncle. From the middle of the epaxial muscle to the dorsal margin the myocomma forms a sweeping curve toward the head, at first at an angle of about 50°, then curving until just at the dorsal margin the angle is about 10° to 15°, measured with reference to the lateral line. The line marking the union between the dorsal and dorso-median curves of the myocommata lies about three-fifths the distance from the lateral line to the base of the dorsal fin.

The surface of the ventral half or hypaxial muscle shows similar curves of the myocommata. The median portion very closely follows the angle formed by the ribs along the sides of the abdominal wall. Posteriorly the inclination is ever increasing, reaching its maximum of about 30° at the caudal peduncle.

[a] Owen, Richard: Comparative anatomy and physiology of vertebrates. vol. I, p. 203. 1866.

[b] Allis, Edward Phelps: The cranial muscles and cranial and first spinal nerves in Amia calva. Journal of Morphology, 1897, vol. 12, p. 487–808.

The ventral limb of the hypaxial portion of the myocomma, like the dorsal limb, is very oblique, curving anteriorly. Directly under the pectoral fin this angle is about 70°, in the neighborhood of the ventral fins the angle is about 40°, and between the ventral fin and the caudal fin it varies from 40° to 20° measured with reference to the lateral line. The myocommata are placed most nearly horizontal just above the base of the anal fin.

The form of the septum, i. e., the myocomma, is more clearly shown from plate II if one follows only the outlines of the most anterior of the four myocommata presented, considering primarily the relations of the superficial margin to the deepest margin. The deep margin is in contact with the skeleton and continuous with the median septum or skeletal membrane. Considering the whole septum the superficial zigzag markings are shallow while the zigzag outlines of the skeletal border are deep. In other words, the skeletal boundaries of the septum in the mid-lateral line are attached several centimeters in front of, i. e., cephalad to, the point at which the septum is attached to the skin on the surface. In a similar manner, the skeletal borders of the mid-dorsal and of the mid-ventral portions of the septum are attached back of, that is caudal to, the corresponding superficial borders. Posteriorly, i. e., over the anal fin (pl. 1), this arrangement of the myocommata and myomeres is much more extended in the longitudinal axis of the salmon. When a given myocomma of the posterior surface of the epaxial half of the body is exposed it is seen that the segment ends in a slender wedge directed caudally, the surface in a particular case being 27 mm. farther back, i. e., caudally, than the surface at the mid-line. The deep or skeletal attachment of the same septum was 55 mm. behind the mid-line surface point. Just at the lateral line the deep portion of the septum dips far forward. The septa of the successive myomeres form long slender conical sheaths extending from the under surface of the skin anteriorly down to the skeleton. This distance amounts in the above case to 90 mm.

The significance of this arrangement can be explained only when one keeps in mind that the individual muscle fibers [a] of the myomeres run in lines closely paralleling the axis of the fish. There are many variations from this rule; nevertheless, the general effect is a relation between the muscle fiber and its septa which gives to the latter the effect of tendons. This relation enormously strengthens the whole mechanism of myomeres and septa as a power-producing machine. Figure 1 attempts to show this advantageous arrangement in a diagrammatic way by a somewhat idealized section through the anterior conelike fold just under the lateral line and of the posterior dorsal fold above the lateral line.

The alternate contractions of the great lateral muscles accomplish the propelling of the body forward in the act of swimming. The skeleton is like a great flexible board. The masses of the myomeres of either side are mechanically so knitted into this support by the complex attachments of the myocommata to the skeleton that when a contraction occurs the force of the act is distributed over an unexpected distance along the

[a] Measurements of length of fibers in the myocommata at points on the surface: At the anterior margin of the dorsal fin at the lateral line, 7.2 mm.; at the dorsal mid curve, 6 mm.; in front of the dorsal fin near the dorso-median line, 3.6 mm.; ventrally 3 cm. below the lateral line, 7 mm.; 6 cm. below, 8.2 mm.; fibers running obliquely down and back just in front of the pelvic fin, 5.8 mm.

Measurements just over the anal fin: Dorsal, 2.5 mm.; at the bend, 6.5 mm.; at the lateral line, 8 mm.; deep fibers directly under this region and 1 cm. dorsal to the lateral line the pink fibers measure 2.8 mm. On the ventral line of the muscle apparently the same general variation in length of fibers occurs. At the point where the myocommata run most obliquely just above the base of the anal fin the fibers measure 3 mm.

length of the fish. In the caudal region, for example, this extent is so great that the contraction of a single myomere, should it occur, would bend the skeleton toward that side through an extent of several segments. The longitudinal extent of a myomere in the caudal region, opposite the anal fin, is 12 centimeters, i. e., 15 myomeres, of the muscle. The alternate cone-like folds of the septa mutually support each other. It

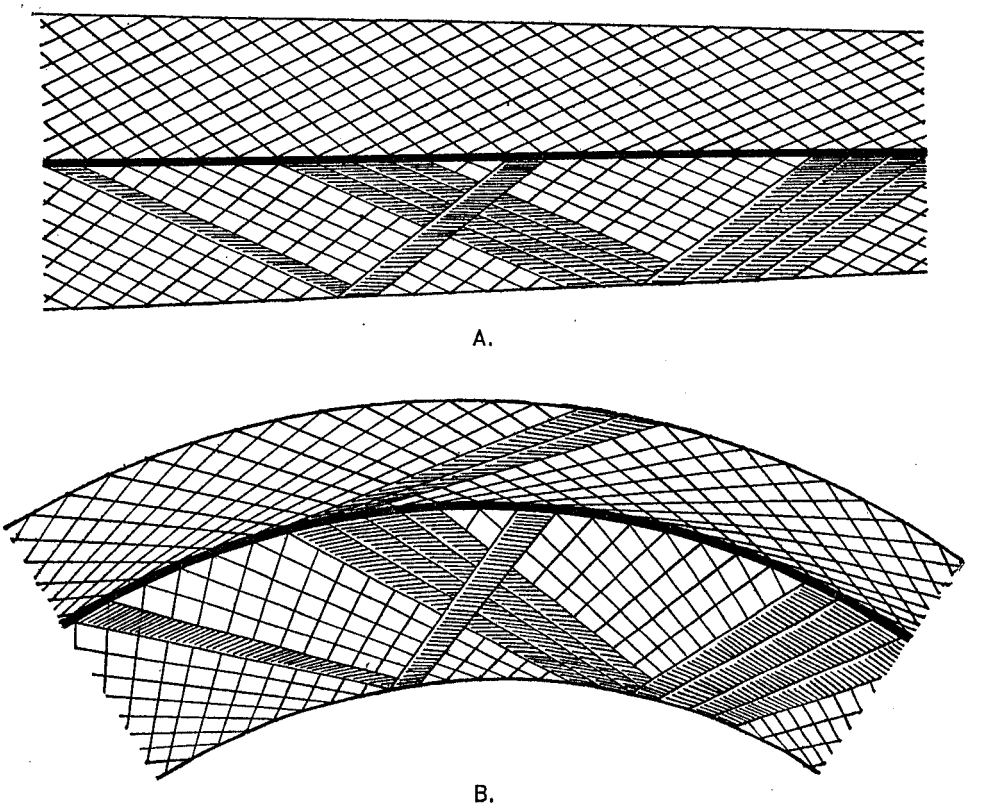

FIG. 1.—Diagrams to illustrate the mechanical relations of the muscle fibers and tendonous septa of the lateral muscles. The diagrams should be considered in comparison with the dissections presented in plates I and II. A, position in rest; B, position during contraction of the left side.

The figures are drawn to represent a composite view of an idealized transverse plane that would cut the individual myomeres and septa through the greatest longitudinal extent. This plane cuts the anterior fold in the median line and the posterior fold through a plane somewhat dorsal to the median line. The posterior folds are less oblique to the skeletal axis than are the anterior folds. More anteriorly the septa will be less oblique, posteriorly more oblique than shown (see pl. 1). This diagram is constructed for the region under the dorsal fin. Note that during contraction of one side the individual fibers on the opposite side are stretched slightly, a condition favorable to the expenditure of contractile energy. Note also that the muscle fibers retain their relatively parallel position with reference to the adjacent skeletal axis. The anterior folds of the septa of the anterior surfaces of the myomeres act as anchors against the posterior folds (dorsal and ventral) of the septa of the posterior surface. As both are inelastic they serve as admirable tendons. Considering the depth of the septa it is obvious that flexion will increase the thickness of the mass of muscle slightly. But the anchoring is such that during flexion parallel septa move or shear over each other in such a way as to produce a maximal amount of movement of the trunk of the salmon by a relatively small amount of muscle fiber shortening, a most advantageous physiological justification of a complex anatomical mechanical relation.

is obvious that the successive septa are very close together and that the fibers from one to the other run very obliquely. In other words, when a contraction occurs every individual fiber is in the best mechanical position to expend all its energy in a much more direct pull on the septal sheet and on the skeleton than would be the case if the myocommata were simple vertical septa placed at right angles to the axis of the fish.

Furthermore, as a contraction progresses and the body of the salmon is sharply curved, i. e., concave, to the side involved, the muscles pull even more directly on the skeleton than at the beginning of the movement, as the figure shows. When the lateral muscles on one side thus bend the ends of the body toward that side, the muscles will pull along the line of oblique attachment of the anterior myocommata on the one hand and the similar attachments of the posterior myocommata on the other, so that these two sheaths serve as direct tendons for the muscle fibers. The arrangement is such that this relation holds for almost every portion of the myomere.

If the septa were simple vertical connective tissue sheaths the mechanical conditions would be wholly changed. In such a case the power expended by the contraction of each myomere would result in a pull on the adjacent myomeres only and from segment to segment and not a direct pull on the skeleton. Only when the great lateral muscles contracted for their full extent would the individual myomere exercise its greatest mechanical possibility. Even then the fibers toward the surface of the myomere would at the time of their maximal contraction soon reach their physiological limit of shortening. The total effect would be to produce tension drawing the superficial part of the muscle away from the skeleton in a relatively inefficient pull. The actual and natural arrangement of the structures in the king salmon is far better and forms a wonderfully efficient and economical mechanical-physiological device.

SUPRACARINALES, THE DORSAL LONGITUDINAL MUSCLES.

Lying along the extreme dorsal margins of the lateral muscles on either side of the body are separate and well developed muscles, the supracarinales. These paired muscles are imbedded in distinct and heavy connective tissue sheaths. In describing the supracarinales the muscles should be considered as made up of two divisions: (1) That portion between the scapula and the anterior portion of the spinous dorsal, and (2) that portion between the posterior margin of the spinous dorsal and the caudal fin. This latter is sharply divided into an anterior and posterior division by the soft dorsal. These two muscle divisions acting together tend to flex the body in the dorso-ventral plane, which in the salmon would seem to be their chief function. Acting separately, each division may be assumed to move the spinous dorsal fin, the first division forward, i. e., in protraction, the second division backward, in retraction. From this latter function the homologous muscles in other fishes have received their names and these names are used here

PROTRACTOR DORSALIS.

This relatively strong muscle extends from the dorsal end of the scapula to the anterior margin of the spinous dorsal and is about 25 cm. long in an 80 cm. salmon. Anteriorly the fibers of the muscle are spread out into a relatively broad fan-shaped mass about 2.5 cm. in width. The mass of the muscle is correspondingly thin in this region. From the middle to the posterior end of the muscle the outline is almost circular, the fibers forming a distinct strong cylindrical bundle even up to the point of insertion. The diameter of this cylindrical mass is from 8 to 10 mm. in an 80 cm. standard fish. Each muscle lies in a tendinous sheath (one on either side of the mid-line of the body). The sheath is less strongly developed anteriorly. The different relations of the walls of

this sheath are as follows: Superficially there is a relatively thin connective tissue sheath separating the muscle from the skin covering it. This portion is heavily loaded with fat. On the ventral surface of the muscle is a thick septum extending from the skin to the median septum into which it is strongly knitted. The median wall is formed by the superior portion of the median septum, in which are imbedded the interneurals of the skeleton. The outlines of a cross section of the sheath are irregular though approximately circular, the outlines being slightly flattened where the septum is strongly developed.

The protractor dorsalis is segmental in the arrangement of its constituent fibers. Connective tissue septa, the homologues of the myocommata of the lateralis superficialis and profundus, extend through the muscle but in an irregular and complexly folded way. In other words, the septa are not simple transverse membranes, but form cone-like spirals. The fibers composing them are strongly interlaced producing in effect a tendinous skeletal framework in which the muscle fibers are imbedded.

The attachments of the muscles are as follows: The anterior end is attached into the posterior margin of the dorsal end of the scapula and by a strong superficial aponeurosis into the skin over the scapula and occiput. This fascia extends forward to the occipital and temporal bones. The tendinous fibers of the posterior end of the protractor are knitted into the anterior and superior margins of the two or three interneurals lying under and supporting the most anterior rays of the dorsal fin. However, all along the median border of the muscle tendinous slips are strongly inserted into the median septum and the interhemals imbedded in this portion of the median septum.

Contractions of the protractor fibers produce traction not only as between the dorsal fin and the occiput, but all along the line of the dorsal margin of the median septum. The whole mechanical effect of the attachments is more favorable for the production of a strong dorsal flexion of the body of the fish than for a protraction of the dorsal fin.

RETRACTOR DORSALIS.

That portion of the supracarinalis lying between the posterior margin of the spinous dorsal and the superior margin of the caudal fin receives the name of retractor dorsalis. This muscle is a cylinder in form. The anterior attachment is by a short tendon inserted into an irregular shaped vertical plate which forms a joint with the last interneural spine, the spine lying under the most posterior dorsal ray. The plate is a modified and enlarged free end of an interneural to receive the tendon of the retractor. The posterior tendon of the retractor is rather broadly attached to the connective tissue enclosing the dorsal ends of the interneural spines of the caudal peduncle which lie under and support the dorsal rudimentary rays of the caudal fin.

The retractor does not seem to be so intimately knitted into its division of the median septum as in the case of the protractor; it is, indeed, free for most of its course. The fact that the muscle is relatively short and smaller in its absolute size than the protractor is probably associated with a development which has separated it from the median septum. That portion of the retractor lying between the soft dorsal and the caudal fin is very slender, 2 or 3 mm. only in diameter. Under the soft dorsal the muscle is wholly tendinous and is closely attached to the base of the fin. Possibly it would be better to consider the two divisions as distinct muscles separated by the soft dorsal.

INFRACARINALES, THE VENTRAL LONGITUDINAL MUSCLES.

Longitudinal muscles lie along either side of the mid-ventral line. These muscles are the homologues of Owen's infracarinales and of McMurrich's fifth portion of the lateral longitudinal musculature. The muscle mass extends from the basibranchiostegal plate to the base of the caudal fin. It is sharply separated from the surrounding muscles for all of its extent except the anterior portion for about one-fourth of its extent.

The infracarinales in the king salmon are divided into three portions, by the interposition of the pelvic arch and of the anal fin. These portions can be described under the names of the protractor ischii, the retractor ischii (protractor analis), and the retractor analis.

PROTRACTOR ISCHII.

This term has been given by Owen to the anterior portion of the infracarinalis. In the king salmon this muscle division extends from the anterior margin of the pelvic arch to the posterior margin of the basibranchiostegal plate, the paired muscles lying on either side the mid-ventral plane. For the greater portion of its length the protractor ischii is inclosed in a cylindrical connective tissue sheath which contains a relatively large amount of adipose tissue. In the mid-line between the two muscles the adjacent portions of this connective tissue sheath form a pretty definite ventral median septum. In the anterior third of the muscle this sheath is less definite and in most specimens scarcely continuous for the full length. In this part of the muscle the form of the muscle as a whole ceases to be cylindrical. The myomeres are not definitely separated from those of the lateral muscle, and the septa are more or less continuous with those of the neighboring lateral muscle. This portion of the protractor is spread out into a slight spatula-shaped terminal mass in the region ventral and anterior to the pectoral fin. The protractor ischii is composed of myomeres, relatively simple in arrangement in the anterior third, and becoming more and more complexly folded into a sort of spiral toward the posterior end of the muscle. In an 80 cm. salmon the diameter of the most cylindrical portion of the muscle varies from 8 to 10 mm., i. e., just in front of the symphysis of the ischii.

The protractor ends in a conical tip which is inserted into the fascias of the skin and of the ventral fin muscles, the median septum, and the antero-ventral border of the ischium itself. The tendons of insertion are formed by the ends of the whorls of myocommatous connective tissue. These are best exposed by a median incision through the skin ventral to the protractor ischii itself.

Contractions of this muscle accomplish two functions. If the axis of the body is rigidly fixed by the action of other muscles then this muscle merely pulls the pelvic girdle forward. It is from this action that it receives its name. However, it seems that a more important function is found in a second action, namely, a strong ventral flexion of the body. Then, too, in the spawning act, if one is to judge by external appearances, the protractor ischii contributes sharply to the pressure that is brought upon the abdominal cavity and which produces the extrusion of the eggs.

RETRACTOR ISCHII (PROTRACTOR ANALIS).

The retractor ischii consists of a cylindrical muscular slip which extends from the posterior end of the pelvic arch directly caudalward and around the anal aperture to be inserted with its fellow into a special bony triangle at the base of the anal fin.

The relation of the pair of muscles and their insertion into this triangle is shown in figures 2 and 8. This bony triangle in its normal position in the body rests directly under and indirectly supports the most anterior rays of the anal fin, with which it is strongly connected by connective tissue fascias. It is a modified interhemal.

The contractions of this division of the infracarinalis contribute to the ventral flexion of the body. It does this by fixing both the anal fins and the pelvic fins. When other muscles are relaxed so that these fins are movable the action of the muscle is to produce retraction of the ventral fins, i. e., the pelvic girdle. If this latter arch is fixed then protraction of the anal fin results, a movement by which the muscle may well receive the alternate designation of protractor analis.

RETRACTOR ANALIS.

On either side of the mid-ventral line of the caudal peduncle lies a slender cylindrical muscle, the retractor analis. The muscle is oval in cross section, about 4 mm. broad by 2.5 mm. thick in an 80 cm. salmon. The fibers run up under the tendinous end of the most posterior erector muscle of the anal fin, to be attached by a broad tendon into the posterior margin of the modified cartilage which supports the most posterior rays as previously described and indicated in figure 14. When the skin is removed and all the muscles are in place this muscle has the appearance of running into the angle formed by the lateral muscles and the posterior margin of the fin.

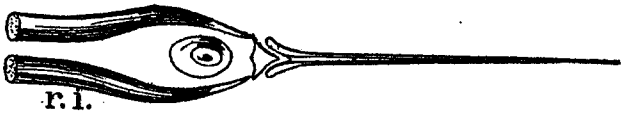

FIG. 2.—An antero-ventral view, somewhat diagrammatic, of the relation of the anterior rays of the anal fin, the supporting triangular cartilage, and the insertions of the pair of retractor ischii, i. e., protractor analis muscles. The figure shows only indistinctly that the three anal rays appear one behind the other, the most anterior of course the shorter.

Posteriorly the muscle runs under the bases of the ventral aborted rays of the caudal fin to be attached into the connective tissue and fascias and the ends of the hemal spines. The muscle is slightly conical in shape, becoming more slender posteriorly. It is only 3 or 4 mm. in diameter at its thickest part.

The function of the muscle is that of retraction of the anal fin, but the muscle is so slightly developed that it can not produce extensive motion.

MUSCLES OF THE HEAD REGION.

The muscles of the head region may be grouped and discussed under the following heads:

A. Superficial dorsal head muscles.
   1. Adductor mandibulæ, (*a*) cephalic portion; (*b*) mandibular portion.
   2. Levator arcus palatini.
   3. Dilatator operculi.
   4. Levator operculi.
B. Deep dorsal head muscles.
   5. Adductor operculi.
   6. Adductor arcus palatini.

C. Dorsal branchial arch muscles.
   7. Levatores arcuum branchialium.
   8. Interarcualis dorsalis obliquus, posterior.
   9. Interarcualis dorsalis obliquus, anterior.
   10. Adductor arcuum branchialium, anterior.
   11. Adductor arcuum branchialium, posterior.
   12. Transversi dorsalis, anterior.
   13. Transversi dorsalis, posterior.
D. Ventral branchial arch muscles.
   14. Interarcuales ventrales obliqui.
   15. Transversi ventralis, anterior.
   16. Transversi ventralis, posterior.
   17. Pharyngo-clavicularis externus.
   18. Pharyngo-clavicularis internus.
E. Mandibular and hyoid arch muscles.
   (18b. Adductor mandibulæ, mandibular portion.)
   19. Intermandibularis.
   20. Geniohyoideus.
   21. Hyohyoideus.
   22. Sternohyoideus.

SUPERFICIAL DORSAL HEAD MUSCLES.

ADDUCTOR MANDIBULÆ (THE MASSETER OF AGASSIZ, OR RETRACTOR ORIS OF OWEN).

This is the largest muscle in the head. It forms the fleshy mass just posterior to the eye which for its delicacy of flavor the fishermen prize under the name "salmon cheeks."

The adductor mandibulæ is in two divisions that are almost though not quite distinct and separate. There is a cephalic division above the angle of the jaw, and a mandibular portion lying chiefly below and along the inner border of the dentary.

The cephalic division of the adductor is in old specimens often more or less indistinctly separated into three divisions, as described by Allis[a] for *Amia*. These divisions are, however, not bounded by more than the thinnest of endomysial membranes and are intimately fused toward the tendon of insertion. In fact they are of lesser importance and scarcely justify the dignity of special designation. The cephalic division will therefore be described as a whole.

(*a*) *The cephalic division of the adductor mandibulæ* has an extensive surface of origin which includes (1) the anterior border of the preopercle for most of its extent, (2) the entire surface of the quadrate, (3) the metapterygoid, and (4) the hyomandibular. Some fibers arise (5) from the connective tissue sheath covering the levator arcus palatini and from the post-orbital septum. The muscle fibers converge in a sweeping curve or general fan-shaped whorl in the dorso-ventral direction to their attachment in the broad tendon at the angle of the jaw. The extreme posterior fibers run anteriorly and somewhat downward toward the ventral margin of attachment. This division of the muscle is attached by a short, heavy, rather broad tendon into the outer margin of the posterior part of the articulare. The tendon is intimately fused with the connective tissue that binds the articulare with the premaxillary and the quadrate bones.

[a] Allis, Edward Phelps, loc. cit.

The cephalic portion of the muscle in the medium sized fish is about 4 cm. broad in the anterior posterior extent and about 5.5 cm. in the dorso-ventral dimension. The thickness is from 1 to 1.5 cm.

(*b*) *Mandibular portion of the adductor.*—Besides the cephalic portion of the adductor there is a stout mandibular portion. It arises from the anterior border of the tendon over the quadrate bone and the angle of the mouth. It extends anteriorly to an attachment along the inner surface of the middle third of the dentary, i. e., from a point directly below the angle of the mouth forward to a point on the jaw. At the origin of this portion the fibers are continuous with the fibers of the cephalic portion. From the origin the fibers diverge slightly as they are distributed to their attachments on the dentary. The lower margin of the muscle takes a continuous attachment along the under and inner surface of the bone. The upper and outer side of the muscle remains free from attachments.

The contraction of the adductor closes the mouth with great power. In addition to its function in feeding it undoubtedly takes part in the motions of respiration.

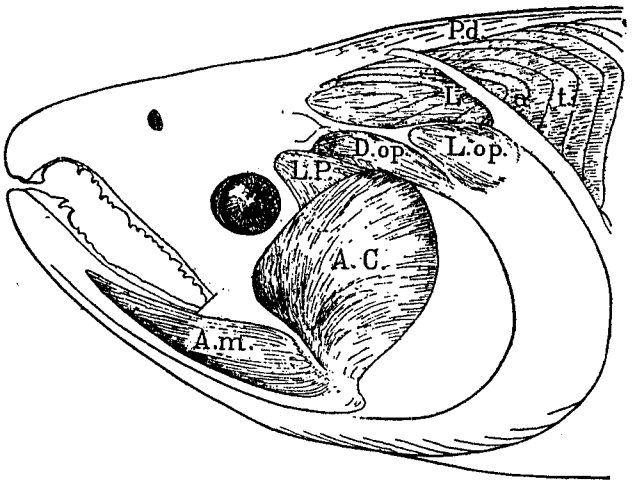

FIG. 3.—Superficial head muscles after removal of the skin and a part of the jaws. *A. C.*, adductor mandibulæ, cephalic portion: *A. m.*, adductor mandibulæ, mandibular portion; *L. P.*, levator arcus palatini; *D. op.*, dilator operculi; *L. op.*, levator operculi; *P. d.*, protractor dorsalis; *Lat.*, lateral muscle.

LEVATOR ARCUS PALATINI.

This is a short, thick, comparatively wide muscle which takes its origin from the angle formed in the external surface of the sphenotic, filling the space just posterior to the eyeball. The fibers run obliquely downward and backward to a broad insertion on the anterior surface of the superior half of the hyomandibular and also into the superior margin of the metapterygoid. The muscle at its posterior dorsal margin is intimately associated with and often inseparable from the fibers at the origin of the dilatator operculi muscle.

DILATATOR OPERCULI.

This muscle has its origin from the anterior margin of the external surface of the pterotic and the posterior border of the sphenotic. Its fibers converge sharply backward and downward to an insertion by a small but strong tendon into the upper margin of the opercle. The attachment is on the knob formed at the junction of the opercle with the hyomandibular. The muscle lies in the groove between the exposed margin of the pterotic and the hyomandibular.

LEVATOR OPERCULI.

The levator operculi is a short, triangular muscle which arises from the posterior spinous border of the pterotic. The fibers converge diagonally downward and backward to an insertion in the upper margin of the opercle. Its contraction leads to an elevation of the opercle aiding in the act of respiration.

DEEP DORSAL HEAD MUSCLES.

When the eye is removed along with the upper portion of the metapterygoid and hyomandibular bones a broad curved sheet of muscle consisting of short thick bundles is exposed. The homologous mass in *Amia* has been divided by Allis into three portions—the levator maxillæ superioris, the adductor hyomandibularis, and the adductor operculi. In *Oncorhynchus* this region can scarcely be divided except for the small group of fibers at the posterior limit of the region. The two parts are better identified under McMurrich's names, the adductor arcus palatini, and the adductor operculi.

ADDUCTOR OPERCULI.

The adductor operculi arises on the ventral surface of the pterotic directly under the origin of the levator operculi. Its origin is overlapped by the posterior fibers of the adductor arcus palatini. The fibers form a short but thick bundle, its length being from 8 to 10 mm. It is inserted into the inner surface of the opercle a little above the insertion of the dilator operculi which it opposes in action.

ADDUCTOR ARCUS PALATINI.

This muscle has an extensive origin along a line from the origin of the adductor operculi to a point in the ventral portion of the eye socket. The fibers are short, thick, and massive for the position in which they lie, but are not readily separated into distinct bundles.

The posterior half of this muscle arises just ventral to the articulation of the hyomandibular and from the ventral surface of the pterotic and sphenotic bones. The fibers are only a few millimeters in length, run directly outward, and are attached into the inner surfaces of the upper half of the hyomandibular and the posterior portion of the metapterygoid. The anterior portion of the mass, which is relatively the larger, arises from the outer surface of the orbitosphenoid. Its fibers extend outward and downward to a broad attachment on the inner surface of the metapterygoid and the dorsal surface of the mesopterygoid.

The contractions of the entire mass tend to elevate the angle of the jaw and to compress the palatine arch.

Allis [a] divides this muscle into the levator maxillæ superioris and the adductor hyomandibularis. No natural division along these lines can be observed in *Oncorhynchus tschawytscha*.

DORSAL BRANCHIAL ARCH MUSCLES

When the palatine arch is removed and the adductor arcus palatini reflected, one can, by trimming away the gill filaments, readily expose the group of muscles of the dorsal half of the branchial arches.

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[a] Allis, Edward Phelps, loc. cit.

LEVATORES ARCUUM BRANCHIALIUM.

This group consists of five diverging muscle slips which are subdivisions of one thin broad sheet. The origin of the muscle sheet is on a line immediately ventral to the origin of the middle portion of the adductor arcus palatini. At the origin the sheet is continuous and its divisions spread out to their attachments somewhat like a miniature fan.

Fifth division.—This, the most posterior slip of the group, is a very slender muscle arising from a point on the skull just in front of the foramen of the tenth nerve. The fibers run posteriorly downward and backward to an attachment on the dorsal margin of the flange of the fourth epibranchial. This muscle is about 2 to 2.5 cm. long, the longest of the group.

Fourth division.—A similar though slightly stouter muscle arises just in front of the latter and is attached on the crest of the corresponding flange of the third epibranchial.

Third division.—The next differentiated strip runs under the tendon of the fourth division and the flange of the third arch and is attached to the dorsal end of the cartilaginous rod corresponding to a fifth epibranchial.

Second division.—The second division is attached on the flange of the second epibranchial. But a tiny slip of this muscle also runs to the dorsal surface of the pharyngobranchial of the third arch.

First division.—The most anterior slip is attached superficially to the flange on the first epibranchial. But its deeper fibers run to the pharyngobranchial of the second arch. These fibers are only a few millimeters long.

The points of attachment of the first, second, and third muscle slips are also common points of union for the connective tissue and septa covering the corresponding gill clefts, i. e., the first muscle is opposite the angle of the second gill cleft, the second opposite the third, and the fourth opposite the fourth.

The pharyngobranchial attachments of the first and second divisions are apparently the homologues of McMurrich's levatores interni, while the five divisions described here would be his externi.

INTERARCUALIS DORSALIS OBLIQUUS, POSTERIOR.

There are two obliqui interarcuales on the dorsal half of the branchial arch. The most posterior dorsal oblique arises from the posterior dorsal surface of the third pharyngobranchial. The fibers run obliquely backward to an insertion on the anterior surface of the flange of the fourth epibranchial.

INTERARCUALIS DORSALIS OBLIQUUS, ANTERIOR.

The second or anterior oblique arises from the second pharyngobranchial near its union with the epibranchial. The fibers run obliquely outward to an insertion on the anterior margin of the flange of the third epibranchial.

ADDUCTOR ARCUUM BRANCHIALIUM, ANTERIOR.

There are two dorsal adductor muscles, an anterior and a posterior. The anterior adductor arises on the posterior surface of the fourth epibranchial plate and is inserted into the dorsal surface of the distal end of the corresponding ceratobranchial. Its contraction approximates the cerato and epibranchials of the fourth arch.

ADDUCTOR ARCUUM BRANCHIALIUM, POSTERIOR.

The posterior adductor muscle arises on the internal surface, i. e., median, of the bony plate of the fourth epibranchial just within the origin of the preceding. It is inserted into the external surface of the cartilaginous cap of the fifth ceratobranchial.

TRANSVERSI DORSALIS, ANTERIOR.

This thin and slightly developed muscle arises from the postero-dorsal surface of the second pharyngobranchial near its junction with the corresponding epibranchial. It runs to a similar attachment on the other side. It is one of the few unpaired muscles.

TRANSVERSI DORSALIS, POSTERIOR.

This unpaired muscle is much more strongly developed than the preceding. It arises from the dorsal surfaces of a part of the fourth pharyngobranchial and the dorsal margin of the central end of the fourth epibranchial. The fibers run to similar attachments on the other side of the body.

Some fibers arise on the dorsal surface of the fifth ceratobranchial and become continuous with the constrictors of the pharynx.

VENTRAL BRANCHIAL ARCH MUSCLES.

The ventral muscles of the branchial arch consist of three groups, the interarcuales ventrales, the transversi ventrales, and the pharyngo-claviculares.

INTERARCUALES VENTRALES OBLIQUI (VETTER).

A group of more or less distinct muscles corresponding to the interarcuales dorsales is present on the ventral side of the branchial basket. In the salmon the anterior three of these muscles are distinct and separate and not divisions of one sheet as in the dorsal group. The posterior two are intimately united. Their dissection should follow that of group E. They are exposed better beginning with the anterior one of the group.

First division.—The most anterior or first division belongs to the first arch. It is a comparatively small slip which has its origin from the ventral surface of the first basibranchial. It extends along the under surface of the hypobranchial to an attachment into the cartilage and ventral tip of the ceratobranchial near its union with the hypobranchial.

Second division.—The second division arises from the ventral surface of the second basibranchial. It runs its course over the second hypobranchial and is attached by a short strong tendon into the ventral surface of the second ceratobranchial. The first and second arch muscles are completely separated at their area of origin. A tendinous band runs over the ventral surface of the basibranchials between the two slips.

Third division.—The third division or muscle of the third arch arises from the third basibranchial and the median portion of the ventral surface of the hypobranchial. Its attachment on the third arch corresponds to that of the first and second divisions.

Fourth division.—This division is continuous with the fifth. They arise from the third basibranchial on the ventral surface somewhat median to and in close contact

with the third. The insertion of the fourth is into the extreme ventral portion of the cartilage of the fourth ceratobranchial.

Fifth division.—The fifth division is regarded as a subdivision of the preceding muscle. It has its origin in a tendinous raphé which is strongly developed at a point ventral to the insertion of the preceding. Some fibers also arise from the cartilaginous plate posterior to the insertion of the fourth division. The muscle is relatively short and thick. It is attached by a short, stout tendon to the fifth ceratobranchial, its tendon being fused with the anterior border of the tendon of the pharyngo-clavicularis externus.

TRANSVERSI VENTRALIS, ANTERIOR.

A short thick triangular bundle of fibers arises on the median surface of the ventral end of the ceratobranchial of the fourth arch. It is an unpaired muscle and runs directly across to an attachment at the corresponding point on the opposite side.

TRANSVERSI VENTRALIS, POSTERIOR.

This stout unpaired muscle is very much like the preceding, but three times larger. It runs from the inner surface of the base of the fifth ceratobranchial transversely under the esophagus to a corresponding insertion on the opposite ceratobranchial.

The transversi ventrales by their contractions approximate the ventral portions of the fourth and fifth arches of the branchial basket.

PHARYNGO-CLAVICULARIS EXTERNUS.

This is a short broad muscle band extending from the antero-dorsal surface of the clavicle directly dorsalward to the lower surface of the fifth ceratobranchial. Its length is only about three times its breadth. Its contractions depress the branchial arch.

PHARYNGO-CLAVICULARIS INTERNUS.

This is a broad thin muscle band arising from the anterior surface of the inner margin of the clavicle at about the middle of its arch. Its fibers run diagonally forward and inward to an insertion on the ventral margin of the fifth ceratobranchial just under the insertion of the pharyngo-clavicularis externus. There is a strong tendinous line along the upper margin of the muscle.

The internus muscle retracts the branchial basket, i. e., draws it backward toward the esophagus.

MANDIBULAR AND HYOID ARCH MUSCLES.

INTERMANDIBULARIS.

A short thick unpaired muscle extends transversely from the left dentary to the right. In cross section it is a rough oval 17 by 6 mm. The muscle is 2 cm. long. It is attached to the inner surfaces of the two dentaries just back of the symphysis. It serves to approximate the mandibles.

GENIOHYOIDEUS.

This is a broad flat sheet of muscle arising from the ceratohyal. The origin is along a diagonal line extending from the postero-ventral border to the antero-dorsal margin of the bone. The muscle joins with its fellow to form a practically continuous

sheet at the insertion into the inner surface of the anterior portion of the dentary around the symphysis. At its insertion the tendon is divided into an external and an internal portion, one passing above, the other below the intermandibularis to its insertion.

HYOHYOIDEUS.

This long thin sheet of muscle arises from the ventral surface of the hypohyal and passes diagonally outward and backward to insertions over the branchiostegal rays. The muscle has attachments to the internal margin of each successive ray. It also has insertions along the ventral margins of the ceratohyal and epihyal. The left hyoideus somewhat overlaps the right at its origin.

STERNOHYOIDEUS.

The name sternohyoideus is applied to a broad and thick sheet of muscle arising on the dorsal surface of the anterior end of the clavicle directly in front of the attachment of the pharyngo-clavicularis externus. Its fibers run forward and are attached to the ventro-lateral surface of the hypobranchial plate. Its differentiation from the ventral portion of the great lateral muscle is apparent and probably it would be better to group it with the longitudinal muscles.

MUSCLES OF THE CAUDAL FIN.

The modifications of the musculature which have come about for the control of the movements of the caudal fin are associated with striking modifications of the caudal skeletal structure. In order to present more accurately the form and relations of the muscles it seems desirable to give the facts concerning the caudal skeletal complex.

CAUDAL SKELETAL COMPLEX.

The caudal fin in the king salmon is regularly bilobed and symmetrical. Externally it appears of the regular homocercal type. The caudal skeleton, however, still shows the heterocercal structure as presented by figure 5. The skeleton reveals the fact that the epichordal component is limited to the rudimentary rays and at most the first two rays of the dorsal lobe. The remainder of the dorsal lobe and all of the ventral represents the hypochordal component. This modification rests on a rather complex caudal skeletal base, as was shown by Kölliker [a] for *Salmo salar*.

The axial region may be considered as composed of those vertebræ entering into the caudal peduncle, and those of the caudal fin proper. Of the three obvious vertebræ that enter into the caudal fin skeleton one only has a well developed centrum. The second and third centra are very much reduced in size, the latter being only a tiny bony nodule. The modifications of the vertebræ of the caudal peduncle begin sharply with the last three vertebræ of the group. However, the spines of the fifth and fourth, counting from the tail, have a median flange on the anterior margin of the neural spines. In the last three vertebræ these flanges are fused each with the spine in front of it. The neural spines of the first and second caudal vertebræ enter into this fusion, the five spines making a firm mass.

[a] Kölliker, Albert von: Ueber das Ende der Wirbelsäule der Ganoiden und einiger Teleostier, taf. IV, fig. 1 and 2. Leipzig, 1860.

Lying on the dorsal surface of the three centra of the caudal group, and extending out over the bases of the neural spines is an irregularly fan-shaped bony plate, the Deckknochen der Chorda of von Kölliker.[a] This plate is coalesced into the dorsal surface of the second, and usually the third, centrum. It has a caudally projecting spine extending in the direction of the axis of the third centrum.

The hemal spines of the last three vertebræ of the peduncle are also sharply modified

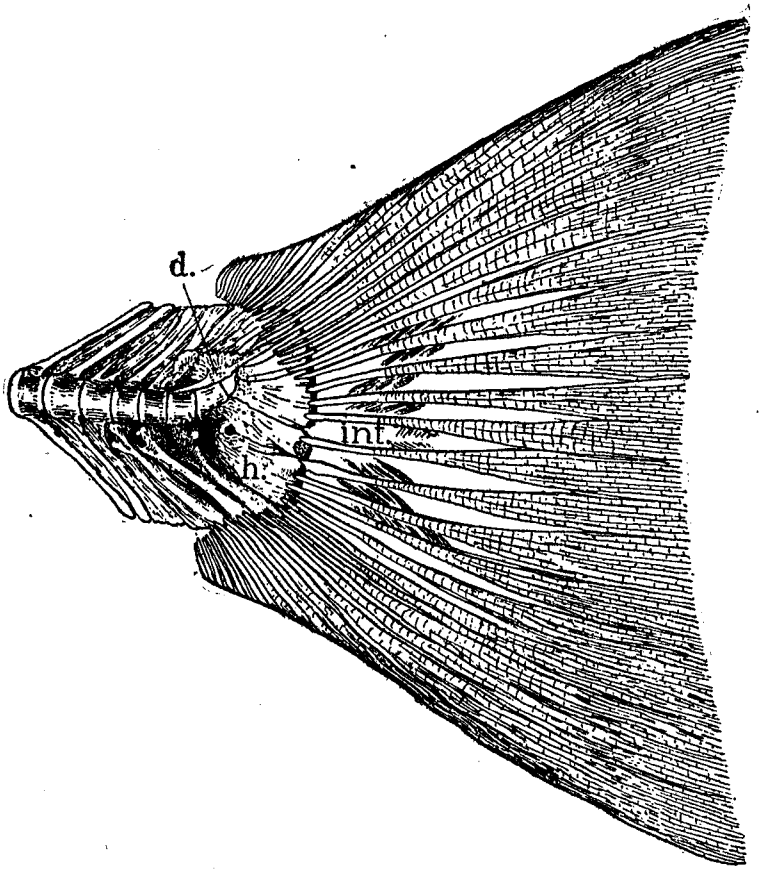

FIG. 4.—Caudal skeleton. Five centra of the caudal peduncle with their modified spines are shown. The three caudal centra are much reduced, the last quite rudimentary. The hemal spine of the basal caudal vertebra is very stout. It bears a transverse spine near its base. The lower one of the five hypurals is marked "*h*." The large irregular plate "*d*" is von Kölliker's "Deckknochen der Chorda" of *Salmo salar*. A few of the deeper fibers of the musculi interfilamenti (*int.*) are shown.

by being greatly thickened and broader. The borders of these plate-like hemal arches are not fused, though they are intimately bound together by connective tissue.

The hemal spine of the most anterior vertebra of the three that belong to the caudal fin proper is very strong and bar-like. It is heavier than the caudal peduncle spines anterior to it, and is especially characterized by its strong and stocky base which carries a well-developed lateral process. This process stands out sharply for 3 to 5 mm.

[a] Kölliker, Albert von, op. cit., p. 12.

from the base of the spine. It is directed somewhat posteriorly and serves for the attachment of a group of the deeper caudal muscles. The last two caudal fin vertebræ are sharply modified. Ventral to the rudimentary centra there is a series of strong and broad hypurals. In the king salmon there are five hypurals, the most anterior one the strongest, and the individuals of the series diminishing in size toward the dorsal lobe of the fin base. The development of the hypurals is commensurate with that of the caudal musculature.

Saddled over the ends of the hemal spines of the last two vertebræ of the caudal peduncle, the spine of the first caudal vertebra, the hypurals, and the bony fusion of neural spines previously described, are the series of paired fin rays constituting the caudal fin. The fully developed rays are 19 in number, with about 12 rudimentary rays above and as many below. The middle ray of the fully developed series represents the axial ray. It is not only in the middle of the series but the interfilamenti caudales muscles are inserted symmetrically with reference to this axial ray (fig. 4). These rays form a joint of limited movement over the end of the skeletal complex to which they are strongly anchored in a firm mass of ensheathing connective tissue.

CAUDAL FIN MUSCLES.

The muscles of the caudal fin are derived from the posterior myotomes of the embryonic lateral muscles. The only probable exception is the interspinous muscle, which is very intimately associated with the dermal fin rays and the skin itself. The muscles are superficial and deep.

SUPERFICIAL MUSCLES.

CAUDAL END OF THE MUSCULUS LATERALIS SUPERFICIALIS.

This is a trunk muscle, but the details of its caudal insertion have been reserved for description at this point. The lateralis superficialis or dark muscle is continued over the lateral surface of the caudal peduncle to be inserted into the base of the tail. It forms a sheath on each side of the mid-line of the caudal peduncle estimated in width at two-thirds the distance from the mid-line of the peduncle to the dorsal and ventral borders respectively. The muscle substance ceases posteriorly in the middle line at a point directly under, i. e., ventral to the base of the first long dorsal caudal ray. The caudal end of the muscle, i. e., marking the termination of the myomeres in its tendon and fascias, is distinctively clavate. The dorsal myomeres are narrowed, and the myocommata run together into a strong tendon that is attached to the bases of the first, second, and usually the third long dorsal rays just exterior to and in the fascia of the profundus lateralis. In a Baird specimen (small male) the last three dorsal myomeres are modified, the last two into a muscular slip running obliquely dorsalward and caudalward to end in a delicate flat tendon or fascia. The dorsal lobe of the superficialis is rendered more prominent by the fact that the dorsal border of the muscle, just at the base of the caudal peduncle, is attached to fascias which are intimately connected with the myocommata between the ventral two-thirds and the dorsal third of the epaxial half of the lateral muscle. There is considerable irregularity in the arrangement of the muscular fibers of the last two or three myomeres of the dorsal lobes of this muscle. A rather common irregularity is that shown in figure 5. The ventral lobe of

the lateralis superficialis forms a similar attachment into the connective tissue over the bases of the first and second long ventral caudal rays.

THE TERMINAL OR CAUDAL PORTION OF THE MUSCULUS LATERALIS PROFUNDUS.

The terminal or caudal portion of the profundus is characterized by the excessive proportion of connective tissue of the myocommata. In fact the myocommata are finally reduced to tendons of insertion.

The epaxial and hypaxial portions are well separated in the region of the caudal peduncle, partly by the greater development of the superficialis which ensheathes the

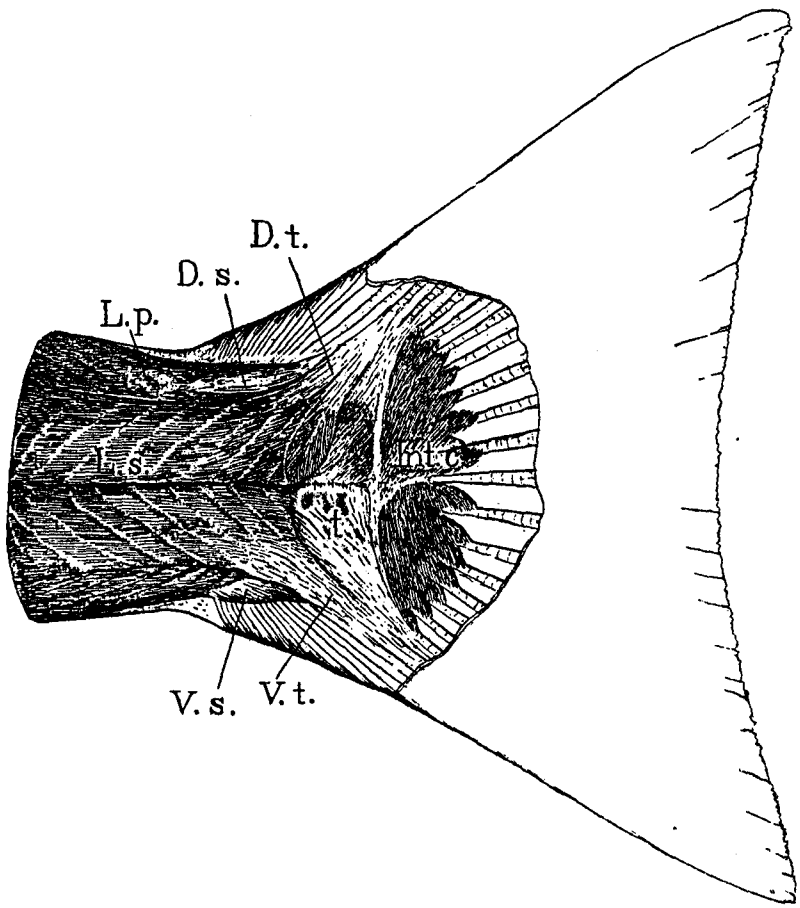

FIG. 5.—The superficial muscles of the caudal fin and the caudal peduncle. *L. s.*, lateralis superficialis; *D. s.*, dorsal slip of lateralis superficialis; *V. s.*, ventral slip of lateralis superficialis; *D. t.*, dorsal tendon of lateralis superficialis; *V. t.*, ventral tendon of lateralis superficialis, *L. p.*, lateralis profundus; *t.*, terminal tendons of the lateralis profundus; *Int. c.*, interfilamenti caudalis.

profundus next the septum. The final terminal tendons run straight back under the clavate margin of the tendon of the superficialis to a very strong insertion into the aponurosis which covers the bases of the rays of both the dorsal and the ventral lobes and includes all of the intermediate series (fig. 5).

The epaxial division covers the deeper muscles presently to be described, but is not strongly fused with their fascias. The hypaxial division is strongly attached into the tips of the hemal spines of the last vertebræ of the caudal peduncle as well as into the bases of the rays. Its superior margin covers the ventral inferior caudal flexor.

The epaxial and hypaxial aponuroses are strongly united across the median line as shown in the area between the terminal myomeres of the superficialis and the interfilamenti muscles.

MUSCULI INTERFILAMENTI CAUDALIS, THE INTRINSIC MUSCLES OF MCMURRICH.

This small superficial double fan-shaped muscle consists of dorsal and ventral portions. The fibers originate in the fascia lying over, or covering, the termination of the lateralis profundus muscle on the base of the tail. They are attached into the admesial margins and the external surfaces of the bases of the seven caudal rays lying on either side the median ray. This middle ray, so far as the muscular arrangements indicate, is axial. The muscle fibers attached to its base above run diagonally upward and caudalward, those below downward and caudalward. The superficial fibers form a continuous layer, while the deep fibers run from ray to ray, as shown in figures 4, 5, and 6. When this muscle contracts it draws the caudal rays together, narrowing the spread of the fin.

The width of the caudal interfilamenti muscles, at the best, is about 10 mm. The dorsal lobe is about 26 mm., the ventral lobe 24 mm. long.

DEEP CAUDAL MUSCLES.

The caudal fin is used by the salmon both as a steering rudder and as a propeller. The deep ventral muscles move the parts of the fin to set its form for a rudder, but the musculature which utilizes it as a propeller is limited to the great lateral muscles acting on the fin as a whole. If, during the movements of the fin as a whole, it is set in some special position or given a characteristic shape, that shape will be utilized for steering the forward motion of the salmon. This activity is accomplished by means of the deep caudal muscles, as can readily be seen by consideration of the effect of the contractions of the muscles singly or in groups. There are six pairs of these deep muscles. They vary considerably in detail of size and position but the usual type will now be described.

FLEXOR CAUDALIS VENTRALIS SUPERFICIALIS.

This is a delicate muscle slip which begins by a small flat tendon attached to the bases of the hemal spines, the third and fourth from the end of the caudal peduncle. A few fibers also arise from the fascia and tendons of the superficialis in the median line. It runs posteriorly to a slender tendon attached to the tip of the transverse spine on the first caudal vertebra. The muscle is continued from this point to an insertion on the base of the third caudal ray ventral to the axial ray. Its attachments lie over the interfilamenti. The ventral margin of the posterior division of this muscle is sometimes fused with the dorsal margin of the next.

FLEXOR CAUDALIS VENTRALIS SUPERIOR.

This caudal flexor is a rather broad group of fibers which arises from the ventral surfaces of the centra and the bases of the hemal spines of the last two vertebræ of the peduncle, also from the base of the spine and the ventral surface of the lateral process of the most anterior caudal vertebra. The fibers run slightly ventralward as they proceed to their insertion into the bases of the fifth to the eighth caudal rays below the axial ray.

The contractions of this muscle lead to a flexion of the lower half of the middle portion of the caudal fin, and of the ventral caudal lobe. The tension in this case is brought primarily on the uppermost rays of the ventral lobe. The muscle presumably acts in conjunction with the next to be described.

FLEXOR CAUDALIS VENTRALIS INFERIOR.

The origin of the inferior ventral flexor is from the surfaces of the last three hemal spines of the caudal peduncle. The attachment is in a line which begins somewhat ventral to the anterior limit of origin of the preceding muscle and runs posteriorly and toward the transverse process of the first caudal vertebra. The fibers of the border of the superior muscle arise under the ventral border of the preceding muscle.

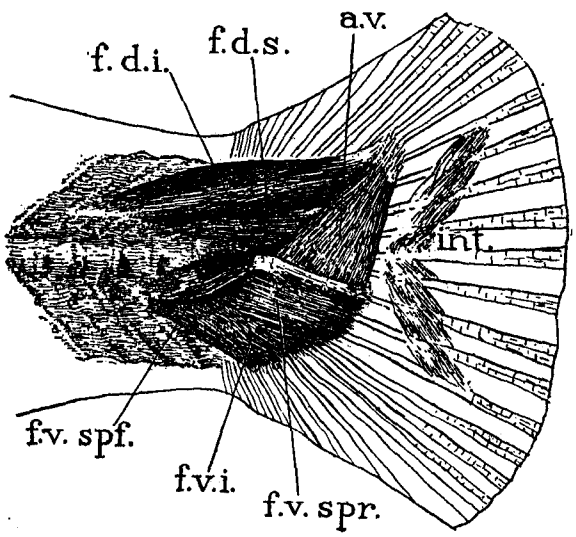

FIG. 6.—The deep caudal fin muscles. *f. v. spf.*, flexor caudalis ventralis superficialis; *f. v. spr.*, flexor caudalis ventralis superioris; *f. v. i.*, flexor caudalis ventralis inferioris; *a. v.*, adductor caudalis ventralis; *f. d. s.*, flexor caudalis dorsalis superior; *f. d. i.*, flexor caudalis dorsalis inferior; *int.*, interfilamenti caudalis.

The fibers of the inferior flexor run ventrally and caudally to insertions into the bases of the last two long ventral caudal rays and into the adjacent series ofrudimentary rays.

Contractions of this muscle sharply flex the extreme ventral border of the ventral caudal lobe. Contraction at the same time with the superior ventral flexor would sharply flex the whole ventral half of the caudal fin toward that side on which the contraction occurred.

ADDUCTOR CAUDALIS VENTRALIS, THE ADDUCTOR OF THE DORSAL CAUDAL LOBE.

This muscle is a relatively thin and broad sheet of muscle fibers lying below but in its body closely parallel with the caudal vertebral axis. The origin of the muscle is revealed by cutting away the posterior portion of the superficialis, the major portion of the interfilamenti caudalis, and the superior border of the superior ventral flexor. It has a rather broad line of origin extending from the lateral spine of the first caudal vertebra directly posterior to the second caudal ray below the axial ray. The tendon of origin is rather thickened at the spine. The line of origin is along the dorsal margin

and the surface of the lowermost hypural bone, and from the connective tissue over the bases of the corresponding caudal spines. The tendon is somewhat stronger at the ends of this broad line of origin. The fibers of the muscle run dorsally and somewhat caudally, converging as they go and ending in a broad and strong tendinous attachment into the fourth, fifth, sixth, and sometimes seventh, long caudal rays dorsal to the axial ray.

The belly of this muscle is 16 mm. wide by 3 to 3.5 mm. thick and the muscular portion is 22 mm. long, i. e., in the 80 cm. fish used as a standard.

The contractions of the adductor lead to sharp flexion and adduction of the dorsal caudal lobe. Since the tension on the lobe is almost directly from the point of attachment of the tendon toward the median axial ray, this would naturally lead to an approximation of the rays and a decrease in the spread of the fin.

FLEXOR CAUDALIS DORSALIS SUPERIOR.

The dorsal flexor is the longest and strongest muscle of the deep caudal series. It lies almost in the axial plane of the fish. It takes its origin from the median septum over the fifth and fourth neural spines and from the third and fourth vertebræ, counting from the posterior end of the caudal peduncle. Some fibers of origin are found along the tips of the second and third neural spines. Fascias of the muscle are more or less intimately attached to the median septum as far back as the first true caudal vertebra. The insertion, in conjunction with the attachment of the adductor caudalis ventralis, is by a thin flat but strong tendon ending on the lateral surface of the bases of the rays of the most superior portion of the hypaxial division of the caudal fin.

Occasionally the muscle is more strongly developed, in which case it has an origin anterior to that described.

Contractions of the superior flexor produce strong flexion of the dorsal caudal lobe toward the side on which the contractions occur. Undoubtedly this muscle and the one preceding it exert the most powerful influence in the control of the rudder-like function of the caudal fin.

FLEXOR CAUDALIS DORSALIS INFERIOR.

The inferior dorsal flexor is a much more slender muscle than the preceding one. Its origin is directly ventral to and lies parallel with the superior flexor. The fibers of origin are from the connective tissue over the basal part of the bony plate formed by the fusion of the neural spines of the last three vertebræ of the caudal peduncle. Some fibers are also attached into the myocommata at its most anterior margin. The muscle belly extends caudally in a line parallel with the general axis of the fish, running under the adductor to a flat tendinous insertion into the bases of the median two or three caudal rays next above the axial ray.

Contractions of this muscle produce simple flexion of the middle portion of the caudal fin.

From the descriptions presented and the accompanying figures it is now more clear that these muscles are the ones concerned in shaping the position and form of the caudal fin during the active movements of forward swimming. The great lateral muscles must be supposed to act on the caudal fin as a whole in the alternate propelling move-

ments. If, during this general propulsive motion, the form and shape of the caudal fin is adjusted as it would be by graduated contractions of the deep caudal muscles, it is obvious that the fin will be the guiding rudder controlling the exact direction of the forward movement. In closing the discussion of this phase of our subject we may reiterate once more the statement previously made, that these deep caudal muscles control the positions of the caudal fin which will adapt it to the purposes of a rudder. The great lateral muscles furnish the power which acts on the caudal fin as a whole, furnishing a piscine propeller seldom equaled and never excelled in the aquatic world.

MUSCLES OF THE PECTORAL GIRDLE.

The pectoral muscles of the European salmon, *Salmo salar*, have been briefly described by Harrison,[a] and more recently described and figured by Pychlau.[b] The development is given by Harrison, and by Vogel[c] for the trout, *Trutta fario*. Many instructive comparative points in the myology of fishes are to be had from the exhaustive papers by Allis[d] on *Amia calva* and *Scromber scrombcr*.

In *Oncorhynchus tschawytscha* the pectoral fin has 14 rays, the basal or external one being markedly heavier and the others successively more slender. The base of each half ray is curved sharply toward the median border of the fin. The two halves of each ray are widely separated at the base. The series of rays is seated like a saddle across the skeletal ridge of the basalia, forming a very mobile joint, as described by Pychlau for *Salmo salar*. This type of joint is also found in all the salmon fins, but with modifications.

ABDUCTOR PECTORALIS SUPERFICIALIS.

This muscle arises from the anterior ventral border and the inner or median ventral margin of the coracoid as far back as the base of the fin. Its surface of origin along the coracoid is widest about one-third the distance from the anterior end of the coracoid, where it covers a surface of about 9 mm. wide in a standard fish. The median line of origin is along the ventral ridge on the coracoid, covering this ridge for one-third its length. The fibers of the muscles run back over the deep abductor to a tendinous insertion in the tips of the processes of the ventral half rays. The ventral surface of the muscle near its origin has its tendon joined by the fibers of the protractor ischii. These occasionally spread fan-shaped over the surface of the angle between the ventral ends of the clavicles. The external fibers of the pectoralis superficialis are in close approximation to, and have tendons intimately fused with, the internal portion of the profundus.

The action of the superficialis is to bend the fin downward and forward and to close the rays.

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[a] Harrison, Ross G.; Die Entwicklung der unpaaren und paarigen Flossen der Teleostier. Archiv für Mikroskopische Anatomie, bd. 46, 1895, p. 500–578.

[b] Pychlau, Waldemar: Untersuchungen an den Brustflossen einiger Teleostier. Jenaische Zeitschrift, bd. 43, 1908, p. 692–728.

[c] Vogel, Richard: Die Entwickelung des Schultergürtels und des Brustflossenskelettes der Forelle (Trutta fario). Jenaische Zeitschrift, bd. 45, 1909, p. 499–544.

[d] Allis, Edward Phelps: The skull and the cranial and first spinal muscles and nerves in Scomber scomber. Journal of Morphology, 1903, vol. 18, 1903, p. 45–328.

Same author: The cranial muscles and cranial and first spinal nerves in Amia calva. Journal of Morphology, vol. 12, 1897, p. 487–808.

ABDUCTOR PECTORALIS PROFUNDUS.

When the superficialis muscle fibers are reflected the abductor profundus is exposed. It arises from the ventral surface of the coracoid. Beginning at a point one-third the distance from the anterior end there is a thick muscular mass intimately attached into the surface of a triangular area on the ventral face of the coracoid. The base of this triangle is marked by a line parallel with the base of the pectoral fin over the union of the coracoid with the basalia. The profundus has a short, heavy tendon divided into slips corresponding with, and inserted into, the inner margins and tips of the curved bases of the ventral half rays of the pectoral fin under the tendons of the superficialis.

The contractions of this muscle draw the fin downward, helping to balance or support the body when quietly resting on the bottom.

EXTENSOR PECTORALIS.

There is a rather thick muscular bundle which arises under the anterior origin of the abductor superficialis and along the margin of the ventral portion of the clavicle.

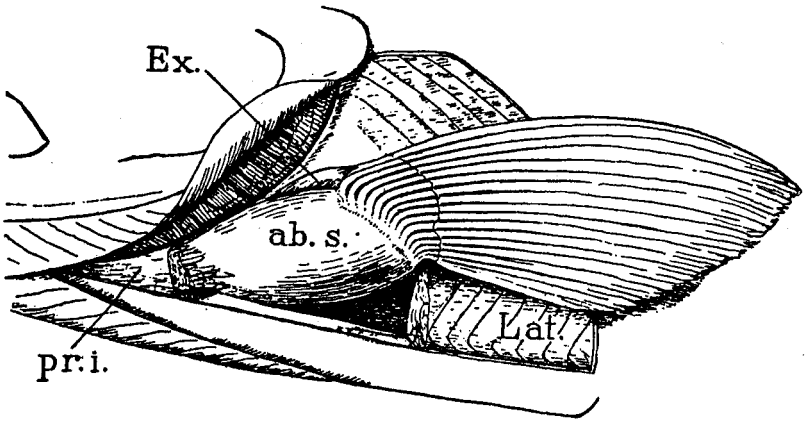

FIG. 7.—Ventral view of the pectoral fin muscles. A segment is cut out of the anterior end of the protractor ischii, *pr. i.*, together with the anterior ventral portion of the lateral muscle. This uncovers the abductor superficialis, *ab. s.*, and its attachments to the ventral half-rays of the fin. The end of the extensor, *Ex.*, with its insertion into the base of the first fin ray is shown.

This muscle lies close within the angle formed in the ventral surface of the clavicle. It is inserted by a short thick tendon into the external surface of the base of the first fin ray.

Contraction of this muscle spreads the fin out in the horizontal position. When the fin is folded back against the body the external ray forms the upper margin of the fin. From this position the extensor pectoralis throws the fin forward, bends it slightly downward and spreads the rays. The muscle tends to support the abductor.

The great lateral muscles are attached by strong slips into the clavicle just dorsal to the insertion of the pectoral fin. There is also a muscular slip from the great lateral muscle running just ventral to the base of the fin and inserted into the fascia of the dorsal wall of the pericardium. This fascia is closely attached to the internal or ventral margin of the coracoid. Undoubtedly contractions of the great lateral muscle would tend to draw the pectoral girdle posteriorly. When the fascia is dissected off a rather

thick triangular muscular mass of the adductors comes into view on the inner and the posterior surface of the coracoid.

ADDUCTOR PECTORALIS SUPERFICIALIS.

This is a short thick muscle lying in the angle between the coracoid and the clavicle and the base of the pectoral fin. It takes its origin from the posterior ventral surface of the coracoid, and the spine on the superior margin of this bone, also from the thin bony plate lying between the superior margin of the coracoid and the clavicle. The muscle fibers converge into a broad tendon which is inserted into the posterior surface of all the dorsal half-fin rays except the first five. When this muscle is reflected a deeper muscle is exposed.

ADDUCTOR PECTORALIS PROFUNDUS.

This muscle arises from the dorsal margin of the extreme ventral portion of the clavicle and the surface included in the angle between this margin and the bony ridge projecting on the side of the clavicle, also from the connective tissue septum joining the clavicle and coracoid and from the dorsal margin of the coracoid including the upper surface of the median spine. The muscle is divided into two parts. Those fibers arising in the angle between the anterior end of the coracoid and the clavicle form a stout tendon inserted into the stout base of the marginal ray. The remaining fibers converge to strong tendons inserted into the bases of the dorsal half rays of the pectoral from the second to the last.

Contractions of the profundus support the contractions of the superficial muscle in throwing the fin back against the side of the body.

INTERFILAMENTI PECTORALIS.

When the skin is removed from the basal half of the ventral surface of the pectoral fin there is exposed a series of very delicate muscle fibers running across the bases of the fin rays. These fibers run from ray to ray, being arranged diagonally so that when they contract they tend to close the rays. No fibers were observed on the dorsal surface.

MUSCLES OF THE PELVIC GIRDLE.

ABDUCTOR VENTRALIS SUPERFICIALIS.

A slender slip of muscle, the abductor ventralis superficialis, arises from the median longitudinal septum of the pelvis beginning at the ventral border of the anterior end of the ischium, also from the adjacent cutaneous fascias. It is surrounded by a strong aponurosis continuous anteriorly with that into which the tendon of the protractor ischii is partially inserted.

The superficialis runs as a slender wedge of muscle to a strong tendinous insertion into the tips of the ventral half rays of the ventral fin. A cross section of the middle of the muscle presents a wedge-shaped surface, the base of the wedge in approximation to the skin, the surface shown in figure 8, and the side in contact with the median (vertical) septum.

Contractions of the abductor ventralis superficialis produce ventral flexions of the ventral fin. It tends to bend the fin downward, i. e., away from the body. If the fin rays are at the time spread then approximation of the rays also occurs.

ABDUCTOR VENTRALIS PROFUNDUS.

This is a large and strong muscle which lies external and dorsal to the superficialis. It takes its origin from the entire ventral surface of the ischial plate, from the septum which connects the external margin of this bone with the skin, and from the similar septum that runs from the internal or median border to the mid-ventral line. This last septum joins the median longitudinal pelvic septum just at the mid-ventral line of the abdominal cavity; hence the peritoneum, the dorsal border of the median longitudinal septum and the internal border of the ischial septum are fused.

The muscle fibers from this extended origin converge in the general caudal direction toward the base of the anal fin. The insertion is by very short tendinous slips into the

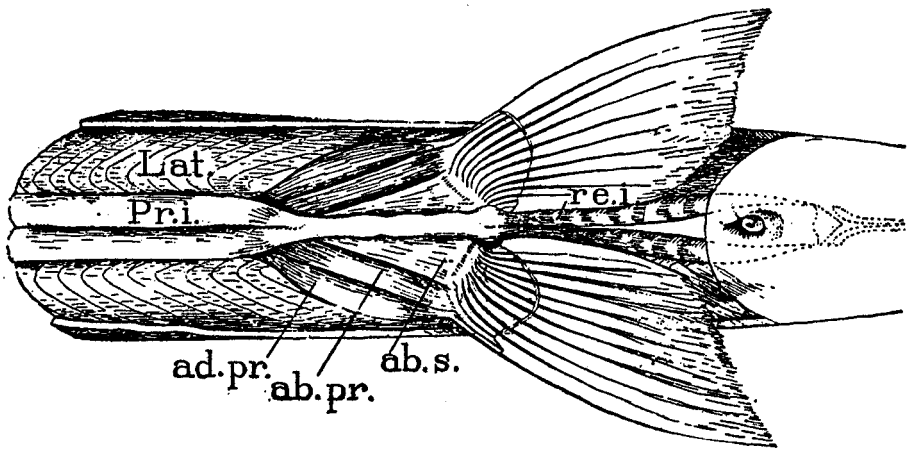

FIG. 8.—Ventral view of the superficial muscles of the ventral fins and of the pelvic arch. *ab. s.*, abductor ventralis superficialis; *ab. pr.*, abductor ventralis profundus; *ad. pr.*, adductor ventralis profundus; *Pr. i.*, protractor ischii; *re. i.*, retractor ischii; *Lat.*, lateral muscle, ventral margin.

ventral half rays of the fin. The tendons run dorsal to the tendons of the superficialis and are inserted into the inner border of the ventral half ray, i. e., the border next the median plane of the fin itself. The fibers arising most anteriorly are inserted into the rays of the external border of the fin. The fibers of the extreme posterior portion of the muscle, arising in the deep angle in front of the ischial thickening, run almost dorsally to the tips of the bases of the rays.

The two halves of the individual rays are more widely separated in the ventral fins than in the unpaired fins, and a distinct synovial joint is formed here as described for the pectoral by Pychlau.[a] The manner of insertion of the abductor profundus and the presence of this very efficient joint insures a strong abduction of the ventral fin on its contraction. There is only a minimal approximation of the fin rays, if any at all, accomplished by this muscle. This latter function seems limited to the superficialis.

[a] Pychlau, loc. cit.

ADDUCTOR VENTRALIS SUPERFICIALIS.

This muscle represents the most dorsal portion of the pelvic musculature. The muscle is separated into two divisions, as described by Harrison for *Salmo salar*.

The *pars anterior* arises from the dorsal surface of the anterior end of the ischium and along the line of aponurosis in which the median longitudinal septum, the abdominal peritoneum, and the ventral attachments of the great lateral muscles meet. The pars anterior division of the muscle runs as a flat band to end in a broad tendinous sheet which covers the dorsal surface of the caudal end of the profundus just over the posterior end of the ischium. It is inserted into the dorsal half rays of the external six or seven rays. The insertion is into the curved bases of the rays at about the middle of the curve.

The *pars posterior* consists of the short and relatively thick mass of muscle fibers which arise from the fascias along the lower border of the lateral muscle immediately dorsal to the posterior part of the ischium and the insertion of the fin. These fibers are well separated from the pars anterior. The fibers run abruptly downward and posteriorly to an insertion into the bases of the last three or four anal rays, i. e., the rays on the median border of the fin. The tendinous insertion does not seem to be sharply subdivided into slips and is extremely short.

ADDUCTOR VENTRALIS PROFUNDUS.

The profundus arises from the dorsal surface of the illium for its full extent, and from the horizontal septum extending from the inner margin of the illium to the mid-ventral line, also from a similar septum extending from the external margin to the skin. The fibers constitute the largest muscle of the pelvic series. In the middle of the belly the muscle is broad and rather thick (22 mm. broad by 6 mm. thick, in an 80 cm. fish). Posteriorly the muscle is somewhat heavier on its median border, the fibers extending transversely out and back over the posterior thickened margin of the illium, and under the tips of the dorsal half rays. When the adductor superficialis is removed the tendon of the profundus is revealed as a broad and relatively long sheath. The insertions of the tendon are on the inner, that is median, borders of all the dorsal half rays. This tendon enters also into the formation of the capsule of the movable joint by which the fin is attached to the posterior end of the illium.

Contractions of the adductor profundus lead to two motions, first, rotation of the fin over the illium at this point, i. e., throwing the fin up against the body of the fish, and second, the spreading of the rays, by throwing the outer fin margin in a lateral direction with reference to the median plane of the fish. These last motions, it will be seen, are directly the opposite of those produced by the abductor group.

The ventral margin of the lateral muscle is strongly attached into the supporting connective tissue of the posterior part of the illium by means of the myocommata. It is evident that contractions of the lower borders of the myomeres lying immediately posterior to the pelvic girdle will have a tendency to draw the pelvic arch as a whole backward. The muscular development does not seem to be of an extent which would lead one to infer that this is a chief function of the muscle. It justifies only the inference that the movement is an incidental but possible one.

MUSCLES OF THE DORSAL FIN.

Harrison [a] has briefly described the muscles of the dorsal fin of *Salmo salar* in connection with his study of the development of the fins of teleosts. The muscles in the king salmon are similar in character and arrangement. The number of dorsal fin rays is greater in *Oncorhynchus tschawytscha* than in *Salmo salar*. The muscles of the fin have a correspondingly greater number of divisions, one for each fin ray. A typical fin ray is moved by three pairs of muscles, (1) an inclinator, (2) an erector, and (3) a depressor. Beside, the fin as a whole is moved forward by the pair of protractors and backward by the pair of retractors described with the group of longitudinal muscles. The specific fin muscles may be described more fully as follows:

INCLINATOR DORSALIS (THE SUPERFICIAL LATERAL MUSCLE OF MCMURRICH).

This muscle in reality consists of a series of short muscles, i. e., independent slips, corresponding in number with the dorsal fin rays. Each tiny slip has its origin in a fascia which is strongly attached to the skin and which covers the dorsal margin of the great lateral muscle. The fibers of each muscle slip converge as a conical mass ending in a short tendon inserted into the postero-lateral margin of the base of each fin ray. These muscle slips are about 20 mm. long in the anterior members of the series and 15 mm. in the posterior. The extreme anterior three or four slips are very rudimentary and may readily be overlooked in the dissection. There are 13 free slips, 16 or 17 in all. In the sea form the spaces between muscle slips are filled with subcutaneous fat. The ends of the deep fin muscles are to be seen just between the ends or insertions of the inclinator slips.

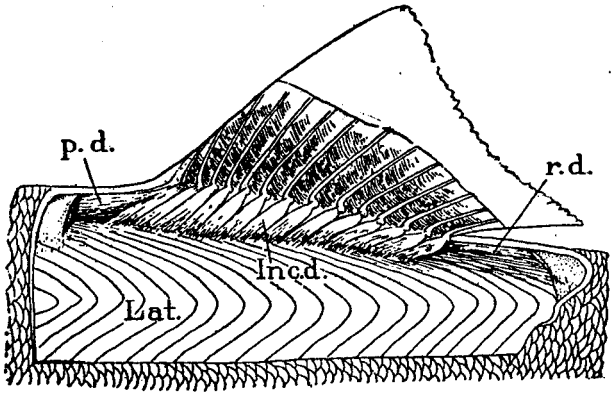

FIG. 9.—Superficial muscles of the dorsal fin after removal of the skin and subcutaneous fat, left side. *Inc. d.*, inclinator dorsalis, one slip for each fin ray; *p.d.*, protractor dorsalis; *r.d.*, retractor dorsalis; *Lat.*, great lateral muscle, epaxial portion.

Harrison was the first to describe these muscles carefully, and to him we owe the name "inclinator."

The contractions of the divisions of the inclinator muscles tend to bend or incline the dorsal fin toward the side.

ERECTOR DORSALIS.

The erector muscle divisions lie between, hence alternate with, the depressor slips of the double series. Each of these arises from the fascia between its interneural spine and the one next in front of it, and from the posterior border of the latter. The mus-

[a] Harrison, Ross G., loc. cit.

cular divisions of the erector dorsalis are separated from those of the depressor by very thin connective tissue septa. But the whole group of muscle slips is encased in a much thicker and tougher sheath. When the muscles are uncovered by removing the lateral muscles the connective tissue sheath is more evident. This is seen to be intimately attached along the line where the neural spines and interneurals are interlocked and embedded in the median longitudinal septum. This sheath, the median septum, and the partitions between muscle divisions serve to form a series of slender glove-finger-like cavities enclosing the pairs of muscle slips on each side.

Each muscle division of the erector is attached by a very short tendon into the anterior margin of the base of the dorsal halfray. The largest erector divisions are 40 mm. in length. At the posterior and shorter margin of the muscle they are about 32 mm. in length. The anterior two or three muscle slips are rudimentary, very slender, and more or less fused.

The contractions of the erector muscle elevate the dorsal fin rays as the name implies. The point of attachment of the tendon of insertion above or distal to the center of movement of the joint favors the erection.

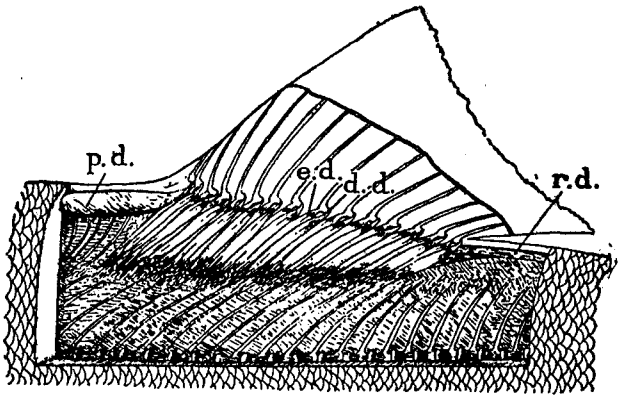

FIG. 10.—Deep muscles of the dorsal fin after removal of the great lateral muscle; *e. d.*, erector dorsalis; *d. d.*, depressor dorsalis *p. d.*, protractor dorsalis; *r. d.*, retractor dorsalis. The skeleton is shown embedded in the median longitudinal septum.

DEPRESSOR DORSALIS.

The depressor muscle of the dorsal fin is intimately associated in position and attachments with the erector dorsalis. The depressor divisions are also segmental in arrangement. They are very slender slips of muscle which arise each along the anterior border of the corresponding interneural spine, and from the fascia separating this muscle from the erector muscle in front of it. The fibers pass across the end of the interneurals to insertions on the posterior border of the base of each dorsal ray. The last muscle division of the posterior border of this series is very strongly developed. It is somewhat broader than its mates and is attached into the bony plate previously described for the retractor dorsalis muscle.

MUSCLES OF THE ANAL FIN.

The musculature of the anal fin is built on the same plan as that of the dorsal fin. The modifications are slight and more or less unimportant. The fin has a protractor, the retractor of the pelvis, and a retractor—both of which are divisions of the infracarinales previously described as the most ventral longitudinal differentiation of the lateral musculature. The proper muscles of the fin are (1) the inclinator analis, (2) the erector analis, (3) the depressor analis, and (4) the interfilamenti analis. These muscles are in divisions

corresponding to the skeletal divisions of the fin itself. Their relations are shown by a consideration of the anal fin skeletal complex.

SKELETON OF THE ANAL FIN.

The anal fin of the king salmon consists of 16 well-developed fin rays, with three rudimentary rays at the anterior margin. There are no spines. A pair of typical rays, say from the middle of the series, serves to show the general skeletal plan (fig. 11).

The ray itself consists of two half rays, the so-called dermal plates, intimately bound to each other except at the base. Near the base the plates diverge slightly and curve sharply posteriorly, not unlike the curve of a hockey stick. The ray is seated cross-saddle fashion over a median cartilage to which it is strongly bound by connective tissue ligaments but with a movable joint.

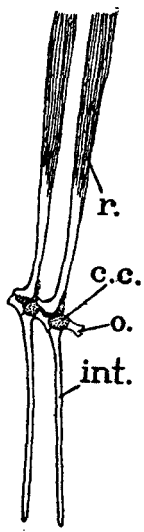

FIG. 11.—Two segments of the skeleton of the anal fin. The lettering is on the cephalic border; *r.*, ray; *int.*, interhemal spine; *c. c.*, cartilages; *o.*, ossicle forming movable joints between the interhemals and the fin rays.

The median cartilage is bound by a movable joint to the head of the supporting interhemal. The interhemal is firmly imbedded in the median longitudinal septum. The position of the interhemals alternates with the hemals but forms an acute angle with the axis of the body, the inclinations being directed caudally.

This skeleton is modified at two points. Anteriorly the whole complex just described is represented by a triangular plate, apparently the homologue of either the median cartilage or more probably of the interhemal. This plate is strongly bound to the anterior margin of the fin base. Its most dorsal angles receive the tendons of the retractor ischii (fig. 2).

Posteriorly there is also a sharp skeletal modification. The last two interhemal spines at the posterior end of the series are fused at their ventral ends forming an irregular club-shaped knob under the second from the last fin ray. This interhemal knob is larger and stronger than the ones immediately in front of it.

Just dorsal to the last rays of the anal fin, and in series with and bound by the enlarged interhemal, is a specially modified cartilaginous plate. It is rather strong and laterally compressed. This plate receives the attachment of the retractor analis. The plate is very strongly bound to the ones in front of it and to the interspinous septum by bands of fibrous connective tissue. Doubtless this modified cartilage is the homologue of one or more intermediate plates or of the interhemals.

INCLINATOR ANALIS.

The constituent serial divisions of this muscle are exposed by removing the skin from along the base of the anal fin and the ventral surface of the adjacent part of the body (fig. 13). There are muscle divisions for each ray including the rudimentary rays at the anterior margin of the fin. The largest and longest divisions are opposite the anterior full rays of the fin. The muscle slips become progressively smaller posteriorly. The muscles of the rudimentary rays are small and imperfectly separated.

The longer muscle slips of the inclinator are about 12 to 15 mm. long and from 4 to 5 mm. wide. They are broad at their origin and the fibers converge to the point of insertion into the rays.

The origin of the inclinator fasciculi is from the skin and the fascia covering the ventral border of the great lateral muscle. This broad origin gives to each slip a base which is seated on the cylindrical border of the lateral muscle. From this broad origin the fibers converge toward a short slender tendon which is inserted into the base of the corresponding ray on its lateral surface, and between the tendons of the erector and depressor respectively. The insertion is in the plane of the axis of the ray joint. The inclinator muscle slips fill the triangular space between the skin, the lateral muscle border, and the erector-depressor group of muscles (see fig. 13). The divisions are strongly embedded in connective tissue sheaths as best shown in formalin-preserved specimens.

Contractions of the inclinator muscle strongly bend the fin toward the corresponding side. This motion is most pronounced at the anterior margin of the fin where the muscle slips are longer and larger. The pull of the muscle is at an angle of about 70°, an angle that decreases with the flexion of the fin in that direction.

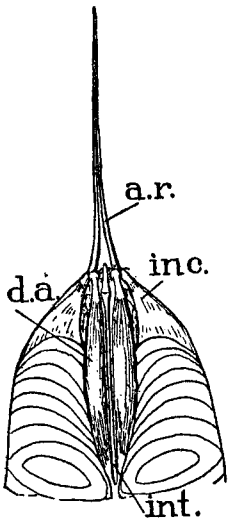

FIG. 12.—Section across the anal fin in the plane of the interhemal spines, the fin rays, and the erector-depressor group of muscles; *int.*, interhemal spine; *d. a.*, depressor analis; *inc.*, inclinator analis muscle; *a. r.*, anal ray.

ERECTOR-DEPRESSOR MUSCLE COMPLEX.

When the great lateral muscles are removed from the region of the anal fin a muscular mass is exposed lying under the superficial and deep lateral muscles and covering the interhema spines of the anal fin. This mass consists of alternate slips of muscles constituting the erector and depressor muscles of the anal fin respectively.

The whole group of muscle divisions is, like that of the dorsal fin, covered with a fibrous connective tissue sheath of considerable thickness. This sheath is continuous with that between the interhemal spines and is especially well developed in the longitudinal line marking the border between the hemal spines and the interhemals.

ERECTOR ANALIS.

The erector muscle of the anal fin is composed of the larger of the alternate divisions mentioned above as constituting the deep muscle complex. There is a muscle slip for each fin ray.

Each erector slip arises from the posterior margin of the interhemal spine in front of the one to which the ray is attached, and from the entire surface of the connective tissue septum between the two interhemals in question. Each muscle division is spindle shaped. It tapers at its ventral end into a short tendon, which runs to an attachment in the anterior basal margin of the corresponding fin ray.

The posterior slips of the erector analis are somewhat modified from the regular arrangement. The last pair of erector and depressor muscle slips is greatly enlarged, or rather the erector is greatly enlarged and the depressor moderately so. The fibers near the tendon of insertion pass over the modified interhemal cartilage to which the retractor ischii is attached, to insertions into the posterior border of the last anal fin ray. This fin ray is itself very small. The tendinous end of the muscle slides over a groove formed by the modified cartilages supporting the ray.

At the anterior margin of the series of erector divisions there is a muscular slip which seems to belong to the series, judging by its origin, but the insertion of which passes into the skin in front of the fin and near the base of the anal papilla.

The contractions of the erector muscles tend to elevate the anal fin and in continued contraction to hold it in the erect position. This is favorable in increasing the

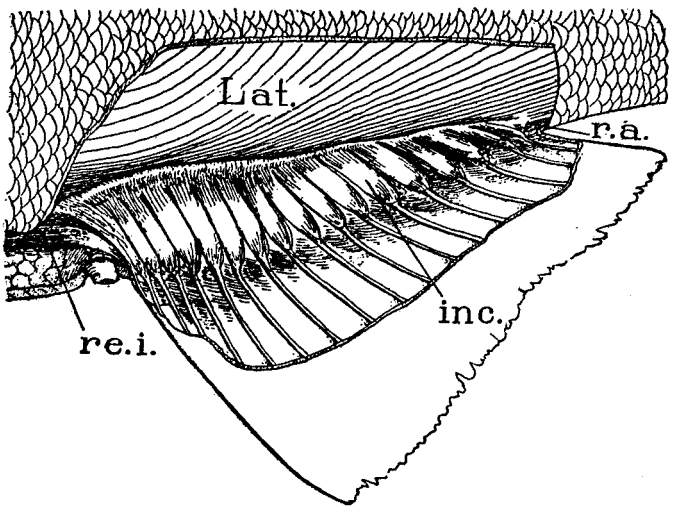

FIG. 13.—Superficial muscles of the anal fin. *inc.*, inclinator analis showing divisions for each ray; *re. i.*, retractor ischii (protractor analis); *r. a.*, retractor analis; *Lat.*, great lateral muscles. The dotted line indicates the ventral limit of the lateralis superficialis.

efficiency of the lateral movements which this fin contributes in balancing the fish in the water.

The contractions of the larger posterior muscle slip described tend to draw the posterior end of the anal fin sharply against the body and to some extent to antagonize the retractor analis. The function of the most anterior slip which has an insertion into the skin in front of the fin would seem to be in connection with the movements of the anal papilla.

DEPRESSOR ANALIS.

The depressor muscle consists of a series of slips which arise from the anterior surface and lateral margin of the interhemal of the segment to which each belongs and from the fascia separating it from the erector muscle attached to the same ray. The fibers of each slip run as a slender ribbon over the shaft of its interhemal to an insertion into the

posterior border and tip of the corresponding fin ray. Each muscle slip is somewhat thicker at its external border.

The muscle slips are from 30 to 35 mm. long anteriorly and 20 mm. at the posterior border of the series. They are about 4 mm. broad by 1 mm. thick in the middle of the muscle belly. They are closely wedged in between the bellies of the erector divisions. The anterior slips attached to the rudimentary rays are very small and slender.

The contraction of these muscles depresses the rays of the fin, tending to close it up against the body.

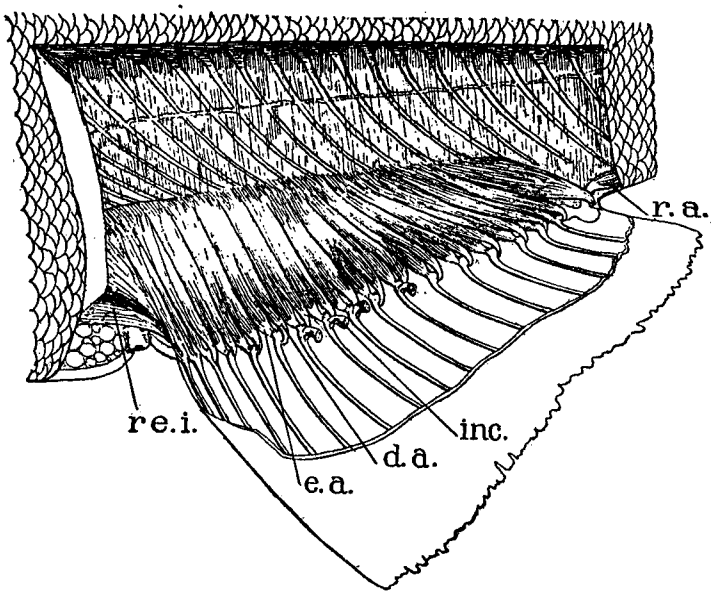

FIG. 14.—Deep muscles of the anal fin. *e. a.*, erector analis; *d. a.*, depressor analis; inc., four reflected tendons of the inclinator analis; *re. i.*, retractor ischii (protractor analis); *r. a.*, retractor analis.

INTERFILAMENTI ANALIS.

Between the fin rays of the anal fin, especially at the base and more strongly developed anteriorly, are found delicate muscle fiber bundles. These muscles are attached to adjacent fin rays, being attached nearer the base of the anterior ray, and more distally to the posterior ray, carrying them in an oblique direction from ray to ray.

Undoubtedly these slips aid in the elevation of the fin rays. They are very delicate and consist of only a very few individual fibers, a fact which easily leads to their being overlooked.

The protractor analis and the retractor analis are longitudinal muscles of the anal fin which have been described on page 34.

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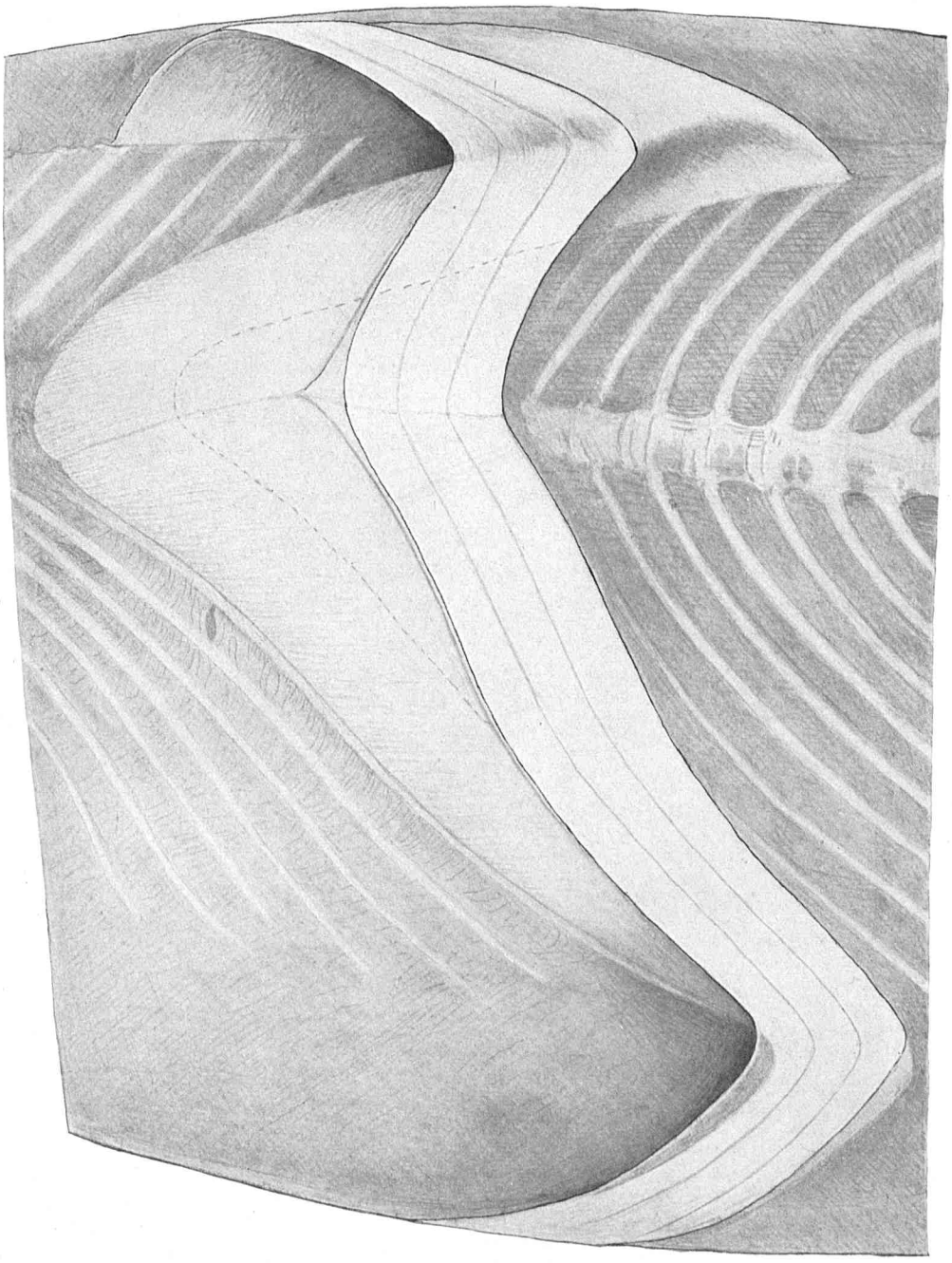
The variations in the form of the myomeres are shown in this figure of the left side of *Oncorhynchus tschawytscha*. The superficial markings of the myomeres and of the myocommata are indicated. There is some little variation, but of no great significance, at the level where the superficialis and the profundus join. It was deemed to detract from the significance of this figure to indicate this separating line, which is indicated in Plate II. The cephalic myocommata are attached to the base of the skull—to the occipital, temporal, and pterotic bones. They are not interrupted by the clavicle, which lies lightly embedded on the surface. The complexity of folding and the longitudinal extent of the myomeres increases posteriorly. The most important variation is due to the division of the anterior fold into two slender cones in the caudal half of the body. Magnification $\frac{1}{4}$.



Three myomeres isolated from the anterior part of the body, i. e., the plane just in front of the anterior border of the dorsal fin. The form of the myomeres and of the septa of the region is revealed. The longitudinal extent of the single myomere is 9 cm., though the length of the longest muscle fibers is scant 7 mm. The skeleton and its dorsal extension in the median septum are well shown. The protractor dorsalis and its supporting tissues lying between the anterior dorsal fold and the median septum have been removed. Magnification $\frac{4}{5}$.

THE DIRECTIVE INFLUENCE OF THE SENSE OF SMELL IN THE DOGFISH

By G. H. Parker

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THE DIRECTIVE INFLUENCE OF THE SENSE OF SMELL IN THE DOGFISH.

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The object of the professional fisherman is to bring fish within his reach. In some instances this is accomplished by direct attack, as in such primitive operations as harpooning and spearing, or by circumvention, as in most forms of seining. But in many cases some deceptive device is set up whereby the fish is lured to its fate. These deceptions are practiced on the fish through some of its various sense organs. Noises are made by the slapping of oars or other such implements on the water and thus fish are driven into gill nets or entangled in trammels. Such influences probably affect the fish through the ear, the sense organs of the lateral line system, or the skin, thus involving hearing and touch. Lights are often carried on boats at night and small fish are thus attracted in sufficient numbers to be easily caught by hand nets, a procedure dependent upon the organs of sight. But fishing in the ordinary acceptation of the term implies the use of some deceptive attraction in the form of bait, which, as originally used, depends on the feeding habits of the fish and has to do with its senses of smell and of taste.

The sense of taste has been almost universally attributed to fish, and most fishermen, naturalists, and even anatomists have assumed that fishes possessed a sense of smell. Of recent years some comparative physiologists have denied this sense to fishes. In their opinion the nasal organ of the fish acts more as an organ of taste than an organ of smell. This conclusion was thrown in question by the observations of Aronsohn (1884) and of Baglioni (1909), and was refuted by the experiments of Parker (1910, 1911), Sheldon (1911), and Copeland (1912). By these studies it was shown that fishes scent their food in essentially the same manner that air-inhabiting vertebrates do and with the corresponding sense organ, the nasal organ.

The following investigation has been carried out with the view of ascertaining the exact method by which a fish finds its food or may be caught by a bait. The work has been done on the smooth dogfish of the Woods Hole region, *Mustelus canis* (Mitchell). The gustatory, chemical, and olfactory senses of this fish have already been studied by Sheldon (1909, 1911), and it was therefore an unusually favorable subject for the investigation of those activities which depend upon the sense organs named.

The experiments were carried out for the most part in a small pen, 24 feet long by 6 feet wide, in one of the pools of the Woods Hole station. The pen was bounded partly

by the walls of the pool and partly by chicken wire attached to poles driven into the bed. It was freely open to the changes of tide. Fish from an adjacent pool were transferred to it from time to time for experiment and observation.

When three or four dogfish were liberated in the pen, they swam leisurely up and down its length in a mid depth of water, occasionally resting on the bottom. If, during their swimming, some crushed crabs wrapped in cheesecloth were thrown into the pen, they quickly changed their method of locomotion. When a fish in the course of its ordinary swimming approached to within a few feet of the packet of crab meat, it usually made a sudden movement to one side with its head, swam at once to the bottom, and in quick circuitous turns, often in the form of a figure eight, swept the bed of the pen. In a short time it narrowed its search to the immediate vicinity of the packet and when at last its mouth was brought close to the bait, this was seized, shaken, and carried off. Occasionally the packet was not found and the fish then resumed its ordinary method of locomotion until again by accident it came close to the packet, when it would repeat the movements already described. The crab meat used in these experiments was always wrapped in cheesecloth to exclude the possibility of the recognition of the meat by sight. The dogfish, as is well known (Sheldon, 1911), has extremely poor vision, at least in daylight, and seldom responds to an object until the latter is within a foot of it.

If the nostrils of a set of fishes to be experimented upon are filled with cotton wool before the animals are liberated in the pen, no attention whatever is paid to the packets of crab meat, though the characteristic reactions return shortly after the removal of the cotton. These experiments, first tried by Sheldon (1911), show that the dogfish is excited by food through the stimulation of its nasal organs and that it subsequently locates the food by the same means; in other words, the dogfish scents its food as air-inhabiting vertebrates do.

The question that I set to decide was that of the precise method by which the dogfish reached its food. After the initial excitement, does the fish move aimlessly about till, by accident, it runs upon the packet of crab meat, or is it directed toward this packet by some special form of stimulation? In working on this problem it proved important to observe two conditions. First, it was found that dogfish of medium size were more favorable for these experiments than larger or smaller ones. In general, fishes about 2 feet in length were found to be most satisfactory, and these were used in most of the experiments. Secondly, the dogfish reacted well only when in companies. A single dogfish in the pen seemed to suffer an inhibition of its activities excepting those directed toward returning it to the school. It swam about with incessant efforts at escape and paid very little if any attention to baits or food. It was therefore found best to work with several fish at once, and usually a set was chosen in which the individuals differed enough in size and markings to make them severally distinguishable. Often in a given set the larger ones were prepared in such a way that they showed no response to the packet of crab meat, and the smallest one of all was made the subject of the special experiment. This one individual was provided with a favorable environment for its own reactions without being subjected to disturbances from others.

If the substances emanating from the packet of crab meat exert a directive influence on the dogfish through the sense of smell, evidence of this might be discovered by inter-

fering with one or other of the nasal organs. This might be accomplished by cutting the olfactory tracts of one side or by temporarily occluding one of the nasal apertures. Fish in which the olfactory tracts have been cut rarely live more than a few days after the operation. They are apparently very susceptible to infection from cuts made in the vicinity of the brain. I therefore abandoned this method of procedure and adopted that of filling the nostrils with cotton wool. But even this method is not without its defects. If a single nostril cavity is plugged tightly with cotton the fish will often fail to respond to the presence of food precisely as it does when both are filled. On removing the cotton from such cavities they are generally found to be inflamed, if not suppurated, showing that their surfaces are decidedly delicate. None of these complications arise when the nasal cavity is only lightly filled with cotton and yet this method seems to be effective in checking the currents of water through the nose. I therefore adopted a light plug of cotton wool as a means of excluding the action of a given nasal organ.

To ascertain the state of the normal dogfish, records were taken of the direction of the head movements, to the right and to the left, in response to the presence of bait, and of the time consumed between the moment when the packet of crab meat was first scented by the dogfish and when it was finally seized and carried off. These records served as a basis for comparison with the reactions of fish especially prepared for tests. The records of the normal fish are given in table 1.

TABLE 1.—RECORDS FROM FIVE NORMAL DOGFISH.

Number of fish.	Movements of the head.		Time in seconds to secure bait.
	To left.	To right.	
1	14	16	186
2	22	20	135
3	27	28	108
4	17	22	161
5	21	18	67
Averages	20.2	20.8	131.4

From table 1 it will be seen that the dogfish tested found the bait in a little over an average of two minutes and that they accomplished this operation by making about as many right-handed as left-handed movements. Many of their movements were combined and resulted in more or less continuous and characteristic courses in the form of a figure eight.

In a second series of tests dogfish in which the left nostrils had been lightly filled with cotton were subjected to the same conditions as were the normal fish whose reactions are recorded in the preceding table. The records of five of the fish with the left nostrils occluded are given in table 2.

TABLE 2.—RECORDS FROM FIVE DOGFISH WITH LEFT NOSTRIL OCCLUDED.

Number of fish.	Movements of the head.		Time in seconds to secure bait.
	To left.	To right.	
1	3	26	132
2	6	32	116
3	0	24	110
4	2	16	121
5	4	32	141
Averages	3	26	124

As table 2 shows, the five dogfish with their left nostrils occluded consumed on the average about the same length of time to find the bait that the normal dogfish did, but they accomplished this by a predominance of right-handed movements rather than by an almost equal number of right and left handed turns. Their movements were essentially circular and seldom, if ever, in the form of a figure eight.

A third series of dogfish were prepared by filling their right nostrils lightly with cotton wool. As might have been expected from the results already recorded these fish found the bait in about the same time as those with the left nostrils occluded, but by means of left-handed movements chiefly. The records of this set are given in table 3.

TABLE 3.—RECORDS FROM FIVE DOGFISH WITH RIGHT NOSTRIL OCCLUDED.

Number of fish.	Movements of the head.		Time in seconds to secure bait.
	To left.	To right.	
1	31	2	123
2	34	1	92
3	21	2	111
4	17	5	116
5	19	3	131
Average	24.4	2.6	114.6

As tables 2 and 3 show, dogfish with one occluded nostril each tend to seek their food by moving over a more or less circular path and in such a way that the open nostril is toward the center of the circle.

Having ascertained that in seeking food or a bait normal dogfish turn about as much to the right as to the left, and that those with an occluded nostril turn predominantly toward the side of the open nostril, I attempted as a check series to repeat all the experiments thus far described on one set of fish. The plan of this series was to test five dogfish, first as normal individuals, then with their left nostrils occluded, next with their right nostrils occluded, and finally with both nostrils free. The fish were allowed to rest one day after each trial with occluded nostrils, so that the whole series of experiments covered a period of about three days. Unfortunately, during this period two of the fish made their escape and the series was completed with only three fish. The records of these three fish throughout the whole set of tests are given in table 4.

TABLE 4.—RECORDS FROM THREE DOGFISH UNDER SUCCESSIVE CONDITIONS, NORMAL, LEFT NOSTRIL OCCLUDED, AND BOTH NOSTRILS OPEN.

State of fish.	Number of fish.	Movements of the head.	
		To left.	To right.
Normal	1	21	26
	2	18	16
	3	31	28
Average		23.3	23.3
Left nostril occluded	1	3	24
	2	4	31
	3	3	30
Average		3.3	28.3
Right nostril occluded	1	26	3
	2	28	0
	3	19	4
Average		24.3	2.3
Both nostrils open	1	19	18
	2	21	17
	3	16	27
Average		18.7	24

It is quite evident from an inspection of table 4 that the conditions recorded in the preceding tables are not due to individual peculiarities on the part of the fish used; that a given fish, which in finding its food normally turns as much to the right as to the left, can be forced to assume either a predominantly right-handed or left-handed course by occluding the appropriate nostril, and that after this treatment the same fish can recover its normal methods of search by the liberation of both nostrils. It must also be evident from the whole series of records that the dogfish does not run upon its food by accident, but finds it in response to an influence more less directive in character.

The consistent and striking circular courses that these fishes can be forced to assume have, in my opinion, more than a superficial resemblance to the so-called circus movements of the invertebrates. These movements are dependent on the differences of intensity of stimulation on the two sides of the body and this explanation holds, I believe, for the circular movements of the dogfish. When a dogfish first enters water permeated with odorous material from its food, it invariably makes a quick turn with its head which, if the conditions of the water have been disturbed by currents, is always toward the bait. This movement is followed by other movements of a like kind whereby the fish eventually reaches the bait. When the normal conditions of the fish are disturbed by the complete occlusion of one nostril, the fish swims as though it were in water that was highly charged with odorous particles on the side of its body corresponding to the open nostril and devoid of these particles on the opposite side. The fish therefore turns toward the side of the open nostril, but since, under the artificial conditions of the experiments, this turn does not equalize the stimulus, the motion is continued and a circular form of locomotion results. Thus, in my opinion, the more or less circular movements induced in a dogfish with an occluded nostril by an odorous bait are to be explained upon the same basis as the circus movements of such invertebrates as crustaceans, insects, etc.

The movements of a dogfish differ from the movements of these lower animals, however, in that they are not pure circus movements. A dogfish with a fully occluded left nostril not only turns to the right but sometimes to the left, and a normal dogfish so often exhibits movements in the form of a figure eight that it is quite clear that the whole figure is a single response rather than two separate acts due to alternate excessive stimulation first on one side and then on the other. Thus, though the responses of the dogfish to the odors of food may be based predominantly on the principle of symmetrical stimulation, it is also clear that odorous material calls forth from this animal what are essentially random movements.

In consequence, the finding of food or of a bait by a dogfish may, therefore, be described as brought about by a combination of movements, partly random and partly directed, which have resulted from stimulations due to the varying concentrations of odorous materials in the surrounding sea water. The dogfish, like other elasmobranchs, has a structure especially favorable for this form of stimulation in that its nostrils are wide apart, a condition which is immensely exaggerated in the hammerhead shark, whose nostrils as well as eyes are lodged at the extreme ends of the transversely extended processes of its head. These conditions make it clear why chumming is so effective with mackerel and other fishes. When a fisherman spreads bait to attract such fishes from a distance the response is undoubtedly directive, especially on the part of sharks, which have been seen to come up to food against the tide from as great a distance as a quarter of a mile. Such fish must keep within the stream of odorous substance in the water by responding to the stimulation of their nasal organs in much the same way as the dogfish was found to do in seeking a bait.

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THE STORAGE OF FAT IN THE MUSCULAR TISSUE OF THE KING SALMON AND ITS RESORPTION DURING THE FAST OF THE SPAWNING MIGRATION

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INTRODUCTION.

To the present no study has been made of the distribution of fats in the musculature of the king salmon, *Oncorhynchus tschawytscha.* We have the classic studies of Miescher[a] on the quantitative chemical variation in the fats of the Rhine salmon, *Salmo salar;* also the studies of Noël Paton and his coworkers on the same species from the Scottish rivers. Miescher found, as did Noël Paton after him, that there was a great decrease in the quantity of fats as the fish ascended the rivers and as the spawning season approached.

Miescher also observed fat in the muscle fibers in considerable quantity. This he considered to be a stage of fatty degeneration preliminary to the use of the fats for the building up of the immense store of food materials present in the salmon ova at the time of spawning. Miescher states that the fat granules increase in numbers in midsummer, and are absent after the salmon have spawned. He also states that the fats are practically absent in the fibers of the muscles of the smaller fins and of the head.

Mahalanobis,[b] as a part of Noël Paton's series of studies, made a special histological examination of the fat in the muscles under the subtitle "Microscopic observations on muscle fat in the salmon." There are six pages of text and seven microphotographic figures presented in his paper. Less than two pages are used to present the findings as to the distribution of fats in the muscles. He contrasts the variations in the fat of "a fish fresh from the sea" with fish taken up the rivers at later dates. The greater part of the text is consumed in a discussion tending to disprove the "fatty degeneration" theory which Miescher has assumed, and Miescher is undoubtedly in error and Mahalanobis right in this particular matter. But Mahalanobis has failed in his special comparisons between two extreme types of fish from the simple fact that he based the comparisons on dissimilar muscles. (See p. 121 of this paper.) The studies on the Atlantic salmon have, therefore, left us without adequate description of the normal histological distribution of the muscle fats. As for the variations in the microscopic

[a] Miescher, Friedrich: Statistische und biologische Beiträge zur Kenntniss vom Leben des Rheinlachses im Süsswasser. Schweizerischer Fischerei-Ausstellung in Berlin, s. 154, 1880. Also reprinted in Die histochemischen und physiologischen Arbeiten von Friedrich Miescher, s. 116, Leipzig, 1897.

[b] In: Paton, D. Noël, Report of the Fishery Board for Scotland, 1898, p. 143.

picture as the salmon proceed up the rivers, one may say, with all due respect to the previous work, that such picture has not yet been given for any species.

The king salmon of our Pacific waters is an entirely distinct genus from that of the Atlantic salmon. It is decidedly unique in its biological characteristics, as shown in a number of fundamental ways. These characteristics have been enumerated at various times,[a] but three facts of peculiar importance may again be stated here as most vital to this investigation:

First, *Oncorhynchus tschawytscha* is an anadromous fish.

Second, it fasts completely during its entire journey from the sea through the tidal waters and up the rivers to the spawning grounds.

Third, *Oncorhynchus* always dies within a short time after spawning.[b] It can not, therefore, return to the sea for a second period of feeding.

These facts, taken in connection with my numerous quantitative chemical determinations revealing variations in the percentages of the fats within wide extremes, form the scientific background of interest in this investigation. There are enormous economic interests involved on the Pacific coast in the catching of the king salmon and the conserving of the flesh as food. There is a well-known great variation in the grade of the commercial products derived from this fish. These facts add to the scientific interest in the problem, and emphasize the necessity of a study of the histological distribution and utilization of the fats in the king salmon, the largest and finest of all the members of the family of Salmonidæ.

At the time the king salmon leaves the ocean for the fresh water it has reached the crest of the life cycle as regards the amount of fat deposited in its tissue. In the Columbia River those salmon caught at the lowest point at the mouth of the river are the fattest of all. The salmon do not feed, i. e., they do not absorb new food materials, during their return passage in fresh water. There is no other adequate storage of food material than this fat. One may, therefore, safely assume with Miescher that the fat is the chief source not only of the fats that go to build up the ovaries but also of the energy liberated in muscular contractions during the migration journey. One may also assume as a working hypothesis that the fat will decrease in amount until the spawning is accomplished and the death ensues. I have followed the variation in chemical composition in the king salmon muscles, including the amount of fat at the different stages of the spawning migration, work that is represented by unpublished results obtained from chemical analytical determinations, and have also followed the microscopic distribution and variations of the fat in fat-stained muscular tissues. It is the results of this latter work to which attention is called in this paper.

MATERIAL AND METHODS OF INVESTIGATION.

The facts presented in this paper concerning the salmon fats have been determined by the microscope.[c] The chief reliance has been placed on the special methods for

[a] Greene, C. W.: Physiological studies of the chinook salmon, Bulletin of the U. S. Bureau of Fisheries, vol. XXIV, 1904, p. 429–456.

[b] Evermann, B. W.: A preliminary report upon salmon investigations in Idaho in 1894, Bulletin U. S. Fish Commission, vol. XV, 1895 (1896), p. 253. Also, A report upon salmon investigations in the headwaters of the Columbia River in the State of Idaho in 1895, together with notes upon the fishes observed in that State in 1894 and 1895, ibid., vol. XVI, 1896, p. 184.

[c] I wish to express my deep obligation to Mr. Thomas J. Heldt, instructor in anatomy, University of Missouri, and to Mr. George T. Kline, biological artist, University of Missouri, for their valuable assistance in the taking of samples and in the making of the numerous field observations on fresh materials, on the excellence of which much of the detail of this paper rests.

staining fats for microscopic determination. The particular technique used throughout the series is the Herxheimer method of staining with scarlet red as modified by Bell. This direct histochemical method has been supplemented by histological preparations fixed and sectioned in paraffin and treated with various differential stains.

Selection of salmon types.—The problem of fat variation in the salmon during the migration is twofold. First, there is variation in any one region of the body at different stages in the migration journey, and second, there is variation in the amount of fat present in different parts of the body at any one time. In order to determine the variations of the first class one must, of course, select typical localities representative of different stages in the journey. For this comparison I have used salmon from the Columbia River Basin, selecting four stations, the first one being at the mouth of the river, working from the town of Ilwaco; the second below the cascades, working at Warrendale; the third at Celilo Rapids and Celilo Falls, working from Seuferts; and the fourth at the spawning grounds on the Clackamas River at Cazadero.

There is a great variation in type among the individual fish present at any one station at any given time. It therefore becomes a matter requiring some considerable skill to select consistent types throughout a comparative series. Only after several years of experience can one select these types with some little assurance that he will be following similar salmon through the variations that occur at the different stations or stages in the migration journey. The attempt was made to secure fish just as early in the migration as possible, i. e., the stage at which the feeding ceases and the migration begins. In several years of collecting at Ilwaco at the mouth of the Columbia River, I have found that this very desirable stage can not be had within the fishing limits of the locality; in fact, the cessation of feeding must begin some considerable time before the fish reach the fishing zone. In order to secure the facts as to the normal fat loading, it was necessary to make a study of the feeding salmon at Monterey, where salmon that are known to reenter the Columbia Basin are caught during the feeding stage.

Salmon from the four stations on the Columbia mentioned above were collected during the months of August and September, 1911, 30 salmon in all being studied in detail. The stations were visited in the order in which they are named above. Invaluable aid and cooperation were constantly received from the local fishermen and from the packers at the various stations.[a] There is an undoubted seasonal variation in the condition of salmon on the Columbia River, but by a rapid collection of material from station to station the effects of the seasonal variations on the series ought to be reduced to a minimum. The finest salmon at Ilwaco, for example, as regards the amount of fat, are reported by the cannery men to come earlier in the season, though the fact has not yet been determined by scientific methods of measurement.

As a routine practice, salmon from each station were chosen of only two classes, the choice or best type from the packers' point of view, and the poorest type, as interpreted by the same standards. Only the choice sizes, ranging in length from 80 to 100 centimeters were selected in this series.

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[a] I am particularly indebted to the P. J. McGowan Co. for aid in securing salmon types, and for numerous material favors in the pursuit of the field work at Ilwaco and at Warrendale. The Warren Packing Co. and the Columbia River Packers' Association granted the use of their receiving house on the Ilwaco Dock for a laboratory at that station. Mr. Frank A. Seufert, of The Dalles, granted an unlimited supply of salmon from his Celilo fish wheels and the Tumwater seining grounds at Celilo Falls. The Cazadero observations were made at the station of the U. S. Bureau of Fisheries at that point on the Clackamas River, where the hatchery force rendered liberal and invaluable assistance.

Selection of tissue.—In order to follow the variations of fat in different regions of the body one must, of course, select typical tissues as regards their relations to fat. For this purpose I have chosen primarily the great lateral muscle, because it represents by far the greatest mass of the fat-storing tissue of the salmon. This muscle is divided into two types; the deep or pink muscle and the superficial or dark muscle. Each extends from the head and pectoral girdle to the base of the caudal fin. There is considerable variation in the amount of fat in different parts of these muscles. Two type regions of the lateral muscle have, therefore, been selected for study. The first is the mid region of the side of the body, in the transverse section which cuts the body just at the front margin of the dorsal fin. This with the muscle anterior to the section is the fattest portion of the great lateral muscle mass. Samples from this region are called trunk muscle, dark or pink, respectively, as the case may be. The second region of the great lateral muscle chosen is that portion at the base of the tail opposite the fifth and sixth caudal vertebræ, counting from the posterior end. This region is called the caudal muscle, dark or pink, according to its type. The caudal muscle always has a relatively less amount of fat than the trunk muscle. It is assumed that there is a more or less gradual variation from the caudal region to the mid lateral or trunk region as regards the percentage amount of fat.

While the primary comparisons are with reference to the two regions of the lateral muscle mentioned, still a considerable number of samples were taken from other muscles; namely, the anal fin muscles, the "belly" muscles, the intercostals, and the cheek muscles.

Fixation of tissues.—In view of Bell's[a] experience, it was thought better to make the examination of the fat in the salmon muscle in as fresh condition as possible, and a full Bardeen freezing microtome equipment was therefore taken into the collecting field. Samples of the tissues from the type localities were thus cut, stained, mounted, and studied with the minimum of delay. It was quickly found to be quite impossible, however, to carry through all the tissues in the fresh condition. There was not time enough, with a field force of three, to do the necessary work before disintegration began. It was therefore found necessary to use a fixative, i. e., 10 per cent formalin.

As a matter of routine practice, tissues that were to be held in the prolonged and tedious processes of working up the material were always fixed in 10 per cent formalin. This fixation was not found to be detrimental to the preservation of the fat, but on the other hand, favorable. The frozen sections cut after two hours or more in formalin were firmer, retained their shape better, and therefore were not so much torn and distorted in the process of handling necessary to staining. In short, the formalin-fixed sections enabled one to arrive at a better conception of the relationships of the fat than is to be had from the tissue cut directly from physiological saline. After a series of observations made along these lines it was decided to pass all the tissues through a formalin fixation. It is not claimed that a long immersion of tissues in formalin is wholly without effect on the fats, yet for salmon muscle we are convinced that the change produced is very slight. In certain samples sections of fresh tissues have been compared with sections made after four months' fixation in formalin. The difference in quantity of fat shown is not easily determined, but there is some variation

[a] Bell, E. T.: The staining of fats in epithelium and muscle fibers. Anatomical Record, vol. IV, p. 199. 1910.

in the picture—enough to prevent one from using any but freshly fixed tissues for close comparative estimates.

In taking samples, in all instances where possible the muscles were quickly cut from the salmon selected while the tissue was yet alive. The muscles were cut in thin pieces with a razor and immersed in a large excess of 10 per cent formalin. Other tissues, such as the liver, cæca, stomach, etc., in which fat studies were to be made were also included. Portions of the material not studied on the collecting grounds were preserved for future use, some of which still retains its fat in approximately the normal quantity and relations (after six months).

Portions of the muscles and of certain other tissues were fixed in different standard preservatives for future study and comparison. But as these studies are not presented in this paper the detail of fixation will be omitted.

Fat stain used and its preparation.—Sudan III and scarlet red are the fat stains on which great reliance is placed in the histological identification of the fats. Since the salmon tissues are so filled with fat it was decided to use the scarlet red as giving the sharper differentiation. Bell has made a series of special studies on the microchemical staining of fats. It is to him I am indebted for the modifications of the Herxheimer method which were followed.

The scarlet red was prepared in saturated solution in alkaline alcohol: Scarlet red, 2 grams; sodium hydroxide, 2 grams; 70 per cent alcohol, 100 cubic centimeters. This was ripened in a 4-ounce wide-mouth vaseline bottle in a water bath at about 75° C, for from 20 to 30 minutes. The ripening took place in a closed bottle. But it was found essential not to heat too strongly lest the reactions lead to the production of a die that would stain general protoplasm. This stain is supersaturated while warm and some crystalization will occur on cooling. Bell's recommendation that the stain be filtered just before using was followed, also the precautions recommended by him against evaporation and sedimentation while staining. This stain retains its properties very well for a very much longer time than one is led to expect from the directions published by Bell.

Technique of sectioning and staining fresh or formalin-fixed material for fats.—Either fresh tissue or tissue fixed in 10 per cent formalin was frozen with carbon dioxide on a Bardeen freezing microtome. The fixed material can be cut as thin as 25–30 μ with comparative ease, but the temperature of the block must be just right. The sections were received from the microtome knife directly into 70 per cent alcohol and stained as quickly as possible.

The sections were handled always with a spatula of proper size. They were stained directly from 70 per cent alcohol. After 5 to 10 minutes in the scarlet red stain the sections were passed as quickly as possible through 70 and 30 per cent alcohol into water. The rinsing was sometimes through the alkaline alcohol of the grade in which the stain was dissolved. This is a safer plan to prevent precipitation of the excess of stain from adhering to the surface of the sections.

When sections were to be counterstained a short washing in acid water, 0.2 per cent hydrochloric acid, preceded the hæmatoxylin counterstain. The acid treatment was finally adopted as a routine procedure for all sections.

The stained sections were mounted directly from the wash water into pure glycerin. The glycerine was of course slightly diluted by the adherent water. The glycerin mounts were sealed with paraffin, or better with paraffin-beeswax cement.

The splendid keeping qualities of many of the scarlet-red preparations made during the investigation is attributed to a thorough neutralization of the alkali in the bath of acid water.

TYPES OF SALMON MUSCULAR TISSUE AS REGARDS THE STORAGE OF FAT.

The musculature of the salmon[a] is relatively simple. The main mass consists of the great lateral muscle of Cuvier. A number of smaller muscles are associated with the various fins and with the structures in the head region. But the storage of fat takes place chiefly in the great lateral muscles.

Great trunk muscles.—The great lateral muscles include the major masses of muscular tissue along the sides of the body from the head and the pectoral girdle to the base of the tail. These masses represent from 60 to 70 per cent of the total weight of the mature fish when in prime condition.

The great lateral muscle of the salmon has lately been described.[b] In *Amiurus*[c] it is described as divided into five longitudinal portions more or less distinct. In the salmon there is a connective tissue septum, the lateral line septum, running the length of the body just under the lateral line. It separates the lateral muscle into a dorsal and a ventral portion, each of approximately the same size. Aside from this there is no further subdivision along the lines designated by McMurrich.

However, the great lateral muscle is divided into two distinct muscles on the basis of the well-marked anatomical and histological differentiations that have taken place. There is a superficial thinner portion which is anatomically distinct and easily identifiable because it is darker in color; and a deeper, wider, and thicker mass which is pink in color. These two divisions are well separated by a thin lamina of fibrous connective tissue bearing an excess of adipose tissue in most of its extent. The lateral line septum separates both the superficial and the deep muscles into dorsal and ventral divisions.

The fin muscles, and to a less extent the head muscles, are the ones in the most constant but slight activity in the daily life of the fish, and, strangely enough, whether for this reason or not, these muscles are not largely loaded with reserve fat.

Musculus lateralis superficialis, or dark lateral muscle.—The superficial dark muscle forms a type of tissue with respect to its loading of fat that has not previous to the preliminary notices of the present writer[d] been described, in so far as I can discover, though it was noted by Miescher.[e] He undoubtedly refers to the superficial lateral muscle when he says: "A thin muscle plate lying along the side of the body just beneath the skin degenerates most strikingly (Hautmuskel)." Miescher makes the reference quoted[e] in the brief discussion of the microscopic picture of the fat in the so-called fatty degeneration.

This muscle is characterized by the following points: (1) Its compact arrangement of fibers; (2) the smallness and uniformity of size of the fibers; (3) the relatively small

[a] It does not seem wise to regard the supracarinales and infracarinales as divisions of the lateral muscle in the ordinary sense of the designation.

[b] Greene, C. W. and Carl H.: The skeletal musculature of the king salmon. Bulletin U. S. Bureau of Fisheries, vol. XXXIII, 1913 (1914), p. 21-60, pl. I-II.

[c] McMurrich, J. P.: The myology of Amiurus catus. Proceedings of Canadian Institute, n. s., vol. II, 1884, p. 328.

[d] Greene, Charles W.; A new type of fat-storing muscle in the salmon, Oncorhynchus tschawytscha. The American Journal of Anatomy, vol. XIII, p. 175, 1912. Also an undescribed longitudinal differentiation of the great lateral muscle of the king salmon. Anatomical Record, vol. VII, p. 99, 1913.

[e] Miescher, op. cit., p. 186 (145).

amount of interstitial connective tissue; and (4) most important of all, by its enormous loading of fat.

Musculus lateralis profundus, the lateral pink muscle.—The profundus, or the deep division of the great lateral muscle, is the pink salmon muscle as it is ordinarily spoken of. It has a totally different appearance from the superficial dark muscle. The fibers of the pink muscle vary enormously in size, from 40 up to 250 μ in diameter in the adult. The average size of the fibers varies somewhat in different regions of the pink muscle even of an adult fish. In the young salmon, 10 to 15 cm. long, there is a greater range of variation than in the adult, as is shown by the outline figure 16, plate X. This is due to the fact that the fibers are undergoing longitudinal cleavage which is very unequal. This cleavage leads to a large number of very slender fibers. It is the method of reproduction of new fibers which leads to the irregular outlines noted in the cross sections of all the fibers of the profundus of the salmon, both young and adult.

The amount of supporting connective tissue in the pink muscle is relatively great. Beside the blood vessels, a large amount of adipose tissue is present. It is the adipose tissue of the pink muscle, which is heavily loaded with fat, that carries the greater part of the fat of the salmon commercial products.

Myocommata of the great lateral muscles.—The myocommata which separate the muscle myomeres are always crowded with fat in the normal adult salmon. These connective tissue partitions are composed of white fibrous connective tissue into which the short longitudinally placed muscle fibers are attached. The tissue is largely filled with adipose cells. Its fat cells are large and relatively uniform in size when filled. They form a considerable mass within the myocommata most thickly studded near its center. There are also large numbers of fat cells on the surface and crowded beneath the ends of the muscle fibers. This fat forms no inconsiderable portion of the storage fat present in the adult salmon.

Supracarinales.—There are longitudinal-paired muscles along the middle of the back of the salmon. These extend from the head to the dorsal fin, the supracarinales, and from the dorsal fin to the adipose fin and to the dorsal lobe of the caudal fin. These paired muscles are cylindrical in shape and about the size of a lead pencil in the thickest part, but spreading out somewhat anteriorly. The muscles are of interest in this connection chiefly because they are imbedded in a relatively thick and prominent mass of adipose tissue. In a prime fish this adipose tissue is crowded with fat.

Infracarinales.—A similar collection of adipose tissue is even more striking along the mid-line of the belly of the salmon. The fat of the belly surrounds the protractor ischii anteriorly and the retractors posteriorly. The mass is from 1 to 2 cm. thick and twice as wide in a prime 80 cm. fish. The cylindrical muscles inclosed will be about 0.8 to 1.5 cm. in diameter, and the rest of the area an almost solid mass of fat cells. In a fish low in fat the fat is taken up, leaving a white fibrous connective tissue mass. These two areas of adipose tissue form a considerable storehouse of salmon fat.

Muscles of the fins.—The storage of fats in the fin muscles has been studied only sufficiently to demonstrate the type. Adequate comparisons have not been made to make the observations complete, except in establishing the normal type. The fin muscles of the pectoral, ventral, dorsal, anal, and caudal fins (the deep caudal muscles) are much alike in general fat loading. Sections of the erectors and of the depressors of the anal fin have been most extensively examined. The two muscles are much alike in general

appearance, are a light reddish-brown color—neither pink nor as dark as the superficial lateral muscle. The constituent fibers are loosely attached to the interhæmal septa, and the pair of muscles between adjacent interhæmal spines are incased each in a stout connective tissue sheath. This arrangement is also characteristic of the corresponding muscles of the dorsal fin.

Muscles of the head.—The masseter muscle is the largest of the muscles of the head. Under the name of "cheek muscle," the masseter is highly prized as a delicacy by the fishery folk. It is of good size, has its fibers compactly arranged, and is not colored like the trunk muscle of the salmon. The cheek muscle of feeding salmon has not been examined, but the Ilwaco and Cazadero types, representing the mouth of the Columbia River and the spawning grounds, respectively, are presented in the proper places. Other muscles of the head region have not been examined for fat.

NORMAL LOADING OF FATS IN THE MUSCLES OF THE KING SALMON AT THE TIME THE SPAWNING MIGRATION BEGINS.

It is exceedingly difficult to secure salmon just at the moment when fasting begins. In the first place, it is not easy to determine just when a salmon ceases feeding; that is, whether a given salmon in hand is one that is still feeding or one that has just ceased feeding. A second and more important difficulty is that of catching salmon at this critical stage in the life cycle. There are only limited regions where the king salmon are captured from the feeding grounds. There is no such place near the mouth of the Columbia River. The lowest point at the mouth of the Columbia where fish are caught is between the Canby Lighthouse on the north bank and the end of the Government jetty on the south shore. Salmon from this locality have already ceased feeding, probably some little time earlier.

Monterey Bay and its immediate vicinity is a popular ground for king salmon fishing. When the salmon schools are in this vicinity they are actively feeding and are readily caught with the trawl. During the spring and early summer months they are taken in large numbers. At this time a considerable business is done in the salmon fisheries at the city of Monterey. There is good evidence that the fish caught at Monterey Bay are from schools which ultimately enter both the Sacramento River basin and the Columbia River basin. This is indicated by the fact that on both rivers specimens are occasionally taken which have in their mouths fish hooks of the type used at Monterey.[a]

Monterey Bay is about 100 miles south of Golden Gate, the entrance to the Sacramento Basin. It is about 800 miles south of the mouth of the Columbia River. A well-developed salmon from Monterey would serve very well as a normal type for the Sacramento Basin, provided one could assure himself that the fish were on its way to and would enter the Golden Gate and the Sacramento. The amount of change that could take place due to additional feeding between Monterey Bay and the Golden Gate would be negligible, assuming a reasonably direct journey. If additional fat were stored it would be too slight to change the average materially. But facts tending to verify these assumptions can not readily be obtained.

[a] Mr. George Warren, of the Warren Packing Co. of Portland, Oreg., showed me a number of such salmon hooks taken from salmon that have come into their packing establishments on the Columbia River.

It is out of the question to assume that a Monterey fish destined to migrate as far north as the mouth of the Columbia River is in the condition that will exist at the time of arrival. Such a salmon is in the growing stage. It will certainly increase in size in so long a feeding journey and probably will also somewhat increase its fat content.

The Monterey fish are the only ocean-feeding fish available as examples of mature specimens typical of the Sacramento and to a certain extent of the Columbia Basins. It follows, therefore, that one must use these specimens as best he can for the purpose. The matter resolves itself, somewhat, into a question of the ability of the investigator to select and judge the type that most nearly approaches the mature one.[a] There is a wide extreme of maturity represented among the Monterey fish. The small fish give all evidences of being relatively young and growing specimens. The larger fish are proven to be the older ones by the work of Gilbert, who finds a close correlation between size and age.[b] His determinations indicate that these larger fish have been feeding in the ocean for four, five, or even more years, according to the size of the specimen chosen. It seems reasonable to assume that such fish will not undergo any very great change in the average fat content during the intervening months between the time of the Monterey feeding and the beginning of the spawning migration. The larger Monterey fish may be taken as the best available examples typical of the disposal of the tissue fat in the late stages of the feeding cycle. On this ground observations and protocols are presented on Monterey specimens.

In the chapter on the types of salmon muscular tissue as regards the storage of fat the muscle characteristics are given in sufficient length to enable one to use them in presenting the picture of the fats and fat variations. It remains now to give the detailed picture of the normal fat content of salmon muscle at the time when the feeding ceases. Under this category will be presented the following muscle types:

Normal fat content of the trunk pink muscle.—The pink muscle, which represents the greater proportion of the total mass of muscle of the salmon, contains an enormous total load of fat at the time the salmon cease feeding. Estimating on the basis of various chemical studies made in other connections, I would say that this fat loading varies between 15 and 25 per cent. This great variation represents the normal variation in fat content.

The fats of the pink muscle are distributed in the connective tissue between the muscle fibers—i. e., they are intermuscular. The pink muscle carries a relatively large amount of connective tissue which supports the muscle fibers and the blood vessels, and this connective tissue has a high percentage of adipose tissue. In it are found enormous numbers of fat droplets, which vary within a wide range of size. The smallest droplets are often not more than 1 or 2 μ in diameter, but there are numerous fat globules of this region that are as much as 100 μ in diameter. No figure is presented of this normal material, but figure 8, plate VI, drawn from an Ilwaco specimen (no. 118), represents very well the average appearance of the intermuscular fat of the normal tissue.

[a] The alternative is to figure back from the first available stage in the spawning migration. For the Columbia River this latter method I believe gives a truer picture of the normal condition. Attention will be called to this fact in the discussion of Ilwaco types.

[b] Prof. Chas. H. Gilbert, who is making extensive studies on the salmon migration and the salmon age, observes that there is, within certain limits, a close correspondence between size and age. It follows that the larger fish have a longer ocean-feeding period, a fact for which we have heretofore had no conclusive proof. Also it is evident that salmon mature sexually at greatly varying ages. (Gilbert, C. H.: Age at maturity of the Pacific coast salmon of the genus Oncorhynchus, Bulletin of the U. S. Bureau of Fisheries, Vol. XXXII, 1912, p. 1.)

There is little or no intramuscular fat in the normal pink muscle. The loading of the fat is intermuscular, in contrast with that in the dark muscle, where it is intramuscular. In the normal feeding, growing salmon there is no intramuscular fat, or at most only a trace of fat in the pink muscle. This condition exists up to the time when the salmon cease to feed. This statement is based on the examination of tissues of the smaller salmon in the rivers and also on the examination of different sizes, including the largest adults coming into the market at Monterey, Cal. In the latter there may be an occasional trace of liposomes within the smallest fibers. Monterey fish that will enter the Sacramento River basin can not be assumed to be wholly typical salmon that have ceased feeding. Yet I think it safe to consider these as sufficiently mature adults to serve for comparative purposes.

In the quite young salmon, from 7 to 16 cm. long, there is no fat in the pink muscle, either between the fibers or in the fibers along the lateral portion of the body. In the ventral or "belly" muscle there is some intermuscular fat at this stage of development. Salmon of this size are still feeding in fresh water. Of the sizes that one obtains at Monterey, which of course are feeding in salt water, fat is beginning to be deposited in the connective tissue between the fibers. This fat is relatively low in amount in the smaller fish. There is great variation in its amount in different individual fishes at Monterey, and while the number of fishes studied is very limited one can say that these indicate that the fat increases in quantity with the size of the fish.

An exception to the above description is found in a narrow zone of pink fibers lying on the surface of the pink muscle. This zone is immediately covered by the deeper layer of the dark muscle fibers. In these pink fibers there is always a slight amount of intracellular fat. This is a special case, the significance of which will be discussed in the chapter on the mechanism of fat transference in the salmon body. (Page 127.)

Normal fat content of the trunk dark muscle.—The trunk dark muscle is described on page 78 as characterized by an enormous loading of fat.

The storage fat is both inter- and intramuscular. It is present between the fibers in a relatively small number of medium-sized drops. These drops vary in size in the adult salmon from 5 to 20 μ in diameter, and are sometimes larger. This fat is most abundant in the immediate neighborhood of blood vessels. In longitudinal preparations it is seen not to be uniformly distributed along the length of the fibers.

The peculiar characteristic of the superficial lateral or dark muscle is its storage of enormous quantities of intramuscular fat. The fat is distributed within the fiber in two general relations. First, in the region between the sarcolemma and the substance of the muscle fiber proper, especially in the young fish (fig. 7, pl. v). It often happens that there is almost a complete ring of fat droplets surrounding the fiber and pushing the sarcolemma out and away from the fiber wall. In a paraffin preparation there will be a series of vacuoles under the sarcolemma, where the fat is extracted. Sometimes these fat drops have grown so large that they have fused or run together into larger masses of fat, but usually the droplets are smaller and remain separated. In the maximum loading these droplets are from 4 to 6 μ in diameter. This sarcolemmal fat is not uniformly present in all regions of the muscle, and in regions where it is absent the sarcolemma is in close approximation to the outer wall of the muscle fiber as usual.

Second, the intramuscular fat is present in large quantities buried within the substance of the sarcoplasm. Especially favorable points for deposit are the angles formed by

Cohnheim's areas. In these locations very large drops, comparatively speaking, are present. They are usually quite uniformly distributed over the surface of the fiber as seen in a cross section. The intracellular droplets vary in diameter from 3 to 6 μ. Beside the larger droplets there are always numerous smaller ones of varying sizes down to a fraction of a micron. Medium to small droplets may be present in close relation to the larger, all more or less evenly distributed among the larger droplets. The smallest droplets are of liposomic size and are deposited in shorter or longer chains between the fibrillæ or groups of fibrillæ. There is evidence that these liposomes[a] are arranged with reference to the striations of the fibrillæ, and it is suggested that such relation is of significance in reference to the function of the fats in the muscle.

Occasionally I have found an enormous fat drop filling up the whole central portion of the dark muscle fiber, the protoplasm of the fiber forming a band-like ring around the drop (fig. 14, pl. IX). Even in these cases the protoplasmic ring is closely studded with smaller fat droplets, in one case as many as 19 droplets 2 to 4 μ in diameter being crowded within the circumference of this protoplasmic ring.

The superficial lateral muscle begins receiving its excess of fat early in the development of the fish, at least as early as the fingerling stage. In this respect the muscle is in marked contrast to the deep lateral muscle, in which there is little or no deposit of intermuscular fat until a considerably later stage in the development of the salmon and no intramuscular fat until maturity.

The main points which characterize the fat disposal in the normal adult trunk muscles may be summarized as follows:

Summary of fat disposal in the normal muscular tissues.—1. The fat is most heavily stored in the superficial lateral muscle, where it is present in enormous quantity both between the muscle fibers and within the fibers. This tissue is heavily loaded with fat at a very early stage, at least by the 7-centimeter stage, and is always found loaded in the feeding fish.

2. The great pink muscle contains little or no fat between the fibers in the fingerling stage, but it has a small amount of such fat in the small Monterey Bay fish. The amount of this intercellular fat increases to its maximum at the time when feeding ceases. The intermuscular fat observed at Ilwaco is relatively high. While it is probably less than at the time of cessation of feeding, it is certainly more than at Monterey Bay.

3. There is no intracellular fat in the pink muscle during the feeding stage, or, at most, a trace of liposomes in the smallest pink fibers. The liposomic fat makes its appearance after the fast begins. An exception is found in the superficial zone next the dark muscle.

4. The fat in the fin and the head muscles is relatively insignificant in amount. It is both inter- and intracellular in its relations to the muscle fibers.

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[a] Various terms have been used to designate the microscopic fat droplets or fat-like droplets. They were first described by Kölliker as interstitial granules. This was before their fatty nature was sufficiently well known. In fact, Kölliker thought they were not true fat droplets. The term liposome was introduced by Albrecht to describe those interstitial granules of muscle which are demonstrated by the scarlet red stain. Bell has used the term "interstitial granules," but he considers the granules that take the scarlet red stain as used in his paper as fat bodies to which the term "liposomes" is applicable. (For historical discussion of the subject see Bell, Internationale Monatsschrift für Anatomie und Physiologie, bd. XXVIII, p. 297; also Anatomical Record, vol. 4, p. 199.) The term liposome is used in the present report to indicate the microscopic fatty bodies staining with scarlet red and of small size, usually under 3 μ, that take the characteristic scarlet red stain. It is not intended to carry any meaning suggestive of the chemical character as regards the specific kind of fat, though it is the opinion of the writer that neutral fats are the ones dealt with in the salmon tissue described in this paper.

5. There is a considerable store of adipose fat in the myocommata, in the adipose tissue around the small longitudinal muscles in the mid-dorsal and mid-ventral lines, and in the connective tissue of the skin. A slight amount of fat in the viscera should be mentioned.

PROTOCOLS.

Male salmon (no. 97), length 25.7 cm., taken at Baird, Cal., July 18, 1911.

This young salmon was caught with hook and line with salmon-egg bait from the deep pool opposite the fish hatchery at Baird, Cal., July 18, 1911. It was a relatively large summer fish derived from the last fall's hatch, as shown by the scale marks kindly identified for me by Prof. Charles H. Gilbert. It was kept alive in a fish can for seven days, after which it was killed and examined for fat. On examination it was found that the testes were well developed, almost mature, and white in appearance. Specimens of the alimentary tract and of the musculature were fixed in formalin for fat staining. Also samples were preserved for paraffin sectioning.

Microscopic examination of the trunk muscle for stainable fat.—Samples of the lateral muscle preserved in 10 per cent formalin were prepared after five months. Freezing microtome sections were stained for fat with alkaline alcoholic scarlet red and counterstained with hæmatoxylin. The fat was present in the trunk dark muscle in large amounts and had not been extracted in any appreciable amount by the long immersion in formalin. As the glycerin mounts were beginning to clear there was a stage of very sharp and distinct differentiation. The fat droplets in the body of the muscle were surrounded or at least partially surrounded with rings of fibrillæ.[a] The clear, brilliant scarlet red of the fat droplets contrasted sharply with the palisade-like bands or rings of fibrillæ. The sarcolemma at this stage of clearing made a clearly marked line inclosing fat droplets between it and the fibrillar areas. These latter fat drops are distinctly outside the areas of fibrillæ, yet some of them press slightly into the interfibrillar spaces. There is not much of this displacement of fibrillæ for the reason that the superficial area of the salmon muscle fiber is bounded by a continuous band of fibrillæ.

The fibrillæ are strap-shaped, i. e., their outlines in cross section are rod-shaped. The fibrillæ are set with their flat sides approximating each other and their narrow edges, therefore, bordering on the surface of the fiber in the case of the superficial area. It is this that gives the palisade-like arrangement in the superficial coat of the muscle. The continuity of the superficial band is occasionally slightly interrupted, since the rows of fibrillæ as seen in cross section here and there turn in toward the central portion of the fiber. Where such turns come there is a slightly greater quantity of sarcoplasm present.

Those dark muscle fibers nearest the skin seem more loaded with fat, although the whole layer is rather uniform in its loading. The striking thing about the material from this fish is the amount of fat which is under the sarcolemma. In general, the fat droplets in this region are fairly uniform in size and are spherical. But often a mass of fat seems compressed and spreads somewhat around the surface of the fiber. Numerous instances are seen in which such masses of fat extend around one-sixth to one-third the circumference of the fiber. If one gets a view of such a fiber isolated from the mass this type of fat droplet or group of droplets stands out like a great blister on the side of the fiber. These droplets are evidently compressed by the pressure of the sarcolemma. They no doubt exist within that sheath under a certain amount of tension.

The fat droplets within the substance of the fiber vary extremely in size and shape; the average of the larger drops is about 4.5 to 6 μ.

Through a typical section four striking variations from the general picture appear. In each of these an enormous fat drop has formed in the center of the muscle fiber. One of these fat drops measures 18 μ in diameter, while the fiber containing it measures 33 μ in diameter. The thinnest part of the muscular ring is 4 μ and the thickest 8 μ. Evidently in this instance an enormous fat drop has formed in the center of the fiber and crowded out the muscular substance into a superficial ring of protoplasm. In this case the ring of protoplasm is filled as full of fat drops as plums in a pudding. There are thirteen such droplets from 2 to 4 μ in thickness. There is not so much fat as usual between the sarcolemma and the muscle substance. Four such fat drops are to be counted in one locality. In another region of the section a fat cavity in the fiber measures 24 μ in thickness. The ring of protoplasm around it is not so thick as in the preceding instance, and the fat drop itself has been pushed to one side, though it is still adherent to the section. Smaller drops of fat are present in the ring of protoplasm. In yet another

[a] Greene, C. W.: A new type of fat-storing muscle in the salmon, Oncorhynchus tschawytscha. American Journal of Anatomy, vol. 13, 1912, fig. 1, pl. 1.

region are two adjacent fibers, each containing an extra large fat drop in the center of the substance of the fiber. One of these drops measures 15 μ in diameter, the other 20 μ in diameter. Here, also, the surrounding muscle substance is loaded down with the usual type of small fat droplets. One can not assume that the large drops of fat arise at the expense of formation of the smaller. Rather is it indicated that these drops are the result of a most active fat storing in this fish at the time it was collected.

The trunk pink muscle is free of fat in the main body of the muscle. There was neither fat between the fibers nor within the fibers. This statement does not hold for a thin layer of pink muscle lying just under the dark muscle. In this intermediate zone the pink muscles show a certain amount of intracellular fat. These fat droplets are never as great in size as in the dark muscle, but are largest in those fibers lying near the dark muscle layer. Passing from fiber to fiber in the direction away from the dark muscle, the amount of intramuscular fat rapidly decreases. This zone is, on an average, only five or six fibers thick. It underlies the whole extent of the thicker portion of the dark muscle.

Microscopic examination of paraffin sections.—These transverse sections were especially fine as giving a negative picture of the fat in the musculature. The sections show a thin membrane or sheath around the dark fibers, the sarcolemma. The interest attaches to the location and relations of this sarcolemma with reference to the substance of the fiber. The sarcolemma is in contact with the sarcoplasm for a portion of its extent round the fibers, but is distinctly separated from it in most of its circumference. The picture is as if the membrane were pushed out and away from the fiber. The space between the sarcolemma and the proper substance of the muscle is subdivided by very delicate strands extending across the intervening space and continuous with the interfibrillar substance. The form of the spaces, their size, and arrangement, all strongly support the interpretation that these spaces are filled with fat in the fresh condition. They are the cavities left when the fat drops are dissolved out, the fat that in the frozen section is so much more difficult to determine as regards its exact relation to the sarcolemmal sheath (fig. 7, pl. v).

The central portion of the muscle fiber presents numerous clear areas around which the fibrillæ are arranged in irregular circles. Where such a group of fibrillæ is unbroken, they usually stand with their broad dimension radial to the center of the clear area. However, there is no particular uniformity about the matter. This arrangement is best shown in figure 7, which is a camera-lucida outline under an oil-immersion lens. The larger angles formed in these whirls of fibrillæ are more or less filled with irregularly arranged and smaller fibrillæ. Between the fibers and forming a slight border along the rows of fibrillæ is the sarcoplasm. In most instances this sarcoplasm is sufficient in quantity to form a very thin sheet surrounding the clear areas already mentioned. The sarcoplasm can usually be distinguished as an extremely thin sheet around the most superficial fibrillæ. It is connected by delicate strands here and there with the sarcolemma.

The pink trunk muscle of these sections exhibits the great variation in size of fibers noted in the frozen sections. The ends of the fibrillæ are very distinct and clear. They are not broad and strap-shaped, as in the dark muscle, but are more thread-like and smaller. In the deeper portion of the pink muscle there is no evidence of interfibrillar spaces. In the intermediate zone of pink fibers, located just under the dark muscle, the fibers are more or less marked by clear spaces. These areas are relatively large and more numerous in the pink fibers lying nearest the dark and decrease in number and size in those fibers further away. Some of the pink fibers show irregular groups of small spaces just under the sarcolemma. In the smaller pink fibers the spaces are more numerous in the center of the fiber. The arrangement of the transparent spaces within the fibers and between the fibrillar portion of the muscle and the sarcolemma corresponds with the distribution of the fat droplets in the fibers of the intermediate zone, as shown by the scarlet-red staining.

Salmon (no. 75 and no. 76) collected at Monterey July 24, 1911, length 100 cm. (estimated).

Microscopic observation of the trunk pink muscle transverse section, oil-immersion lens.—The material was studied after three days' fixation in formalin. The striking picture is that of the intermuscular fat, which is present in large quantity. The fat drops vary in size from 3 μ up to 45 μ in diameter, the smaller drops being very numerous. The fat is far greater in amount than in the young specimen (no. 97) from Baird, on the McCloud River.

This section is well fixed by its three days' immersion in formalin. It shows a splendid picture of the fibrillar structure. The muscle fibers are without intracellular fat, or, at best, have only a trace. The large and most of the medium-sized fibers are perfectly free of fat. There are a few of the

smaller fibers and occasionally a medium-sized one which show a trace of fat around the superficial ring of protoplasm. Such fibers are surrounded with fat droplets massed on the surface of the fibers in the connective tissue. Some droplets are undoubtedly under the sarcolemma. It is this fat which gives the show of color at the superficial coat of fibrillæ. In the smallest fibers of the section some scattered liposomes of minute size are present between the fibrillæ of the surface of the fiber.

A longitudinal section of pink-trunk muscle (slide H81) shows numerous fat droplets of comparatively small size adherent to the surface of the fibers. The sarcoplasm shows the striations in splendid contrast, but no liposomes were to be found within the fiber.

The intermediate zone of pink fibers.—The line of separation between the pink and the dark trunk muscle is marked by a connective tissue septum. Occasionally a microscopic group of small fibers may be found on the dark-muscle side of the septum (sec. H82). These intermediate pink-muscle fibers have in their protoplasm a few liposomes, which are limited to the small fibers. There is not so broad a zone of intermediate fibers as was noted in the young muscle—for example, protocol no. 97. Well out in the field of pink fibers of section H82 there is an abundance of intercellular fat, but no evidence of intracellular fat.

Notwithstanding these exceptions, the general picture is that of muscle free from intracellular fat.

Microscopic examination of the trunk dark muscle of fish no. 75 (section H70).—Observation with one-twelfth oil immersion. The section shows an abundance of fat both between the fibers and within the fibers. The fat between the fibers is in droplets from 6 to 10 μ in diameter. The muscle fibers themselves are only from 25 to 50 μ in diameter and somewhat irregular in outline. The fat droplets are rather uniformly distributed among these fibers, though not so great in amount as in the same type of muscle from Ilwaco.

The intramuscular fat is present in large amount and very uniformly distributed through the protoplasm of the fibers. The droplets, strictly within the fiber, vary around 4 μ in diameter.

It is difficult to determine whether the fat droplets around the superficial zone are under the sarcolemma, and therefore intracellular, or lie outside this membrane. Certainly in a number of cases the former is the fact. In comparison with the dark muscle of the younger fish it is noted that the intracellular droplets of the Monterey muscle are more uniformly distributed through the protoplasm and have a more uniform size.

The intermuscular fat of the dark muscle of fish no. 76 (sec. H73) is in relatively large drops, 30μ on an average in diameter; but there is only a small proportion of the intercellular fat present in the finer droplets. The intracellular fat is rather uniformly distributed through the sarcoplasm; but the droplets are smaller than in fish no. 75, 2 μ, or slightly larger, in diameter.

VARIATION OF THE AMOUNT OF FAT IN THE SALMON DURING THE SPAWNING MIGRATION.

It is to be expected that the amount of fat present in different portions of the musculature of the salmon will vary sharply at different times during the migration. Whether this variation will be directly proportional to the time since the migration began remains to be discovered. The attempt in this paper is to present the normal distribution of fats at the end of the feeding period—i. e., the beginning of the migration phase—and to follow the variations through four typical regions of the Columbia River Basin. The regions chosen represent (1) the tidewater stage of the migration, (2) an early intermediate stage in the migration, (3) a later intermediate stage, and (4) the condition at the spawning ground and at the time of death.

As representing an early stage I have chosen a station at Ilwaco on the Washington side of the mouth of the Columbia River. At this point P. J. McGowan & Sons have a canning establishment, the lowest on the river. It is in the midst of the Bakers Bay field of traps and is the most accessible point to the lowest channel fishing done on the Columbia River.

For the second stage Warrendale, Oreg., about 6 miles below the cascades in the canyon of the Columbia, was chosen. The upriver cannery of P. J. McGowan & Sons

is located here. The region is accessible to fisheries which depend upon the catch of salmon below the cascades of the Columbia. The samples of this series were chosen from the fisheries at the seining grounds on the Washington side about 1½ miles below Warrendale.

The third stage was chosen at the Frank A. Seufert's fishery at The Dalles, on the Columbia. The fish wheels and seining grounds along the course of the Columbia below the Celilo Falls furnish splendid opportunity for salmon which have run the lower mountain course of the Columbia River through the cascades and through the lower portion of the rapids of The Dalles.

The spawning-ground stage was that on the Clackamas River, Cazadero, Oreg. A United States fishery is located here. This is the most accessible, in fact, the only point where spawning salmon can be had during the time of the year in which the field work was done.

DISTRIBUTION OF THE FATS OF THE SALMON MUSCLE AT TIDEWATER.

At the mouth of the Columbia River the salmon have already ceased feeding and the muscles have begun to show the first stages of change in the amount and distribution of the fats. This change is readily detected in the pink muscle, though not so in the dark muscle. In the dark muscle the amount of fat is so great that one has no adequate microscopic comparisons for showing the variations. But it is easy to convince one on general comparisons that the storage of fat is even as great in amount as when the salmon first cease to feed, as they do at some considerable time before this locality is reached in the migration journey.

Trunk pink muscle.—In the trunk pink muscle the most striking change consists in the fact of the appearance of intramuscular fat not noted previous to this stage. This seems to be one of the first histological evidences of the cessation of feeding. At this time the central core of the pink muscle fibers, and especially of the smaller fibers, is dotted through with extremely small fat droplets. These fat droplets are rarely as much as 2 μ in diameter, usually not more than 1 μ, and from this size down to droplets so small as to be scarcely visible by the 1/12 oil immersion. All evidence that I have points to the fact that this microscopic salmon fat reacts uniformly to the Herxheimer stain whether the droplets be large or small. The pink muscle fat at this stage is quite evenly distributed through the cross section of a fiber except in the outer circle of fibrillæ. In this circle there is no intramuscular fat. This gives the fibers the appearance of having a clear surface border as distinguished from the inner portion of the fiber, which is of course slightly pink from the presence of stained fat. At this stage I can distinguish a few small and scattered fat droplets between the sarcolemma and the muscle substance. The intramuscular liposomes are largest in the smallest pink fibers, usually from two to three times greater in diameter on the average than in the very large fibers.

The trunk pink fibers show the details of liposome arrangement best in teased preparations. The liposomes are in short chains consisting of a few individual droplets in each. At this stage the liposomes in the middle of the chain are largest and they decrease quite uniformly from the middle toward each end. These chains are loaded in the interfibrillar spaces. They are present only in certain, not all, spaces between groups of fibrillæ. The number of such spaces occupied by the chains of liposomes,

therefore the relative number of liposomes, varies in preparations from different individual salmon. The amount of fat in the pink muscle fibers is measured therefore by two microscopic factors; first, the number of chains in a given mass of fiber; second, the size of the individual liposomes in the chains.

The pink muscle fibers vary within a wide range of size of fiber, from 25 to 250 μ in diameter. This variation is illustrated in the figure 17, plate X. In the larger fibers of the Ilwaco fish (notably no. 111 and no. 118, the latter of which is figured in figs. 8 and 9, pl. VI) the chains of liposomes are quite evenly distributed throughout the mass of the fiber. However, they are characterized by the relatively small number of liposomes in the chains and the comparatively small size of the liposomes. In the large fibers of no. 118 the largest liposomes in the centers of the chains are about 0.5 μ in diameter and the smallest ones which form the ends of the chains are just identifiable with the oil immersion. In the smaller fibers of this same fish the liposomic chains are somewhat larger, the largest liposomes in the chains about double the diameter of the largest in the large fibers. The liposomes in the small fibers are more thickly distributed around the central core of the fiber. This variation is not noted in the relatively large mass of the fibers whose diameters run over 200 μ.

Fish no. 117 seems an exception to the group from the Ilwaco station. It is certainly very far below the average of the other specimens as regards the amount of fat revealed by the microscope. Reference to the protocol will show that this fish came from a trap some little distance up Bakers Bay. The whole appearance of the salmon, both its gross appearance and the microscopic appearance, suggests the type of fish characterized by a certain degree of retrogression. The weight is much below the standard for the length, as much below the average as certain farther advanced salmon taken from stations higher up the river. These comparisons lead to the deduction that salmon no. 117 has been in fresh water some time. Although it has not gone up the river, the probability is that it has undergone as much migratory change in fats as specimens that have gone farther up the river. The chemical quantitative determination of the fats abundantly confirms the above deductions. (See page 92.)

In this salmon the amount of intermuscular fat in the trunk pink muscle is very much reduced. The number of fat drops is less and the size smaller. The intramuscular fat is present in all of the trunk pink, but the number of liposomic chains and the size of the liposomes themselves is reduced. In the very largest fibers there is almost no liposomic fat. Another point associated with the amount of fat is the decrease in the intermuscular spaces, so that the fibers themselves seem more compact in arrangement.

Caudal pink muscle.—The pink muscle from the caudal peduncle in each Ilwaco specimen examined has a strikingly smaller quantity of intermuscular fat than the muscle from the middle of the body of the same animal. It would seem from the Ilwaco fish that the intermuscular fat is never laid down in the caudal region in as great quantity as in the lateral or trunk region of the body. The fat drops are relatively smaller and, in general, fewer in number than from the fatter region of the body. The intramuscular fat of the caudal pink muscle in the specimens from the Ilwaco station is less than in the trunk pink muscle. Those conditions which at the beginning of the migration lead to an infiltration of fat into the muscle cells do not result in as great a deposit in the caudal pink muscle as in the trunk fibers.

In the fattest fish the caudal pink muscle is characterized by the smaller size of the liposomes; also by a smaller number of liposomes in the center of the fiber. This gives the impression of a somewhat greater quantity of fats around the superficial layer of the fiber.

In those Ilwaco fish which have less fat than the average, for example, no. 117, the amount of fat in the caudal pink muscle is very strikingly less than in the middle of the body. In this particular fish the fat between the fibers is very noticeably less in quantity; in fact, it is practically absent except in those areas which have a relatively large amount of connective tissue. The caudal intramuscular fat of no. 117 has almost disappeared, or at least is present in extremely small amount.

The caudal pink muscle also shows that fish no. 117 has already passed well into the retrogressive stage which comes with the migration fast, an indication noted in connection with the discussion of the trunk pink muscle. This is apparent from the character and arrangement, particularly the arrangement, of the fat in the connective tissue, as well as in the spaces between the muscle fibers. It is certainly true that the amount of fat present sharply increases as one proceeds through successive segments from the caudal peduncle to the mid-lateral region of the body. Light on the significance of the above observations is had by considering the condition of the fat in the dark muscle of the two regions.

Trunk dark muscle.—The dark muscle forms a distinct type of muscle, as previously announced. In this case the fat has been loaded into the muscle in enormous quantities, both intermuscular and intramuscular. At the Ilwaco station the amount of fat in the dark muscle is enormous, as illustrated by fishes no. 111, 113, 115, 116, and 118, in all of which the fat deposited in the dark muscle has reached its maximum.

The intermuscular fat is relatively much less than in the pink muscle. This is due among other things to a structural factor. The muscle fibers are very compactly arranged, forming a much denser mass than is formed by the pink fibers. The interstitial connective tissue is correspondingly reduced in mass, hence there is not so much fat carried. On the other hand, the muscle substance has received so great a deposit of intramuscular fat that one must regard this muscle as a definite fat depot. Attention has already been called to the fact that deposit in this muscle begins in embryonic life. It increases in amount up to the time of the cessation of feeding and, we assume, has not appreciably changed when the good-conditioned fish reach Ilwaco. The trunk dark muscle contains so much fat in the muscle substance that one can not make adequate comparisons showing slight variations.

When this tissue is examined in teased preparations, so that a side view is had of an individual fiber, it is found that the fat droplets are so large and so numerous that the fibers are difficult to distinguish as individuals. This is shown in figure 1, plate III, where a transparency is figured of a fiber from fish no. 115. Often in the examination of these teased fibers one notes elongated fat drops or rods. These have formed in the interfibrillar spaces owing to the fact that the droplets have increased so much in size that adjacent ones have run together, thus fusing into the mass noted.

Caudal dark muscle.—The superficial or dark muscle from the caudal region in all these Ilwaco specimens has a very considerably less amount of fat than the corresponding muscle from the lateral region. Even fish no. 113, which is as fat as any in the series, presents a sharp contrast as regards the comparison of the amount of fat in the

caudal dark and in the trunk dark muscle. The size of the intermuscular fat droplets has sharply decreased, though the number of droplets is as great or even greater than in the lateral region.

The sharpest contrast lies in the intramuscular fat. In the caudal region this fat is very markedly less, especially in the size of the larger droplets. Even in the fatter fish the larger droplets seem to be congregated around the superficial border of the fibers. The superficial fat droplets are under the sarcolemma, though this fact is often very difficult to determine. There is a large supply of the finer fat droplets and liposomes scattered through the protoplasm of the caudal muscle substance. The contrast between this and the fatter regions is not so much a matter of the number of the liposomes as in the size and arrangement, especially of the larger droplets. The largest droplets in the caudal muscle will not average more than one-half as great in diameter as in the trunk muscle.

In a salmon like no. 117, which is poor in the general amount of fat of the body, the caudal dark muscle presents the sharpest contrast in comparison with the standard of this station. Under the low magnification, sections of the caudal dark show that in the regions bordering along the blood vessels there are areas which by contrast with other portions of the section are relatively free of fat. These areas are faded. This is a condition undoubtedly indicative of the removal of stored fat. The retrogressive process has already gone so far that one can distinguish the regions in which the active process of fat resorption is going on with most vigor. This is the first clear-cut picture showing the process of fat resorption. The appearance of the section is exactly the reverse of that shown for the dark muscle in the growing stage, also of that in certain pathological processes wherein fat is being very rapidly laid down.[a] In discussing later stages it is argued that these contrasts are due to the irregularity of resorption of the fat from the tissue. In other words, the fat is being taken up from the tissues and transported to other parts of the body, to be utilized by the body in the construction of new tissues (egg yolk, etc.) or in the production of energy. This movement is best facilitated in the neighborhood of the small blood vessels, and is expressed microscopically by these contrasts in fat content. These facts are in further confirmation of the deduction that fish no. 117 has been for some time on the migration phase of its life cycle.

Fat in the fin muscles.—A few examinations were made of the small muscles of the fins at the Ilwaco station. The samples selected were the pairs of erector and depressor muscles located in a single interspace between two interhemal spines. These muscles are made up of fibers rather loosely bound together. There is a small amount of interfibrous connective tissue with a tolerably thick sheath around each muscle slip. The fibers themselves are of a type somewhat like the cheek muscle of the head.

The intermuscular fat is present in droplets of good size, but not in very large numbers. In the connective tissue sheaths around the muscles the amount of fat corresponds more nearly to that of the myocommata of the lateral muscle. In general, the amount of intermuscular fat is considerably lower than that of the pink lateral muscle of the same salmon.

[a] My colleague, Dr. W. J. Calvert, tells me that in his unpublished work on the plague he often noted a striking deposit of fat in the parenchyma along the immediate border of the blood vessels of the liver. This deposit in the early stages of the disease extends out only a short distance into the parenchyma of the liver. The course of the smaller blood vessels is easily followed through the parenchymatous tissue by the bordering deposit of fat. This is, of course, the reverse picture of that described above. In the salmon the fat is in process of removal; in the plague liver the fat is in process of rapid deposit, but in each case the histological picture is that of the early, therefore differential, stage in the process.

The intramuscular fat is relatively small in amount. Many of the fibers show liposomes of extremely fine size, often so small that one can trace them with difficulty. There are no rings of fat droplets under the sarcolemma such as characterize muscle that is beginning to show fat exhaustion. There are certain groups of fibers in these sections which show a relatively larger amount of intracellular fat. In such fibers the liposomes will average as much as 2 μ in diameter. The liposomes are quite evenly distributed through the cross section of the fiber and are occasionally quite numerous under the sarcolemma. This latter type of fiber is suggestive of the dark muscle type. Not enough comparative work has been done in studying these muscles to determine whether or not the dark muscle fibers are present in portions of these muscles. There is some indication that the superficial muscle of the anal fin, the inclinator analis, contains fibers of the dark type, whereas the erector and depressor muscles are more nearly of the pink type. If the inclinator contains fibers of the dark type it would suggest that that muscle is more nearly homologous with the superficalis lateralis, a homology that needs further investigation.

Fat of the adductor mandibulæ or cheek muscle.—The fibers of the muscle are more compactly arranged and different in appearance from the other portions of the salmon musculature. They are, however, most like the great lateral pink muscle. At Ilwaco the intermuscular fat is distributed in scattered but relatively large fat droplets, 60 to 70 μ in diameter. There is also a comparatively large number of small droplets not over 20 μ in diameter.

The intracellular fat is always present. The large fibers in the muscle carry a few scattered chains of extremely minute liposomes. On the other hand, the smallest fibers have liposomes about 0.6 μ in diameter.

Considering the muscle as a whole at the Ilwaco station the fat distribution is most nearly like that of the great lateral pink muscle, though both the intermuscular and intramuscular fat is very much less in quantity. This muscle, like the fin muscles, carries a relatively small amount of intramuscular fat. This fat is more than adequate for the uses of the muscle, but the striking fact shown by the sections is that there is never an excessive accumulation of the fat.

ANALYTICAL DETERMINATIONS OF THE PERCENTAGE OF FATS IN SALMON FROM THE MOUTH OF THE COLUMBIA RIVER.

When this study was projected it was planned to take a full set of samples of the muscles studied and make fat determinations by accurate chemical methods. Such a full set of determinations would have been very valuable in itself but of inestimable value as corroborative evidence in connection with the microscopic comparisions. It turned out to be impossible to carry through the full program of the work and the sacrifice fell on the chemical series. Chemical samples were taken, however, whenever it could be done, though the analyses were reserved to be made not in the field but in the home laboratories. The few samples secured were not analysed until after the microscopic work was completed and the results sent off for publication.

The fat determinations secured on samples from Ilwaco are inserted at this point. Considering the fact that the eight Ilwaco fishes were chosen to represent the entire range of types present in the lower Columbia at the time of the expedition, this showing of fat percentages is most significant. The salmon were taken, first, from the main

channel as far out toward the end of the jetty as the fishermen go (111, 112); second, from the main channel south of Sand Island (113, 114, 115, 116); third, from the north channel leading out of Bakers Bay at a point near Fort Canby (118); and fourth, from Bakers Bay at the Whitcomb trap (117).

The two chief types of muscle described, the pink muscle and the dark muscle, were the only ones selected for analyses. The samples were taken in the mid-lateral region just in the plane that cuts the front of the dorsal fin, the same region from which histological samples came.

The greater amount of fat in the lateral dark muscle as compared with the pink was revealed by the microscope. But this fact is even more strikingly shown by the quantitative percentages given in the table below. A glance shows that the percentage of fat in the dark muscle is roughly twice as great as in the pink. There is no law to be deduced about it from so few samples. The fattest salmon have relatively the highest quantity in the pink muscle. The intermediate salmon from this station have a greater reduction in the fat of the pink than in the dark.

Particular attention is directed to the two females, no. 112 and 117. The former is from the channel of the Columbia from the farthest point out toward sea. The latter is from Bakers Bay, quite out of the main channel of the river. Undoubtedly the great difference is due to the fact that no. 112 was just coming in from the sea. No. 117 had undoubtedly lost most of its fat and is quite comparable with the salmon in better condition from the spawning grounds of the Clackamas River at Cazadero.

TABLE I.—ANALYTICAL DETERMINATIONS OF FAT IN THE TISSUES OF CERTAIN SALMON OF THE 1911 SERIES.

Date.	No. and sex of fish.	Muscle fats in per cent of wet weight.		Caught in Columbia River, Ilwaco, Wash.
		Pink muscle.	Dark muscle.	
1911.				
Aug. 3	111 ♂	15.680	28.018	Outer channel opposite the end of the jetty.
Aug. 3	112 ♀	19.655	30.813	Do.
Aug. 4	113 ♀	14.662	.....	Mid-channel, off the upper end of Sand Island.
Aug. 4	114 ♀	20.179	29.080	Do.
Aug. 7	115 ♂	10.062	27.420	Do.
Aug. 8	116 ♀	5.395	23.248	Do.
Aug. 10	117 ♀	2.727	14.324	Bakers Bay, Whitcomb trap.
Aug. 11	118 ♂	10.507	.....	North channel (from Bakers Bay), McGowan's trap near Fort Canby.

Significance of the fat in Ilwaco salmon with reference to the normal quantity of fat at the beginning of the migration.—In discussing the normal salmon type, the type at the beginning of the migration deduced from the study of feeding salmon secured at Monterey Bay, it was suggested that one might arrive at a better conception of the normal type by figuring back from the first migration station. But Ilwaco fish show a number of signs of physiological change presumably due to the cessation of feeding. Among these changes the most striking are to be had by the examination of the alimentary tract, where, in the stomach, and especially in the intestine and cæca, one finds extensive evidences of

degeneration. These changes are almost wholly in the direction of retrogression of structure.

In the muscular tissue there are two factors mentioned above which are interpreted as changes that have come on in the amount and distribution of fat in the muscle since the beginning of the migration fast. These are, first, the abundance of intracellular fat laid down in the pink-muscle fibers; second, the evidence in the dark-muscle fibers of removal of fat. Interpreting these two phenomena broadly one may assume that there has occurred already in the best conditioned channel fish a using up of a certain amount of fat. Considered from the standpoint of percentages this amount has not reduced the total storage enough to be readily measured by the microscope except in the second case. In the poorer fish it is quite obvious that fat is disappearing, undoubtedly due to the prolonged fast. This is especially shown in the Bakers Bay type illustrated by no. 117. This interpretation is supported by the quantitative chemical determinations of fat.

There is absolutely no microscopic evidence which can be legitimately interpreted as meaning a fatty production from the disintegration of protoplasm. If, therefore, one could follow back the physiological condition of an Ilwaco salmon to that point in its history where it first ceased to feed, and would examine its tissues for the loading of fat, he would find that this time represented the maximum amount of fat present in the animal. In other words, this stage of the beginning of the fast, i. e., end of the feeding period, represents the climax of fat storage during the salmon's history. The microscopic picture obtained by a study of the Ilwaco specimens is therefore applicable to this normal stage, provided, first, that the intramuscular fat of the pink muscle be omitted, and, second, that all the areas of dark muscle which appear to be losing fat be considered as uniformly filled with fat. Figure 8, plate VI, representing the fat in a cross section of pink muscle of salmon no. 118 from Ilwaco, would, if the intramuscular fat were eliminated and the intermuscular fat increased in quantity, represent very well my conception of the quantity of fat in this tissue when the fast begins. So also in the dark muscle, figure 3, plate I, would serve as a type for the dark muscle at the beginning of the fast. These figures fail in the fact that they have too little intermuscular fat to represent the normal, but the percentage difference is one which can not easily be estimated by the microscope. Salmon no. 114 has nearly twice the amount of fat in the pink muscle shown by no. 118. This fat is wholly intermuscular and would show in the microscope in the form of larger drops rather than in a greater number. Judging wholly by the microscopic comparisons, one would never judge that the difference is as great as that revealed by the chemical determinations of the fat percentages.

PROTOCOLS.

Male salmon (no. 111) length 950 mm., weight 13,776 grams, taken between the jetty and the black buoy at the mouth of the Columbia River, August 3, 1911.

This was a clean, bright, silvery salmon of the short, deep type. It is a perfect looking specimen of the sea type. It was caught with a gill net by Mr. Cliff Sweeney. This salmon had all the appearance of a first-class, very fat specimen. Its flesh looked oily and there was a thick layer of cutaneous fat.

Microscopic examination of trunk pink muscle, teased (slide J6).—These isolated fibers of pink muscle are simply crowded with liposomic fat. The fat is arranged in longitudinal rows or chains of liposomes between the fibrillæ which bear relation to the striations. The liposomes are from 0.2 μ or less to 2 μ

in diameter, rarely larger, as observed in a certain large fiber under examination. This fiber is 200 μ in diameter. The liposomic chains are comparatively uniform in their disposition throughout the mass of the fiber. The liposomes themselves are not uniform in diameter in the rows. Adjacent droplets may alternate between small and large sizes, though in some of the rows the droplets are fused, thus making an oval droplet extending across the intervening striation membrane. In some of the smaller muscle fibers the fusions are much more extensive, extending over four or five striations. In the smaller fibers the fat droplets are relatively larger, averaging between 1.5 and 2 μ in diameter. Over the surface of the fibers and under the sarcolemma there is a sprinkling of small fat droplets from 2 to 5 μ in diameter. These are irregularly placed.

The caudal pink muscle was not prepared in this fish.

Trunk dark muscle (longitudinal section, J1).—The preparation is so filled with fat that the structure is obscured. The intermuscular fat is in the largest drops observed for the trunk dark muscle, the average diameter being about 30 μ. These drops are often compressed into oval outlines by the pressure of the fibers. The muscle fibers themselves are only about 40 μ in diameter.

There are large quantities of intramuscular fat, the droplets being simply crowded throughout the whole structure. The larger intramuscular droplets are from 15 to 20 μ in diameter. These large droplets often appear in rows along the course of the fiber, giving the appearance of splitting the fiber. However, they only press the fibrillæ apart. There are relatively few of the smallest liposomes present, though the bundles of fibrillæ all show a certain number of these small liposomes. The quantity of fat in this preparation is the greatest for any dark muscle noted in the whole season's work. The droplets are larger, relatively more numerous, and have so distorted the relations of the fibrillæ as to break up the regularity of the structure.

Another section (J33), fixed 18 hours in formalin, gives a much better view of the outlines of the fibers. The fibers are crowded thickly with relatively large intracellular droplets. They are so numerous as to form almost a continuous layer of drops. A section (J35) stained in sudan shows the same crowding of fat as those stained with scarlet red. The contrasts are less sharp.

The myocommata of the trunk dark muscle are filled with adipose cells which are crowded with fat. Many of the cells have ruptured and the fat has run together, but the fat drops of those cells still intact measure from 50 to 70 μ in diameter.

Caudal dark muscle (transverse section, J20).—The dark muscle of the caudal peduncle is very fat, but not so fat as in the trunk muscle. The drops are relatively smaller. Those between the fibers are from 6 to 15 μ in diameter. The fat within the fibers varies extremely in different parts of the section. I notice one region in which the fibers are almost free of large drops of fat; only smaller liposomes are present. In the near neighborhood of this group the fat is gathered around the surface of the fibers, apparently just under the sarcolemma, where the drops vary from 5 μ down. The centers of these fibers have liposomes averaging only about 1 μ in diameter. In that portion of the section which is fattest the central portion of the fibers has larger droplets, not averaging more than 3 μ, however. From this section it seems that the caudal dark muscle must have a greatly reduced amount of fat in comparison with the trunk region. Slide J21 shows relatively more fat than slide J20. The fibers are cut somewhat obliquely, and this brings out the fact that the drops are oval in shape, as in the lateral line region.

Intercostal muscle (longitudinal section, J7).—This section has a large amount of intermuscular fat. There are numerous large drops averaging 30 μ. The connective tissue of the whole section is jotted full of very fine fat droplets, from 1 to 10 μ. There is a trace only of intramuscular fat, nothing comparable to that in the teased trunk muscle. This fat is in extremely fine liposomes, averaging only a fraction of a micron in diameter. It seems quite uniformly distributed throughout the substance of the fibers.

Muscles of the anal fin (transverse section, J23 and 31).—This section was across the group of muscles between the interhemal spines and should therefore be of the erector and depressor muscles. There is a small group of fibers on the outer margin of the section different from the main body, which probably belongs to the inclinator muscle of the fin.

There is a very small quantity of intermuscular fat. The drops are scattered but relatively large. The main portion of the muscle has only traces of liposomic fat in extremely fine granules. There are no rings of fat droplets under the sarcolemma of the type which characterizes fat-exhausted muscle.

The group of fibers on the outer margin of the section has a uniform distribution of intracellular fat in comparatively large liposomes. These liposomes average 2 μ in diameter. They are quite evenly distributed throughout the substance of the fiber and under the sarcolemma, where they are somewhat

more numerous. The liposomes appear as rows running between the fibrillæ which the oil immersion lens shows have an arrangement with reference to the cross striations. In one fiber six striation segments have a length of 8 μ, an average of 1.3 μ per striation. The liposomes that are spaced with reference to these particular striations are from 0.4 to 0.6 μ in diameter. The arrangement of fat in this group of fibers is very like the arrangement in the dark muscle of the lateralis superficialis.

Masseter or cheek muscle (transverse section, J9).—The fibers of the cheek muscle vary considerably in diameter, running from 30 up to 110 μ. This transverse section has a medium amount of intermuscular fat distributed in large droplets from 60 to 70 μ in diameter. There are also numerous fat drops from 15 to 20 μ in diameter.

The muscle fibers themselves are much split up by ice crystals in sectioning, yet it is clear that the fibers contain intracellular fat. This fat is greatest in amount in the smallest fibers, where the droplets are about 0.6 μ in diameter. The larger fibers also contain intramuscular fat, but the droplets are smaller.

Male salmon (no. 115), length 940 mm., weight 12,225 grams, caught in the Columbia River channel just above Sand Island, August 7, 1911.

This salmon was a clean fish, free of sea lice; testes slightly developed and dark venous red in appearance; stomach relaxed, 5 cm. in diameter with mucous content. Dark muscle teased immediately in physiological saline and figured.

Microscopic examination of trunk pink muscle teased in physiological saline.—These muscle fibers show extremely fine liposomes within the fiber. In larger fibers the liposomes are difficult to distinguish, but are readily seen in the small ones. The amount of intramuscular fat is not so great as in the trunk pink muscle of no. 113. Section J99, stained with hæmatoxylin only, differentiates the interfibrillar sarcoplasm in such a way as greatly to emphasize the outlines of transparent liposomes. The liposomes themselves appear highly refractive and have not taken more than a trace of the stain (1/12 oil immersion).

Microscopic examination of trunk dark muscle teased in physiological saline (J46).—A drawing of a fiber from this slide is presented (fig. 1) showing the fat throughout the dark muscle. Practically all the fibers in this slide are loaded down with fat. There is an enormous quantity of fat present, more than one can adequately represent by any graphic method. The fat drops within the fiber are relatively large and are so numerous that they push out the sarcolemma, making its outlines irregular. The drops are somewhat oval in shape, measuring 8 by 13, 5 by 6, 7 by 16 μ, and smaller. The diameters of some of the fibers are 35, 36, 40, and 45 μ. The fat droplets are in rows. They are relatively large in almost all parts of the field. This is due to the fact that the liposomes have grown in size until adjacent ones have fused, a condition throughout the fiber.

With the fusion of droplets the resultant is an oval mass with the long axis with the interfibrillar space. As the fat mass has grown the fibrils have been forced out of their normal relations. Where the drops lie outside the sarcoplasm and under the sarcolemma this membrane is seen to be pushed out in numerous irregular protuberances. This section is unusually clear and transparent, probably because it was not subjected to formalin.

Female salmon (no. 116), length 975 mm., weight 14,530 grams, caught in the channel of the Columbia River opposite the lower end of Sand Island, August 8, 1911.

A bright silvery fish, no sea lice, stomach small and contracted with thick walls; intestine one-half as large as in no. 115; ovaries relatively large, weighing 965 grams.

Microscopic examination of the trunk dark muscle (slides J77–91).—The trunk dark muscle of this fish has less fat than either no. 111 or no. 115. The larger fat drops are between the fibers. They measure 12 to 14 μ, but the average is not much over 7 μ.

The intracellular fat is in smaller droplets, from a fraction of a micron to 3 and 4 μ in diameter. There is a massing of the fat droplets around the surface of the fibers.

A series of teased preparations were treated in various ways to test the method The fresh muscle teased in physiological saline is more transparent than the other preparations and the fat gives the appearance of a greater quantity, largely because it is more clearly distinguished. Fibers teased in formalin were very opaque. Those teased in alcohol were somewhat intermediate in character between the saline and the formalin preparations.

Female salmon (no. 117), length 940 mm., weight 8,245 grams, taken from the Whitcomb trap located in the bend of Bakers Bay.

This salmon was more slender than no. 111, was a clean fish, but did not appear in as prime condition. The ovaries weighed 510 grams. The flesh appeared less oily and was very pale in color, especially in the caudal peduncle.

Microscopic examination of the trunk pink muscle (transverse section, K31).—This section is taken ventral to the lateral-line septum. There is very little intermuscular fat. Even along the myocommata there are only a few droplets and these are of small size, from 1 to 10 μ in diameter. The very largest drop seen was only 15 μ in diameter.

The substance of the muscle fiber contains a supply of liposomes arranged in chains throughout the mass of the fiber. These liposomes run from 0.3 to 0.5 μ in diameter. The section is cut obliquely, making it difficult to interpret the point, yet it is obvious that the liposomes are in greater numbers along the superficial region of the fibers while the deeper portion of the fibers is relatively poor in liposomes. This gives the section as a whole a mosaic-like appearance. The point is not absolutely certain, yet I am convinced that this increased quantity of liposomic fat is under the sarcolemma and between the more superficial layer of fibrils.

A section taken from the dorsal division of the deep lateral muscle has a less amount of fat than that from the ventral. The fibers of this section are very compactly arranged and the outlines are correspondingly sharp and angular, similar to that shown in later stages. (See salmon nos. 125 and 126.) The diameters of the fibers themselves vary between 30 and 150 μ, rather smaller than the average pink muscle fibers.

There is a very small quantity of very fine liposomes within the substance of the fibers. In many fibers no liposomes are to be distinguished. In the smallest fibers shown in the field, those 30 μ in diameter, the liposomes are present in the middle of the fiber. In the medium-sized fibers the liposomes are largely around the superficial area of the fiber and are from 0.2 to 0.3 μ in diameter, rarely larger (1/12 oil immersion). In the middle of these medium-sized fibers and in most of the body of the large fibers, liposomes are much fewer and exceedingly small, scarcely discernible.

The smallest fibers often have rings of very small fat droplets, the droplets running from 2 to 3 μ in diameter. These droplets are just under the sarcolemma. In areas where they are more numerous there are occasional fat drops 15 μ in diameter located between the muscle fibers.

Microscopic examination of the caudal pink muscle (transverse sections, K19 and 20).—These sections of caudal muscle show a markedly less amount of fat than from the dark muscle. The type of arrangement is that of the trunk muscle except that there is less intermuscular fat.

The intramuscular fat is also conspicuously less in quantity. The liposomes are practically absent from the larger fibers and are very minute and few in numbers in the smaller fibers.

Microscopic examination of the trunk dark muscle (transverse section, K7).—The fat droplets are relatively small in this preparation, notably smaller than in salmon no. 115. The intracellular fat is evenly scattered through the substance of the dark fibers. The liposomes vary from 2 to 5 μ in diameter (tissue cut fresh). In the regions which have the smallest amount of fat the number of droplets of the larger size which are so prominent in fish no. 111 are greatly reduced in size, running from 1 to 5 μ in diameter. In certain areas of the section lying near the connective tissue partitions there are small groups of dark fibers which apparently have their fat reduced greatly below the average. Such fibers will contain irregularly placed liposomes from 1 μ down to a just visible size, 0.2 to 0.3, μ, while adjacent fibers will have a more prominent loading of fat in which the droplets average from 3 to 5 μ in diameter. Also the number of droplets in the latter fibers is greater than in the former. This picture suggests the thought that the fat of the dark muscle is being removed along the course of the larger blood vessels.

Microscopic examination of the caudal dark muscle.—Insufficient study was made of the caudal dark muscle, but the rather poor sections bring out one point, namely, that the fat is reduced much below the average and that the fat droplets are massed around the surface of the fiber.

Male salmon (no. 118), length 940 mm., weight 12,470 grams, taken in McGowan & Co.'s trap at the mouth of Bakers Bay near the Fort Canby Dock.

This fish was a deep smooth specimen, skin bright and clean, no sea lice, head shaped like the female, medium depth, a splendid specimen apparently comparable to no. 111. The testes were quite small and immature.

Microscopic examination of the trunk pink muscle (sections K38, 45, K55-58).—The intermuscular fat is crowded in every angle formed by groups of fibers. The drops vary in size from small ones to as high as 100 μ in diameter. The muscle fibers themselves vary greatly in size, from 50 to 300 μ in diameter. In cross section the muscle fibers are oval to round in outline, the round contour of the individual fibers being in sharp contrast to the polygonal shaped outlines of fibers of salmon no. 117.

The two sections on slide K45 were made free-hand and thick in order to show the relations of the intermuscular fat. The section includes a tendinous myocomma. Where the muscle fibers are very close together a single row of large fat drops extends down the length of the fiber from the myocomma. In two or three regions the intermuscular space is filled up with two or even more rows of such fat drops. These fat drops are from 50 to 60 μ in diameter. They are somewhat compressed, having their long axis in the longitudinal axis of the fibers. The myocomma itself is crowded with fat.

The intramuscular fat is present in all the fibers. It consists of extremely fine liposomes, being most minute in the large muscle fibers and greatest in amount in the small fibers. They are uniformly distributed throughout all of the body of the muscle fiber, with the exception of the narrow ring of band-shaped fibrils which forms the surface layer.

The chains of liposomes are rather evenly distributed throughout the substance of the large fibers, but consist of very small liposomes from those just identifiable up to 0.3 μ. In the medium-sized fibers the liposomes are somewhat larger and in the smaller fibers considerably larger than in the ones just described. In the latter the liposomes reach the diameter of 1.5 μ, though the average is less than 1 μ. In one fiber 56 μ in diameter the liposomes were in unusually long chains and large in size, similar to the arrangement in dark muscle at a late stage in the resorption. Several liposomes in this muscle were measured which were 2 μ in diameter, but the average was from 1 to 1.2 μ. Figure 8, plate VI, shows the distribution of fat in the trunk pink of salmon no. 118.

Pink muscle from the belly shows an even greater amount of intermuscular fat; also minute liposomes in chains throughout the substance of the fibers.

Microscopic examination of the trunk dark muscle (K41 and 42, transverse sections).—There is a large amount of intramuscular fat in the lateral dark muscle of fish no. 118. The size of the fat droplets in this region is from 9 to 12 μ in diameter. In a certain interseptal region the fat drops are large, running as much as 60 μ in diameter. This fat belongs to the adipose tissue proper. A noticeable difference in the staining character is present between it and the fat in general; i. e., the large fats are less red. The muscle fibers of this section are so compact that it is often difficult to determine whether a given fat drop is within the sarcolemma or without. It is judged that a rather large proportion of the fat which is massed around the surface of the fiber is under the sarcolemma. The section throughout its whole extent shows an enormous quantity of fat massed along the lines which separate the fibers.

The teased dark muscle (slide K52) shows numbers of relatively large fat droplets along the sides of the fiber wall and adherent to the protoplasm. These droplets are smaller on the average than those of the cross section which were judged to be intermuscular.

Within the dark muscle fibers of this teased material the fat is present in masses—no other word seems adequately to express the condition. There are numerous fibers, in fact nearly all of them, in which many chains of liposomes are displaced by long masses or rods of fat. Undoubtedly, these rods of fat have been produced by the fusion of liposomes in the loading of the fiber with a higher percentage of fat than is found when liposomes are typically present, as, for example, in salmon no. 132. In the present section there are four such rods in one microscopic field. In another similar field there are six. In a fiber 40 μ in diameter these rods continue unbroken for as much as 126 μ. They are located in the areas between the bundles of fibrillæ, where one finds in the ordinary loading either chains of liposomes or, at most, short oblong droplets.

There are fibers in this teased material that have a somewhat less quantity of fat than that described in the last paragraph. In one such typical case the smallest liposomes observed measure 1.5 to 2.5 μ. In close proximity to this last chain of liposomes there is a chain of fused liposomes, i. e., a rod, which is continuous for 120 μ. This rod has, however, several partial constrictions which undoubtedly represent points where in the earlier stage of fat deposit the rod is discontinuous.

In the transverse section (1/12 oil immersion) the fat is crowded into the fiber in a way comparable only to no. 111. The whole surface of the field is taken up with fat droplets almost as thick as they can stand. There are relatively few liposomes that measure less than 1.8 μ in diameter and the size varies up to 5 μ. There are chains of these smaller liposomes throughout the protoplasm, even in fibers obviously distorted by the long rods of fat.

DISTRIBUTION OF THE FATS AT AN EARLY INTERMEDIATE STAGE OF THE SPAWNING MIGRATION.

The first station above Ilwaco where salmon were collected was at Warrendale, Oreg. This station is about 6 miles below Cascade Locks and is in the midst of an extensive fishing field. Salmon taken here have not yet passed the swifter runs of the river, but have already made a run of about 135 miles above the mouth of the river. The station was located at the cannery of P. J. McGowan & Sons, and I am particularly indebted to the superintendent, Mr. Charles A. McGowan, for many special favors. This company has a seining ground on the sand bar on the Washington side about 1½ miles below Warrendale. Our specimens were chiefly taken from this point, as the fish captured there were fine conditioned channel fish.

Fish nos. 120, 121, 122, 125, and 126 were taken at this station during the month of August. In August one secures salmon which clearly show stages of the removal of fat from storage localities. There is at this time of the year considerable variation in the grade of fish at this point. The fatter salmon, for example, no. 120, have their tissues well loaded with a reserve of fat. The poorer salmon, no. 126, show marked stages indicative of retrogression as regards the loading of fat.

Trunk pink muscle.—There is wide variation in the microscopic appearance of the fats in the trunk pink muscle of the fishes at this station. The fattest observed was no. 120 and the poorest no. 126.

The intermuscular fat is disposed in the muscle according to the same general plan as in salmon from Ilwaco. However, there is a very great diminution in the amount of this fat. This is indicated by the decrease in the size of the larger droplets, and to a less degree in a decrease in the number of droplets. A striking fact in comparison is that in these Warrendale fish the fat is very much less uniformly distributed among the fibers than in either the Ilwaco or in the normal tissue. The comparison between two stations is difficult to make. One can not microscopically measure the number of fat droplets and compute their diameters and thus the volume of material from the two stations. Rather he is limited to impressions made by placing the slides side by side. It is largely on this type of evidence that the above comparisons are made. However, as regards the intermuscular fat drops, a comparison of the diameters of the largest drops is illuminating. At Warrendale these largest drops seldom measure over 50 μ in diameter (see the protocol for salmon no. 121), whereas at Ilwaco they often measure 100 μ and more in diameter. In observations made at the time the material was collected and sectioned on the grounds at Warrendale it was judged that the amount of intermuscular fat in fish no. 120 was about one-half to two-thirds as great as in fish no. 118 from Ilwaco. Each of these fishes is among the best represented at its station. In those fishes which were relatively poor in fat, as no. 126, the amount of intermuscular fat is very greatly reduced. The amount of this reduction is best shown by comparing figures 8 and 10 of plates VI and VII. Judging from the comparison of a large number of preparations, I would say that the intermuscular fat of this poorest salmon could not be above 25 to 30 per cent of that of the normal type.

The intramuscular fat is abundant in all of the fibers of the pink muscle from the trunk region. The smaller fibers as usual are more heavily loaded with fat than are the larger. This is shown chiefly by the larger size of the liposomes in these small fibers.

In the fatter specimen the largest fibers are relatively thickly filled with numerous chains of liposomes. These chains are more numerous than in fish no. 115 and no. 118 from Ilwaco, and the size of the liposomes in the chains is, if anything, comparatively greater. When the poorest fish are examined, it is found that the large fibers are strikingly low in fat, for example, no. 126, fig. 10, pl. VII. In fact, it is difficult to distinguish liposomes in the largest trunk pink fibers of this fish. In numerous instances observed there were tiny groups of very small liposomes ranged near the surface of the cell, chiefly under the sarcolemma. If liposomes were present in the body of the large fibers at all, they were too small to be distinguished with the 1/12 oil immersion. In many sections of this poor fish the intermediate-sized fibers had their liposomes chiefly at the surface, whereas the central portion of the fiber was comparatively free of liposomes. Disappearance of fat is not accompanied by any signs of degeneration at this stage. The structural detail is clearer and very sharp and distinct, as shown in figure 13, plate VIII.

In teased preparations where one has a view of a fiber for some considerable length it appears that the Warrendale pink muscle is relatively rich in liposomic fat. In the best salmon there is even a greater amount of intracellular fat in the pink muscle than at the Ilwaco station. The chains of liposomes are more continuous and the size of the individual liposomes relatively greater. In the small fibers particularly this comparison holds. In fact, it often happens that in the smallest fibers the liposomes have reached a size at which adjacent ones coalesce, a phenomenon the significance of which is discussed in another connection.

While the above comparison is true and striking it is also true that at this station the range of variation in the amount of liposomic fat in the pink muscle is far greater than at Ilwaco. The fattest muscles have a greater amount of intracellular fat, the poorer muscles have a much smaller amount of intracellular fat.

Caudal pink muscle.—The caudal pink muscle of salmon from the Warrendale station shows the sharpest contrast as regards the amount and arrangement of the fat. In salmon no. 120 the intermuscular fat is all gone except along the connective tissue septa where it is present in scattered but medium-sized drops. In the poorer salmon the amount of intermuscular fat in the caudal pink is practically nil. Here and there in the thicker septa between bundles of fibers one will find an individual droplet or a group of three or four droplets not more than 4 or 5 μ in diameter.

The intramuscular fat of the caudal pink muscle is very slight indeed even in the fattest fish. The smallest fibers are fairly well supplied with liposomes which run in chains comparatively evenly distributed throughout the sarcoplasm. In these instances, however, there are distinct groups of liposomes under the sarcolemma, but at the surface of the sarcoplasm. There is a distinct difference in size between the surface liposomes and the deep ones. The former range from 1 to 1.5 μ in diameter, while the latter are only from 0.2 to 0.4 μ in diameter in fish no. 120. In salmon no. 126 the liposomes are still present in the small fibers, having much the same arrangement as that just described and averaging about 0.4 μ in diameter.

In the intermediate and in the larger sized fibers the amount of intracellular fat is very small. In the larger fibers only an occasional group of very tiny liposomes at the surface of the fiber can be seen. In the intermediate fibers there are now and then fibers which have a comparatively even sprinkling of tiniest liposomes throughout the mass of the protoplasm with somewhat larger liposomes at the surface of the fibers. On

an average for the station, however, one must say that the presence of liposomes is very greatly reduced, both in size and number for all the intermediate fibers, while for the larger fibers it is present only in traces.

Trunk dark muscle.—In the dark muscle of salmon from the Warrendale station there is even wider variation as regards the loading of fat than in the pink muscle. In fish nos. 120 and 121 the amount of fat in the trunk dark muscle is very great, while in no. 125 it is low. In the fatter salmon the loading of fat is almost as great as in the specimens from Ilwaco, with the exception of Ilwaco specimen no. 111 which was an extraordinarily fat fish. On the other hand, in the poorer specimens the amount of dark muscle fat is only a small percentage of that at the Ilwaco station.

The intermuscular fat is comparatively plentiful, is located in the connective tissue septa and in the myocommata. However, the fat droplets average much smaller in size than in the Ilwaco specimens. Oftentimes the number of these fat droplets, especially of the smaller ones, seems relatively greater. In Warrendale fish the individual fibers are usually somewhat more definitely separated and this fact makes it easier to determine the relation of the intermuscular fat. In fish no. 125 the amount of this intermuscular fat is very low, but occasionally individual drops are as large in this fish as in those that have more fat. The amount of intermuscular fat varies in different regions of one and the same muscle. This variation undoubtedly is associated with a process of fat erosion which was first observed in certain Ilwaco specimens. In Warrendale fish the erosion process has gone much further and is more readily followed. In areas in which the fat has been most fully eliminated the intermuscular fat is reduced to tiny droplets.

The intramuscular fat of the dark trunk muscle is abundant in all of the fibers of the fatter fish. In no. 120 the cross sections and the teased preparations show that the fibers are especially richly supplied with liposomes in their sarcoplasm. The liposomes are of large size and in relatively long chains. There is considerable fusion of adjacent liposomes. Especially in fish no. 121 the liposomes are so large that one might better describe them as droplets. The diameters run from 1 to as much as 4μ. Certain of the fibers in this fish and also in fish no. 122 show fusion of liposomes into long rods of fat. These slender rods usually appear more or less constricted at points corresponding to the striations of fibrillæ.

The most striking thing about the fat in the trunk dark muscle of fish from the Warrendale station is its great irregularity in different microscopic areas. This has been spoken of in connection with the very fat fish no. 120, but it is an appearance that marks every fish examined. If the specimen is one of low grade, as in no. 125, then these irregularities are most prominent. Certain groups of dark muscle fibers will appear richly loaded with fat while other areas will be almost free, certainly will not contain more than from 30 to 50 per cent as much as in the fatter areas. In these clear areas the reduction in fat is due to two factors: First, the great reduction in the average size of the liposomes; and second, the great decrease in the number of liposomes. In numerous areas where muscle fibers are in close contact with small blood vessels the fat is very low in amount. This condition is described in the protocol of fish no. 125. The characteristic picture presented where a group of fibers lies along the blood vessel is as follows: First, that portion of the fiber next the blood vessel will have no intermuscular fat; second, the intramuscular fat will be either absent or greatly reduced in the corresponding area; third,

the portion of the fiber opposite the blood vessel will have a relatively high content of fat; fourth, as a rule the intermuscular fat in contact with the opposite outer border of the fiber will still be present.

The teased fibers of this dark muscle show instructive variations. Different lengths of one and the same fiber show wide variation in the loading of fat. This is expressed especially through the variation in size of the liposomes. But if the fat is very light in amount there will also be a variation in the number of liposomes. Careful focusing will always bring out the fact that the richer portions of the fiber will have a relatively large amount of fat under the sarcolemma. In many instances the liposomes in the chains will have fused, forming slender fat rods showing constrictions at the point of fusion. The poorer areas in the fiber will show a small amount of fat under the sarcolemma, smaller liposomes in the chains and little or no fusion. Where the fat is almost completely eroded the number of liposomes will be obviously reduced. In this case the reduction takes place more completely near the center of the fiber as compared with its superficial area.

In numerous instances at the other stations, as well as at Warrendale, I have noticed that while the fat is being eroded there will appear variations in number and arrangement of fat droplets under the sarcolemma. In a teased fiber a rather definite pattern will often be noted in this subsarcolemmal fat, a pattern which coincides with the blood vessels, the pattern being marked by rows of very small droplets along what would correspond to the border of the capillaries. Also small rings of droplets will appear at various points, sometimes in groups. These rings of droplets are arranged around a clear center. They are interpreted as part of the process of erosion of large intermuscular fat drops. As lipolysis goes on, the fat that is dissolved away from the large fat drop will often be redisposed in small droplets within the sarcolemma around the area which is being compressed by the large drop.

Caudal dark muscle.—The caudal dark muscle of the Warrendale fish varies through even a wider range of fat content than the corresponding muscle from the trunk region. There is always considerable fat in the myocommata, but the amount of intermuscular and intramuscular fat varies exceedingly.

In the fatter fish the intermuscular fat is reduced in the number of droplets present, but particularly in their size. In no. 125 there is practically no fat in the caudal dark muscle.

In this salmon certain of the dark fibers are absolutely clear of fat within the fibers, and the fattest fibers observed contained only a sprinkling of liposomes around the superficial areas with a trace in the center of the fiber. The whole muscle is as nearly fat free as any dark muscle examined. It is noticed here also, as in the trunk muscle, that the fibers freest of fat lie in the neighborhood of small blood vessels.

In a few scattered fibers in the caudal dark muscle of no. 125 an appearance is noted for the first time that is suggestive of a disintegrative process. We have not been able to convince ourselves that these fibers are actively breaking down, but they certainly do show appearances suggestive of the initial stages of water absorption characteristic, for example, of cloudy swelling. The fibers stain lightly in a way which characterizes an early stage of muscle degeneration. These fibers also contain small transparent, highly refractive and lightly stained granules. The stain does not have the usual appearance of fat stain—that is, the color is not the brick red of the scarlet

red dye. Rather it is a more brilliant and dark appearing neutral red. The amount of stain taken is only slight. These granules do not contain any pigment, as was noted in degenerating cheek muscle of fish no. 140, to be described later.

PROTOCOLS.

Male, salmon (no. 120), length 937 mm., weight 11,480 grams, Warrendale, August 16, 1911.

This fish was taken from the McGowan seining grounds, 1½ miles below Warrendale. It was a fine fish, in splendid condition; the nose slightly hooked, no large teeth, the testes two-thirds developed, color normal, but a trace darker than fish at the mouth of the river; back darker but not rusty; fins perfect.

The muscles were pink and oily. The fish was received fresh from the seining grounds, and the fin muscles were still alive when samples were taken.

Microscopic examination of the trunk pink muscle (K87, 88, and 90).—The intermuscular fat is about one-half to two-thirds as great as in no. 118 from Ilwaco. Its disposal between the fibers is similar to the fish taken from the mouth of the Columbia. There is less intermuscular fat from the middle of the dorsal portion of the great lateral muscle.

The intramuscular fat is abundantly present in all of the fibers. The smaller fibers are more deeply stained, showing the greatest amount of fat. The small fibers of the teased preparation are filled with chains of liposomes, the individual liposomes being larger than in the large fibers. In the fibers of large size the chains of liposomes are not quite so numerous, and the liposomes themselves are relatively small. Two fibers, side by side, one large and the other small, are in sharp contrast.

Microscopic examination of the caudal pink muscle (transverse section, K91).—In this section the intermuscular fat is all gone except along the connective tissue septa, where it is present in scattered but medium large drops (1/12 oil immersion). The substance of these fibers is well fixed in formalin, and the fibrillar outlines show clearly. In the large fibers of the section there is no fat stain in the body of the fibers. Occasionally at the very surface there are tiny groups of liposomes. In the smallest fibers there are in the body of the fibers between the fibrillæ numerous extremely small liposomes. There are distinct masses of liposomes on the surface of the sarcoplasm and under the sarcolemma. The liposomes within the fiber are from 0.2 to 0.4 μ in diameter, those on the surface from 1 to 1.5 μ in diameter. The intermediate-sized muscle fibers have a few scattered groups of liposomes immediately under the sarcolemma, but none in the body of the fiber. These observations are confirmed on fragments of fibers in which the fibrillæ are turned in a horizontal position.

Microscopic examination of the trunk dark muscle (sections K72–76).—The muscle fibers in this material, both in the transverse sections and in the teased preparations, are especially richly supplied with fat. The fat is crowded, both between the fibers and throughout the sarcoplasm of the fibers. The intermuscular fat droplets are numerous, of medium size, but not so numerous nor so large as in fish no. 111 from the Ilwaco station.

Certain areas in the transverse section have an appreciably smaller quantity of fat. These areas are associated with connective tissue septa carrying blood vessels, and are similar to those noted in salmon no. 117 and no. 118, from the mouth of the Columbia. This appearance is undoubtedly due to the beginning of fat erosion from this type of muscle, and is greater in this section than in the two Ilwaco fish referred to. The erosion areas have a much less quantity of fat than in fish no. 118. The fat droplets are not so numerous and are smaller.

On the whole, the amount of fat is somewhat less than in fish no. 118, though the comparison is difficult to make. In the transverse section of one fiber 100 μ in diameter, 46 droplets were counted. They were from 3 to 6 μ in diameter. In the spaces around the particular fiber and in the same focal field were 12 droplets oval in shape, 20 μ long, but from 4 to 6 μ thick.

The intramuscular fat is remarkably uniform in its distribution through the muscle fiber, the larger droplets averaging from 4 to 6 μ in diameter. The disposal of the fat is similar in character to that noted in previous fish and is shown in figure 3, plate III.

Microscopic examination of the caudal dark muscle.—The muscles in this section have very much less fat than the trunk dark fibers. The intermuscular fat is smaller, 6 to 10 μ in diameter, but the droplets are numerous.

The largest intramuscular liposomes average 3 μ in diameter, but there are many smaller liposomes. There are rings of small droplets around the border of the muscle, these averaging 4 μ in diameter. This superficial fat sharply marks the boundaries of transparent cross sections forming a definite mosaic under the low magnification. It is almost wholly intramuscular fat lying under the sarcolemma.

Male salmon (no. 121), length 950 mm., weight not given.

A first-class fish from the McGowan seining grounds, 1½ miles below Warrendale on the Columbia River. The testes two-fifths developed.

Microscopic examination of the trunk pink muscle (transverse section, L1).—The amount of intermuscular fat is intermediate between salmon no. 115 and no. 117 from Ilwaco. The fat droplets between the muscles are many of them relatively large but not so numerous, and do not average so large as in no. 118. The largest drops are from 45 to 55 μ in diameter. The fibers themselves are somewhat more compact in arrangement, but the outlines of the fibers in cross section are less smooth and circular than in no. 115, but not so angular, and the fibers do not seem so much compressed as in fish no. 117.

The surprising fact is the great amount of intramuscular fat. This fat is most thickly deposited through the small fibers, where the liposomes have a size from 1 to 2 μ in diameter. These liposomes are quite uniformly distributed through the substance of the small fibers. An occasional fiber will have its liposomes more thickly set around the superficial border. In certain of the smallest fibers, an example 75 μ in diameter, also in other regions of the section, there is fat in relatively small droplets just outside the surface of the sarcoplasm and under the sarcolemma.

Liposomes are present in the largest fibers also, but are exceedingly small and not so plentiful in the body of the fiber. In these very large fibers many liposomes are found between the fibrillæ near the surface of the fiber. They appear as if the liposomes were formed just under the sarcolemma and between the fibrillæ of the most superficial or band-shaped layers. In the inner borders of the band-shaped fibrils there is a second zone where the liposomes are present in relatively greater numbers. The liposomes are not larger but more numerous in this zone.

Microscopic examination of the caudal pink muscle (L9, 10, and 11).—Sections were preserved for 18 hours in formalin. The fibrillæ show well indeed. The surface layer of band-shaped fibrillæ are in contrast to the smaller fibrillæ of the body of the muscle. The fibers are compact in arrangement, but retain a certain amount of round contour which characterizes muscular tissue in prime condition. The following points characterize the tissue: (*a*) There is very little, almost no intermuscular fat in the section. Here and there a small droplet is found in the angles between the fibers. The largest one observed is only 18 μ in diameter. (*b*) The outlines of the fibers of the caudal pink muscle are definitely marked by very small fat droplets, measuring from 1 to 2.5 μ in diameter, many of them even smaller. The point is difficult to determine, but the droplets seem to be within the sarcolemma. (*c*) The caudal pink muscle fibers are relatively low in liposomes. The smallest fibers contain only a few liposomes. The fibers measuring from 50 to 100 μ in diameter have easily identified liposomes, but the larger fibers are free of liposomes in all but the extreme superficial part of the fiber (1/12 oil immersion.) The liposomes in the smaller fibers are chiefly around the outer third of the muscle. In the central portion of the fiber there is not more than one-fourth as much stainable fat as in this superficial rim.

Microscopic examination of the trunk dark muscle (transverse section, L5 and 6).—The lateral dark muscle shows an amount of fat greater than in no. 117, but not so great as in no. 111. The fibers are compactly arranged everywhere in the dark, and under the low magnification their outlines are marked by the excess of fat in that zone, the fat droplets averaging from 4 to 6 μ.

In some of the angles between fibers and in certain regions where the connective tissue is greater there is unquestioned intermuscular fat. The size of these drops runs from 10 to 15 μ in diameter. Along one exceptionally thick septum this intermuscular fat is absent. The section has the appearance which indicates the process of resorption of fat (under the 1/12 oil immersion).

The sarcoplasm of these fibers is full of small fat droplets almost as large as those in no. 115. The droplets are too large to be called liposomes, though every gradation exists between liposomes 1 μ in diameter up to these larger droplets which average 3.6 to 4 μ in diameter. There are certain fields of the section which contain relatively less fat in the sarcoplasm, the fat droplets being almost gone and the liposomes relatively smaller but thickly distributed. In these fields there is also relatively less fat around the border of the fiber, i. e., less fat under the sarcolemma.

Microscopic examination of the caudal dark muscle (L14 and 15).—This transverse section of the dark muscle from the tail is quite well supplied with fat. I do not see fat droplets that are unquestionably between the fibers, but groups of droplets that lie just under the sarcolemma were noticed, the largest of which were 7 μ in diameter.

The intramuscular fat is greatly less than that in the dark muscle of the portion of the body where the largest liposomes measure 3.6 μ in diameter, but this larger size is rare. The largest of the liposomes run about 2 μ in diameter. The smaller are from this size down to 0.3 μ and less in diameter. Judging from the intensity of the stain, one would say that the caudal dark does not contain more than one-fourth, possibly one-third, as much fat as the trunk dark.

There are areas, especially along certain septa, which have a strikingly less quantity of fat. This appearance is associated with the more vascular areas.

Female salmon (no. 122), length 890 mm., weight 8,980 grams, taken at Warrendale, August 17, 1911.

This was a good conditioned fish, taken at McGowan's seining grounds, 1½ miles below Warrendale. The weight of the ovaries was 680 grams, stomach quite small, appearing one-half degenerated.

Microscopic examination of the trunk pink muscle (transverse section, L38).—This section shows a relatively large quantity of fat between the fibers, not so much, however, as in no. 115 and no. 118 (1/12 oil immersion). The larger drops measure about 20 μ in diameter. The distribution is similar to that in the fish just mentioned. In this section the smallest fibers, 40 μ in diameter, are thickly set with liposomes distributed rather uniformly through the fiber. These liposomes vary in size from 0.6 to 1.3 μ in diameter. In the medium fibers, 75 to 100 μ in diameter, the number of liposomes diminishes in the center of the fiber; also there is a marked diminution of the size of those present. In one fiber, 100 μ in diameter, the liposomes in the middle of the cross section measure from 0.1 to 0.4 μ in diameter.

In the larger fibers of the section, those above 100 μ in diameter, there is a marked diminution of liposomes. This diminution is most apparent in the main body of the fiber, i. e., exclusive of the superficial area. This contrast in amount of liposomes between the deep and superficial part of the fiber is sharp, giving the fiber the appearance in cross section of having a superficial ring of fat. In certain of the larger fibers, the central liposomes are absent, or, at any rate, one can not distinguish them with the oil immersion. In these same fibers liposomes around the superficial border will vary greatly in size, measuring from scarcely identifiable liposomes up to as much as 1.4 μ in diameter.

An examination of the longitudinal sections (L33), brings out the fact that there is a relatively high content of fat near the surface of the fiber, both external to the fiber and just under the surface. The external fat is in the connective tissue, the endomysium, therefore, intermuscular.

Microscopic examination of the caudal pink muscle (section L51).—The fibers are almost free of fat. There is no intermuscular fat hanging to them, but the connective tissue, myocommata, of the caudal pink muscle (slide L54) is crowded with adipose fat.

Scattered over the surface of the fiber, all apparently under the sarcolemma, is a good deal of fat in droplets, from 2 to 3 μ in diameter, not uniform in size. The small and intermediate sized fibers have tolerably evenly distributed chains of smallest liposomes. In the larger fibers the central portion is relatively free of chains of liposomes, which, in many instances less than 0.2 μ in diameter, are so small they are difficult to identify. Certain of these teased fibers show the striations clearly. In one such example there are 13 striations in 36 μ (slide L54, 15 striation to 36 μ). The diameter of the fiber showing these striations is 63 μ. The chains of finest liposomes in the largest fibers are not always perfect. The irregularity is due to the dropping out of individual liposomes. In some chains there are more liposomes than fibrillæ. This is due to the presence of two liposomes in the space opposite certain striations. There is not always perfect correspondence in the number of striations and liposomes in the chains in these pink fibers.

This slide gives examples of the cone-like ends of the fibers. These ends are supplied with liposomes just as in the body of the fiber. All the teased material of the caudal pink fibers shows an increased quantity of fat at the surface of the fiber. This fat is in tiny droplets varying from the smaller liposomes 0.2 μ in diameter up to 2.5 and even 3 μ. Many of the droplets are in regular rows, but not so regular as those deep in the muscle fiber. Undoubtedly this fat is just under the sarcolemma, a fact confirmed by the appearance when the optical section cuts the middle of the fiber.

Microscopic examination of the trunk dark muscle (sections L18–25).—These transverse sections show a relatively large quantity of intermuscular fat. The fibers are more widely separated than is usual

for dark muscle, and it is correspondingly easy to determine whether the fat is free or under the sarcolemma. The longitudinal section (L25) gives a fine confirmation of the cross section.

The intermuscular fat is present in large quantity, the larger drops averaging from 12 to 15 μ in diameter. There are many smaller droplets interspersed among the larger.

The fat under the sarcolemma shows well both in the cross sections and in teased preparations. It is seen best when the focal plane cuts the surface of the fiber. The drops are irregularly placed over the surface, being held in position by the delicate sarcolemma. When the focal plane cuts the center of the fiber one can see that the sarcolemma incloses the fat drops.

The intramuscular fat in the body of the fiber is uniform in its distribution, as viewed in cross section. The droplets average from 2 to 2.5 μ in diameter, many of them smaller, but some larger. Rarely does one see a droplet greater than 3 μ in diameter.

In the longitudinal sections the chains of liposomes are more numerous and longer than in any tissue examined. In some fibers these chains extend across a whole microscopic field. Several fibers show chains in which the individual liposomes have fused. In such case the fat is in long slender rods, showing constrictions corresponding to the fibrillæ (slide L46). In comparison with salmon no. 111 and no. 115 from Ilwaco, this fish has as many, even more, intramuscular fat droplets, but the droplets are relatively smaller. The larger droplets, which in the Ilwaco salmon measure as much as 6 μ in diameter, are absent here.

Microscopic examination of the caudal dark muscle, teased (L58).—These teased caudal dark fibers show considerable variation in the amount of loading of the fat in the different fibers present (1/12 oil immersion). One fiber contains a relatively large amount of fat on the surface of the fiber, interpreted as under the sarcolemma. The larger fibers show but little fat in this subsarcolemmal region. If present at all, it is relatively small, running 4 to 5 μ.

The fat within the substance of the fibers is greatly reduced in amount in comparison with the fibers from the middle of the body. The liposomic chains are not so numerous and the average size of the liposomes not so great. In these caudal fibers one often finds a chain of liposomes which has become fused, as in the trunk dark. In the muscle fibers least filled with fat the number of chains of liposomes is very much less and the size of the liposomes does not average over 1.2 μ.

A large salmon (no. 125), length and weight not recorded, taken at Warrendale, Oreg.

Microscopic examination of the trunk pink muscle (transverse section, 1/12 oil immersion).—The amount of intermuscular fat is relatively small, the drops are often large, as much as 50 μ in diameter, but they are few in number—not more than 1 to every 3 or 4 fibers.

The amount of fat within the fibers is obviously greater in the smaller fibers than in the larger. In the small fibers, 50 μ in diameter and less, the liposomes are fairly uniformly distributed in size, ranging from 0.6 to 2.5 μ in diameter, but averaging about 1 μ in diameter. The intermediate fibers have the fat collected around a superficial zone about 8 to 10 μ beneath the surface of the fiber. Some of the fat droplets in this region are relatively large, 4 μ in diameter, though these are comparatively rare. In the center of the fibers the liposomes are smaller, the larger ones averaging 1 μ. In a fiber 100 μ in diameter the liposomes are quite uniformly distributed over the surface of the cross section, but run only 0.2 to 0.6 μ in diameter. The liposomes in the largest fibers are of practically the same diameter, but not so numerous. In the largest fibers examined and in those most free of liposomes there is a noticeably greater number of liposomes near the surface of the fiber. In certain of the fibers these liposomes are just under the sarcolemma, between the fibrillæ of the surface layer and in the zone at the inner border of the superficial or palisade fibrillæ.

Microscopic examination of the caudal pink muscle.—The intermuscular fat is limited to the myocommata and to the thick connective tissue septa.

The intramuscular fat is present as liposomes in the small and intermediate fibers and just under the sarcolemma of most of the large fibers. Liposomes are distinguished with difficulty in the central body of the large fibers.

Microscopic examination of the trunk dark muscle.—The intermuscular fat is not so great in amount as in no. 122, though the fat drops run up to 20 μ in diameter. There are areas over the section which have practically all the intermuscular fat as well as much of the intramuscular fat removed.

The fibers are thickly studded with chains of liposomes 0.6 to 2 μ in diameter. Only occasionally are adjacent liposomes fused as in no. 121 and no. 122. The longitudinal section shows a greater quantity of fat along the borders than in the bodies of the fibers.

The fat under the sarcolemma is in drops measuring from 4 to 5 μ in diameter, occasionally 6 μ. The number of these fat droplets around any given fiber varies greatly, due to the fact that the fat is being removed in the neighborhood. Choosing an area containing the most fat, the intramuscular fat is in liposomes from 0.4 μ in diameter up to as much as 4.3 μ. The number of the largest droplets is relatively small, but when present they are evenly distributed through the fiber. There is great variation in the size of the liposomes in different portions of the length of one and the same teased fiber.

In the areas referred to above the intramuscular fat is reduced in amount. The size of liposomes is affected more than the number of chains. There are several fields through which small blood vessels go in which that portion of the muscle in contact with the blood vessel is strikingly free of fat. In the neighborhood of a blood vessel where the fat is most removed the droplets lying under the sarcolemma are reduced to a few liposomes lying on the side farther from the vascular area. The largest of these liposomes measure 1.6 μ in diameter. Through the body of the same fiber in the half opposite the blood vessels the liposomes are fewer in number and relatively smaller in diameter (0.4 to 1 μ) than in portions of the fiber not in contact with blood vessels. In the third of the muscle lying next to the vascular area the liposomes are still present, but small—too small to measure accurately. There are numerous areas in this section (L74) showing contrast as regards the degree of removal of fat.

Microscopic examination of the caudal dark muscle.—There is quite a little fat in the myocommata. Through the body of the dark muscle, however, there is no intermuscular fat.

Under the oil immersion certain fibers of this section are absolutely clear of fat within the fiber. In other fibers there are traces of liposomes too small to measure. These traces are confirmed by fibers which have been turned to a horizontal position in the handling. In still other fibers there are scattered and irregularly placed groups of liposomes at points near the surface, but none deep down in the substance of the sarcoplasm. The fattest fibers observed contained a fairly uniform sprinkling of liposomes around the superficial area of the fiber and a somewhat smaller quantity in the middle of the fiber. The whole preparation presents as nearly a fat-free section as has been observed of caudal dark muscle.

A number of fibers contain numerous spherical bodies measuring approximately 2 μ in diameter and having a dark-red color (1/12 oil immersion). These bodie are irregularly placed through the substance of the fiber, as are the brown pigment granules of degeneration. The stained bodies are spherical, and one might take them for fat bodies. However, if they are fat bodies then the color of the stain is distinctly different from the type. This color is a brilliant dark neutral red as against the usual lighter brick red characteristic of this stain. It is possible that we are dealing here with the reaction of some special fat which stains differentially, according to the observations of Bell.

Female salmon (no. 126), length 780 mm., weight not taken, Warrendale, Columbia River, August 24, 1911.

This salmon was fresh from the McGowan seining ground and was chosen as representative of the group of fish which show an advanced stage of migration change at this station.

Microscopic examination of the trunk pink muscle (transverse sections M1–3, L78).—The intermuscular fat is in relatively small amount. The myocommata contain a small amount of fat arranged as a narrow band of droplets on either side of the tendon. The larger fat drops measure 30 to 40 μ in diameter, seldom more. The intermuscular septa still contain a small amount of intermuscular fat. In the larger of these sheets of connective tissue a few fat droplets measure as much as 15 μ in diameter, most of them less. These are in the areas where in fish no. 118 the droplets were as much as 100 μ in diameter. Most of the fat drops are small and in relatively small group of 9 or 10 droplets in a group.

The intramuscular fat is very low in this specimen. It is present in the small and intermediate fibers, but difficult to distinguish in the larger fibers. The smallest fibers have their intracellular fat tolerably thickly sprinkled over the microscopic field. Most of these fibers show a somewhat greater amount of liposomes near the surface. The medium fibers show great variation. Certain ones are almost clear of liposomes, while others have a liberal sprinkling. In muscles of this size there is a condensation of fine liposomes under the sarcolemma. The same arrangement is true for the smallest fibers. These small intracellular liposomes are 0.4 to 0.6 μ in diameter.

In the largest fibers in the section one can scarcely find any liposomes in the body of the fiber. Around the border and immediately under the sarcolemma, especially in regions which have intercellular fat in the neighborhood, there are groups of liposomes.

These groups are shown in the figure 10, plate VII, and are characteristic. In one fiber 144 μ in diameter very delicate liposomes are rather thickly dispersed in the superficial layer and more scattering in the central portion of the fiber. This fiber is smaller than the average of the large size and has relatively more fat.

The teased pink fibers are filled with liposomes. These are in long chains, which are quite uniform in appearance. In the small fibers the liposomes are from 1 to 1.5 μ and occasionally 2 μ in diameter. In a number of instances observed adjacent liposomes have fused into oblong droplets. In certain isolated fibrillæ the liposomes are adherent but irregular in position. In a fiber about 150 μ in diameter I find that the central portion has only scattered chains of liposomes, while near the surface the chains are more numerous. In either case the liposomes are of irregular size in the chains and not of regular arrangement, as in the type from down river. The striations in this material are very narrow. I have not examined carefully enough to determine the exact relation between the liposomes and the striations. The number of liposomes in a given area varies in different portions of the fiber. There are irregular patches of fat droplets of liposomic size at the surface of the fiber. From this appearance and that noted in the cross section one comes to the conclusion that these patches of liposomes are the ones shown under the sarcolemma in the transverse section. The arrangement of these patches of fat under the sarcolemma is partly dependent on some pressure factor. At any rate, there is a fairly definite map shown by them. In some instances this may correspond with the capillary net. Also there are numerous areas, oftentimes two or three in the same field, in which the fat is arranged in a definite ring around a clear area. This suggests a relation to some relatively large intercellular fat drop. There is no other structure present with which such a definite arrangement of fat drops coincide, and the number of instances observed is too great to be a mere matter of chance. This pattern-like arrangement of fat under the sarcolemma was often noticed in preparing fresh material in the field. In one of three fibers of a group examined in this connection there were a number of fused liposomes in the chains. These fusions have taken place in the chains of liposomes near the surface, but are not of the subsarcolemmal group.

Microscopic examination of the caudal pink muscle (section M11).—The amount of intermuscular fat is insignificant in this section, almost exclusively limited to the myocommata. The intramuscular fat is also very small. In the very smallest fibers there is still present quite an appreciable amount of fat in small liposomes. These liposomes vary in size, about an average of 0.4 μ. They are not so numerous as in the same size of muscle fiber in the trunk region. There are small fibers which show groups of liposomes under the sarcolemma. In the medium-sized fibers such liposomes as are present are limited to the superficial layer of fibrillæ and to the space under the sarcolemma. In the large fibers the only trace of fat is under the sarcolemma, and that is present only in isolated regions where the liposomes are of scarcely visible size.

Microscopic examination of the trunk dark muscle (sections M7, 16, 25).—Section M7 shows a comparatively slight amount of intermuscular fat. That is chiefly along the thicker connective tissue strands. Among some of these strands which are more vascular the low magnification shows areas in which the bordering muscle fibers are almost free of fat. The appearance suggests that the fat is in process of removal. Under the 1/12 oil immersion it is noted that in the compact areas of the muscle there are scattered droplets of intermuscular fat. The drops are comparatively small in size, 3.5 to 4 μ, but occasionally as much as 12 μ in diameter.

The intracellular fat is present in medium amount. It is distributed less uniformly over the surface of the section of the fibers. It is noticeably less in amount in the center of the section of many of the larger fibers. The diminution in the amount of the fat in the middle of the fiber is primarily due to a great reduction in the size rather than in the number of the liposomes. In the center of a given fiber under observation the liposomes are from 0.4 to 1 μ in diameter, while at the surface of the same fiber they are 1.6 to 2 μ in diameter. In this fiber there are fat drops under the sarcolemma which measure 2.5 to 3 μ in diameter.

The examination of a fiber bordering on one of the lightly stained areas mentioned above shows no large fat droplets, and the liposomes are reduced to an average size of 0.3 μ in diameter. There is a group of liposomes about 1.2 μ in diameter under the sarcolemma of this fiber at the point farthest

from the blood vessel. Bordering on the opposite side of the same area there are two fibers which show a very marked reduction in the number of liposomes next the vascular area, although the liposomes are not altogether absent. The parts of the fibers opposite the area contain larger liposomes, 1.6 to 2 μ in diameter.

There is a very great variation in the amount of fat in different parts of this section (M25, teased), if one is to judge by the microscopic size of the liposomes. Oil immersion examination of the dark fibers shows numerous chains of liposomes very similar in arrangement and appearance to slide M20 of trunk pink. The liposomes are more numerous than in the trunk pink, but the average size for the center of the fiber is about the same. Many of these fibers show fat droplets on the surface under the sarcolemma. Certain of the fibers show that the surface of the fiber has relatively large liposomes. In a certain case near the superficial focus are liposomes 2 to 2.4 μ in diameter and near the center of the fiber numerous smaller liposomes not over 0.6 μ in diameter with an occasional larger one 1.2 μ. The loading of liposomes varies along the length of the fiber. This might easily happen in a tissue where the fat was being eroded, since the arrangement of blood vessels can not be uniform with reference to the surface of the whole fiber (fig. 4, pl. IV).

Microscopic examination of the caudal dark muscle, slides M 16 and 17.—The intercellular fat has disappeared, or is limited to a tiny droplet here and there in the connective tissue (1/12 oil immersion examination). There are no larger drops or groups of droplets as in the trunk pink muscle. The myocommata still have some fat drops.

The intracellular fat is present in the dark caudal muscle, but the liposomes are extremely small in size. There are no fibers with the larger liposomes characteristic of the normal dark muscle. The smaller liposomes average only 0.4 to 0.8 μ. In a small area which contains more fat, the liposomes are larger, from 0.4 to 2 μ in diameter. These liposomes are in a group toward one side of the fiber in an area about 20 μ square. The center of the fiber has the smaller liposomes, and there is also a very marked irregularity in the number in different parts of the field.

In the above fiber and in four others in the immediate neighborhood there are small fat droplets under the sarcolemma, measuring 2 to 3 μ, but in each case these droplets are on the side opposite the adjacent blood vessels. The liposomes throughout the central portion of the fibers in fields in which the fat is evidently sharply removed are reduced to scarcely distinguishable size, but are comparatively numerous. On the surface of such fibers the liposomes are about 0.6 to 0.8 μ in diameter and also numerous. Certain portions of the section show the fibers turned horizontally. Liposome chains can be distinguished in these fibers. In one such fiber the liposomes of the chains are about 0.6 to 0.8 μ in diameter. There are no fused liposomes in this case. This caudal muscle does not have more than one-half to three-fifths the fat of the trunk muscle.

DISTRIBUTION OF THE FATS AT A LATE INTERMEDIATE STAGE OF THE SPAWNING MIGRATION.

A study was made of the amount of fat present in the tissues of salmon from the Columbia River at the Celilo Rapids. These fish have passed through a longer stretch of fresh water and through the relatively swift currents of the canyon of the Cascades. The famous fishery of Mr. Frank A. Seufert extends along the full extent of The Dalles of the Columbia.[a] The numerous fish wheels adapted for the different stages of the water make it an ideal collecting ground for scientific material. At the time of the visit to the fishery in August, 1911, active fishing was in progress on the lower Dalles, at Celilo Falls, and at the Tumwater seining grounds. The samples that were studied came from a point known as the Cement Wheel, also from the seining grounds at Tumwater immediately below the Celilo Falls. The Cement Wheel is about 300 yards above the mouth of the Government canal.

The Cement Wheel salmon will have battled only a short distance of the swifter portion of the rapids of The Dalles. Two salmon were taken from this point, a male and

[a] Mr. Seufert has always taken an active interest in the scientific questions concerning the propagation of the salmon and in work tending to develop and protect the industry. He aided the present work by putting at our disposal every facility for securing material in the best of condition.

female. The protocol for no. 127 is the male. By reference to the protocol it will be seen that the trunk pink muscle contains a medium amount of intermuscular fat. The fat droplets have a distribution that is normal, but the relative size of the droplets is small so that the amount of fat represented is evidently greatly decreased as compared with the standards from Ilwaco.

The intramuscular fat of the trunk pink muscle was present in much lower amount than in the fatter specimens from Warrendale but also in greater amount than in the poorer specimens. The characteristic of these Celilo fish in this regard is in the relatively low fat in the larger pink muscle fibers. Only scattered traces of intramuscular fat were found in any of these fibers, and those traces usually in the neighborhood of intermuscular fat.

Keeping in mind the condition of the dark muscle from the salmon from Warrendale it was to be expected that the corresponding tissue in the Celilo salmon would have a considerable loading of fat. This was found to be the case. The specimens from the Cement Wheel agreed in that the dark muscle fat was definitely diminished in quantity in comparison with average fish from Warrendale, and sharply diminished in comparison with Ilwaco specimens. The samples chosen were the average of the type running at that time, August 22 and 23. The grade of fish at this season is very much lower than at an earlier date, but this is a factor which characterizes the entire series studied and does not interfere with the comparison.

The caudal pink muscle was practically free of fat in each of the specimens studied. The caudal dark muscle had both intermuscular and intramuscular fat, but in each instance about 40 to 50 per cent as much as in the trunk muscle of the same specimen. The female of the Cement Wheel specimens showed less fat in both the trunk and caudal dark muscle than was present in the muscles of the male, notwithstanding the fact that the male gave other evidences of a greater retrogression in general than did the female.

Little or no variation could be shown between the salmon taken at the Tumwater seining grounds and those taken at the Cement Wheel. In each instance there was a fair showing of intermuscular fat in the pink muscle, and an amount of fat within the fibers of both the pink and dark muscle which characterize an approximately average grade running below the Cascades.

At the time of this visit no fish were running which were judged to be of as high a grade as no. 120 and no. 122, described in the protocols from Warrendale.

PROTOCOL.

Male salmon (no. 127), length 800 mm., taken at Seufert's Cement Wheel, The Dalles of the Columbia, August 22, 1911.

The fish was silvery in color, with dark dorsal surface; shape that of a half-exhausted specimen; flesh oily in appearance and to the touch; visceral mass small and degenerated.

Microscopic examination of the trunk pink muscle, I.—The intermuscular fat is medium in quantity. There are a few large droplets, the largest 40 μ in diameter, but many smaller droplets, especially of the size from 3 to 6 μ in diameter. The amount of fat is much less than in the specimens like no. 118 from Ilwaco, estimated at 40 per cent. The distribution of the intermuscular fat varies in different parts of the preparation. In certain parts the amount is not more than 25 per cent that described above.

The intracellular fat varies extremely in the different fibers. In the small fibers, size 40 to 70 μ in diameter, the fat droplets are fairly numerous but of larger size and greater number around the surface of the fiber. These fibers (1/12 oil immersion) show that the fat droplets aggregated around the surface are within the sarcolemma and superficial. In the center of the fibers the liposomes are much smaller,

about 0.3 to 0.5 μ. There are droplets under the sarcolemma in practically all these pink fibers. They measure from 0.8 μ up to 2 μ in diameter. The average amount of intramuscular fat in the small fibers is not more than 40 to 60 per cent of that found at Warrendale. Many of the intermediate-sized fibers show only traces of fat in the center of the section. Around the circumference there is a somewhat greater quantity of fat, especially just at the surface. The largest fibers in the section have irregular outlines, look compressed and are very clear of fat, at least these fibers do not contain fat that stains in the usual way. I notice an occasional small group of liposomes at some point on the surface of the section, though these groups are few, often not present at all.

An appearance that is difficult to interpret is due to the presence of very small highly refractive granules in the protoplasm of the large fibers. These granules do not take stain, at least, if they are stained at all, it is very different in appearance from the normal, and they do not appear to be uniformly present in all the large fibers.

Teased muscle (M38 and 39) shows a comparatively small amount of fat. The smallest fibers and some of the intermediate fibers have chains of liposomes. The chains in the small fibers are not so numerous as one usually finds. The liposomes themselves are very small, and the picture is one of low content of fat. In the intermediate fibers the chains of liposomes are more numerous near the surface of the fiber. Just under the sarcolemma there are groups of small fat droplets rather irregular in arrangement. In the larger fibers of these slides it is difficult to distinguish the chains except at the very surface.

Microscopic examination of the caudal pink muscle.—The fibers of the caudal region are closely packed together and are very free of fat (section M46). The connective tissue septa have strands of fat droplets, most of them small but some medium in size. These measure from 2 to 15 μ, chiefly the former size.

The caudal pink muscle fibers are practically free of intracellular fat. Certain ones show traces of fat at points on the surface, but these are only traces and are limited to areas bordering on the fat-bearing septa. Extensive areas with no septa between the fibers are free of fat.

The fibers are so compact that their outlines in cross section are irregular polygons.

The caudal pink teased muscle shows practically no fat in the fibers, traces only appear. Certain of the fibers have a slight bluish tinge and through their substance are opaque granules which are difficult to identify. These granules are in the interfibrillar spaces.

Microscopic examination of the trunk dark muscle.—The trunk dark muscle still retains a large amount of fat (slide M41 under the oil immersion). Drops between the fibers measure on an average from 6 to 9 μ, occasionally as much as 15 μ.

Fat is distributed throughout the substance of the fiber. The droplets vary greatly in size. The largest ones run from 2 to 2.6 μ in diameter. These are more numerous around the superficial portion of the fiber in most of the material, though groups of fibers are found in which these large liposomes are quite evenly distributed through the substance. In numerous fibers the central portion is relatively free of liposomes and the fibers look lighter in color under the microscope. In the light areas, however, there are liposomes present, though they are very small for dark fibers, 0.6 to 0.8 μ, and they are not as numerous as in the superficial border. Different portions of the section vary greatly in the amount of fat. The muscles freest of fat are those which lie along the septa which carry blood vessels. Under a low magnification these areas are sharply limited.

Teased fibers (section M44) give a good view of the amount of fat along the course of individual fibers. There is much variation in different lengths of one and the same fiber. In the fatter areas numerous groups of fat droplets lie over the surface of the teased fibers. These groups have a configuration such as was noted in similar muscle from specimens from Warrendale. Undoubtedly the arrangement of fat bears a definite relation to the arrangement of blood vessels. The chains of liposomes are continuous in some areas for long distances. The individual liposomes will measure 2 μ in diameter in the larger chains, but vary through a range of much smaller sizes according to the relative amount of fat present. The larger chains are obviously near the surface of the fiber. Occasionally a chain is noted in which the majority of the liposomes have fused, forming a fat rod such as has previously been described. These rods, as observed, lie near the surface of the fiber.

Microscopic examination of the caudal dark muscle.—The dark tail muscle has enough fat to give it a relatively deep stain (M56, oil immersion), but this fat is much less in quantity than in the trunk muscle of this salmon. The fat is condensed around the superficial areas of the fiber. Apparently there is some intermuscular fat in droplets 3 to 6 μ in diameter.

The intramuscular fat is arranged practically the same as in the trunk muscle, except that the size of the droplets is smaller throughout. Droplets 2 μ in diameter are relatively rare and the liposomes in the chains in the middle portion of the fibers run about 0.5 to 0.8 μ in diameter, about the size of the liposomes in the relatively fat-free fibers of the trunk.

The teased fibers give an explanation of the diffuse appearance of the stain in this section (M58–59). It is due to the extremely small but numerous liposomes. Liposomes are also present in groups just under the sarcolemma in many places. In the largest chains observed in fibers that seem relatively better supplied with fat the liposomes measure from 1 to 1.2 μ in diameter.

FAT IN THE TISSUES OF SALMON FROM THE SPAWNING GROUNDS.

Salmon at the spawning grounds in the Clackamas River, at Cazadero, Oreg., have been fasting from three to four months. During the entire time there is no food source for the energy which the salmon have expended other than in the tissues and in the stores of fat. One would certainly expect the great fat storehouse to be sharply drawn on if not exhausted by the time this stage of the life cycle is reached.

We have taken fish from the spawning grounds so emaciated and so weakened that they were scarcely able to maintain equilibrium in the river currents. One knows that some of these fish would die in a day or so even if they were kept in the water in the most carefully protected condition.[a] Fat is still present in the muscular tissues of such salmon. Its percentage is low, yet the microscope reveals an unexpected quantity.

There is considerable variation among the different individuals present at Cazadero. All of the early September spawners are undoubtedly of the so-called spring run of salmon. They have been in the river at least since May or June (estimated). Yet there are specimens that have very much more fat than the dying salmon mentioned above. These variations are shown best in a comparative study of the various types of muscle.

Trunk pink muscle.—The preparations of pink muscle show a sharp contrast with the loading of fat observed in the normal fish and in fish from the mouth of the Columbia River. Instead of the large intermuscular fat globules there are only small droplets present and these are few in number. They are located in the angles in the larger connective tissue masses which mark the points where several fibers are grouped. If two fibers are compactly pressed to each other there are no fat droplets between them. Where several fibers are separated by masses of connective tissue there may still be small groups of fat droplets. In such cases the fat droplets are small in size and few in number. In one specimen, no. 131, the number of intermuscular fat droplets averages about one droplet to ten fibers, and the largest droplet present measures only 6 μ in diameter. This quantity of intermuscular fat is insignificant.

The presence of a significant amount of intramuscular fat in these most exhausted specimens from the spawning grounds is in striking contrast to the disappearance of the intermuscular fat. The intramuscular fat is in minute liposomes less than 0.5 μ in diameter. The largest liposomes are in the smallest fibers. They are scattered throughout the substance of the fibers except in the surface band of fibrillæ, where the absence of interfibrillar fat gives the appearance of a clear band of fibrils around the superficial border of the muscle fiber as seen in transverse section. Around the surface of the pink fibers and under the sarcolemma are groups of fat droplets. Where

[a] Males were selected from those retained in the spawning pens. In one instance salmon for study were selected from those most advanced in retrogression yet with no obvious fatal lesions. Of those not used three had died by the next morning. This is positive evidence that the salmon selected were at the dying stage.

two fibers touch each other a double row of droplets slightly separated can be easily distinguished. The separating line, of course, is the section through the sarcolemma. The largest fat droplets in this series under the sarcolemma measure as much as 2 μ in diameter. Often the droplets are slightly compressed, evidently by the pressure of the sarcolemma, since the radial diameter is a little less than the diameter tangent to the fiber.

Taking the pink fibers as a whole it seems that in the better specimens the liposomic fat is present in greater number of droplets, also in larger droplets, than in the poorest specimens from Warrendale (compare fig. 10 and 11). Certainly this fat is greater than in no. 125 and no. 126. In fact, the comparison is close to those fish which have the highest quantity of intracellular fat at stations lower down the river. In the poorest fish taken from the spawning grounds the fat is almost completely eliminated both from the intermuscular and intramuscular regions. This is true for fish no. 140, which has the lowest amount of fat observed in the lateral pink muscle.

The pink muscle fibers themselves are not plump and round in the fish from the spawning ground. On the other hand, they form irregular polygons in cross section. Even the smaller fibers have lost their cylindrical shape. The fibers are more compact and the whole appearance suggests a diminution in volume (fig. 19, pl. XI).

In teased preparations there is one rather striking deviation from the typical arrangement of liposomes, namely, the deep-lying liposomes are no longer in such regular spindle-shaped rows as are found in the normal. The chains have the appearance of broken rows, in which the smaller liposomes are absent, thus giving the chains an irregularity that is rather constantly observed in the fish of this station. Those chains that are most definite and least interrupted are clearly located near the surface of the fiber. In the teased material from no. 139 there is a marked difference in the appearance of the liposomes present in the small fibers as compared with the larger. In the larger fibers the chains are less numerous and the droplets in the chains smaller. In this fish the small fibers, 40 to 50 μ in diameter, have very evenly distributed liposomes, the diameters varying from 0.3 to 0.7 μ. In certain fibers of this section there are irregularly placed highly refractive bodies which (1/12 oil immersion) are only lightly stained. These granules are probably associated with an early stage of degeneration.

These teased fibers also show irregular patches of liposomes over the surface of the fiber and under the sarcolemma. These fat droplets are a trifle larger than those within the sarcoplasm. In salmon no. 140 we still have a small amount of fat under the sarcolemma. In the larger fibers the intracellular fat is present only in traces, the liposomes being not over 0.2 μ in diameter and in very short, irregular, and scattered chains. In the smaller fibers of the material the number of liposomes is still relatively slight, but the size of the individual liposomes is somewhat larger. Where a liposomic chain is present it is noted that the arrangement of individual liposomes is very irregular, giving the chain the appearance of being broken.

Caudal pink muscle.—The pink fibers of the caudal region have as nearly no fat as in any specimen examined. The intermuscular fat is completely eliminated, while only a trace of intracellular fat is to be found. The teased preparations show that this trace is made up of definite but tiny liposomes which are only sufficient in quantity to give a faint stain to the section. Now and then in a fiber near the surface one can note

a few small liposomes in irregular groups. At best this quantity and distribution can be called only a trace.

Trunk dark muscle.—In the dark muscle there is no intermuscular fat, or if any is present at all it is in tiny droplets in those localities which contain the greatest amount of connective tissue. In a cross section and in a low-power field one might see two or three such areas.

The intramuscular fat in the dark muscle has markedly changed in its appearance and arrangement. The relatively large droplets characteristic of the normal tissue, often measuring as much as 6 μ in diameter, have practically disappeared in this stage. In place of the large droplets the dark muscle now contains a much greater number of relatively very small sized droplets of the type described as liposomes. Also the fat droplets under the sarcolemma are now reduced to liposomic size, not larger than 2 μ in diameter and averaging about 1 μ. In a cross section these tiny fat drops around the surface of the fiber and within the sarcolemma form definite rings which mark the outlines of the fibers. Examination under the 1/12 oil immersion shows that many of the droplets entering into the composition of this superficial layer of fat are wedged in between fibrillæ. Within the central substance of the muscle the liposomes are now interfibrillar in arrangement. They are small in size but numerous and comparatively evenly distributed through the sarcoplasm. This description applies to the trunk dark muscle of the fatter spawning salmon. The poor salmon do not have so many liposomes in the deep sarcoplasm and different individual fibers vary greatly in their fat content.

In the teased dark muscle fibers from the trunk it is noted that the most fat lies just under the sarcolemma, but that it is very irregularly placed. The droplets are small and seldom exceed 2 μ in diameter. There are a few chains of liposomes in the body of the fiber, but these chains are widely separated and consist of extremely small liposomes. The largest liposomes are about 0.4 μ in diameter in fish no. 139. In no. 140 the surface of the fibers has irregular fat droplets often running as much as 3 μ in diameter. But distributed through the substance of the fiber there are only traces of fat except at the very superficial sheet of sarcoplasm where the liposomes are measurable.

Caudal dark muscle.—In the caudal dark muscle there is still some considerable quantity of intercellular fat, especially in salmon no. 138, although this fat is less than in the lateral dark muscle of the same specimen.

Within the sarcoplasm of the fibers there are numerous areas in which there are only traces of fat. Even at the surface of the fiber there is often only a trace of fat. Under the sarcolemma the fat is in isolated groups of liposomes measuring only a fraction of a micron.

In fish no. 139 there is more fat in the caudal dark muscle, especially under the sarcolemma, where the droplets measure from 1 to 3 μ. Different portions of the section show great variation in the amount of intracellular fat. These areas are similar to that noted in Warrendale fishes no. 125 and no. 126. In fibers bordering on these vascular areas the fat droplets are removed from under the sarcolemma, and are absent except for traces in the body of the fiber.

In comparing the amount and distribution of fat in the Cazadero specimens it is obvious that the total percentage amount of fat is profoundly reduced. On the other hand, it is apparent that this reduction has taken place chiefly in the intercellular fat. The extreme case of exhaustion shows practically no fat either between or within the

fibers. Yet the majority of the salmon taken at Cazadero show on the average as much intracellular fat as is shown in those salmon taken from the Columbia River at a much earlier stage in the migration. Certainly they show as much fat in the fibers as all but the very fattest of the earlier specimens. It is this showing which presents such a striking factor in the comparison between the pink muscle of different salmon at the various stages. The pink muscle maintains a surprisingly large amount of intracellular fat throughout the whole series of stations, even when the fat is practically eliminated from the great storage depots.

In the case of the dark muscle, which at the early stages is surcharged with fat, there is an obvious gradual diminution from the mouth of the river to the spawning ground. On the other hand, there is no complete elimination of fat below that stage of smallest liposomes which characterizes the pink muscle as a type. The fat may be eroded from the dark muscle; that is to say, the large drops will gradually decrease in size but will never be completely eliminated. There is some factor operating which maintains a supply of liposomes in the active muscle of the major portion of the body. It is true this supply is not kept up in the caudal muscle, but this undoubtedly is due to the great and continuous activity of that musculature.

Cheek muscle.—The amount of fat in the cheek muscle has been described for fish from Ilwaco, but this particular type of muscle has not been studied in all the intermediate stations. However, in one specimen, no. 140 from Cazadero, this muscle has been carefully reexamined. The fibers of the muscle of this fish are even more compact than noted at Ilwaco. There is only a small amount of interstitial connective tissue and this carries a few scattered but small fat droplets.

The intracellular fat is present only in traces. In a large proportion of the fibers no fat can be distinguished; yet in a few of the smallest fibers merest traces are discernible.

The striking characteristic of the cheek muscle of no. 140 is found in the evidences of degeneration. Certain of the fibers take a definite stain not due to the fat, but due to characteristic degenerative changes in the fibers. These fibers stain a light rose pink. Under the oil immersion the fibers that take this special stain show signs of disintegration or atrophy. The bodies of the fibers have greatly shrunken. Their outlines show that they are compressed as if between adjacent fibers. The fibrillar structure has likewise disappeared. Slight vacuoles are present. The most diagnostic feature of the change consists in the pigment granules of muscular atrophy. These pigment granules are irregularly placed and vary greatly in size. Measured with the 1/12 oil immersion they vary between 0.1 and 0.2 μ in diameter. The degenerative changes noted are typical of simple atrophy. The changes in this particular muscle are the most advanced that have been noted. There is some slight indication of atrophy in the trunk musculature even at an earlier stage of the journey, but nowhere else have I found definite degenerative pigments, unless the highly refractive bodies noted in no. 125 and no. 139 be such.

The further details of these degenerative changes are being studied and will be presented later in a special report.

ANALYTICAL DETERMINATIONS OF THE PERCENTAGE OF FATS IN THE SPAWNING SALMON.

The three Cazadero salmon of this series from which samples were taken for fat percentage determinations reveal a larger per cent of fat than one would, a priori, expect.

On the whole, they confirm the microscopic findings. It is unfortunate that no fat percentage determinations were made on no. 140, the only salmon that presented in the masseter muscle definite and unmistakable evidence of muscle degenerations. This series should be compared with the Ilwaco series in table 1, page 92.

TABLE II.—ANALYTICAL DETERMINATIONS OF FATS IN THE TISSUES OF CERTAIN SALMON FROM CLACKAMAS RIVER, CAZADERO, OREG., TAKEN SEPTEMBER, 1911.

No. and sex of fish.	Muscle fats in percentage.		Remarks.
	Pink muscle.	Dark muscle.	
134 ♀	2.870	6.719	Spawned.
137 ♂	6.139	9.662	Spawning.
138 ♂	3.332	7.962	Spawning, one-half to three-fifths spawned.

PROTOCOLS.

Spawning female salmon (no. 132), length 960 mm., weight 6,840 grams (after artificial spawning).

External appearance first class, body slender in form. An appreciable oedema of the inner surface of the body cavity walls. Visceral mass exceptionally small.

Microscopic examination of the trunk pink muscle.—In a small portion of section N47 there is a very small quantity of intermuscular fat, but in general neither the connective tissue septa nor the thicker strands separating the fibers have more than tiny liposomes. There is a sharp contrast between this fish and no. 127 from Celilo in this regard. These fat droplets in the connective tissue measure a maximum of 5 to 6 μ. The average size is very much smaller, between 1 and 2 μ. The trunk pink fibers of this salmon are somewhat more plump and round in outline than in no. 131. All the larger fibers, however, are compressed and irregular in outline, suggesting the same type of change noticed in other relatively fat free tissues.

There is intramuscular fat in the smaller fibers and in the medium in the form of liposomes. These liposomes are distributed rather uniformly through the substance of the smaller fibers. In the medium-sized fibers there are not so many liposomes and they are much fewer in the center of the fibers. Around the circumference of the fibers and under the sarcolemma there is more fat, especially under the sarcolemma. These small and intermediate fibers have sometimes almost complete rings of fat droplets under the sarcolemma. The largest fibers have a greatly reduced quantity of fat. The fat is in very much smaller liposomes, often scarcely visible. Around the surface and under the sarcolemma there are groups of fat droplets, but not so plentiful as in the medium fibers.

This material on the whole is characterized by the small amount of intermuscular fat and the relatively great amount of intramuscular fat. In some portions of the sections the intramuscular fat is as great as in fish no. 118 from Ilwaco, far greater than in no. 126 from Warrendale or no. 127 from Seuferts Cement Wheel. The uniformity of distribution of the fat is not so great as in the Ilwaco specimens.

Microscopic examination of the caudal pink muscle.—There is no intermuscular fat in this section (N59). In the myocommata shown there is a trifle of fat just at the surface.

The muscle fibers have some intramuscular fat, but the relative amount in the different types of fibers is difficult to determine, on account of the excess of precipitate present. Some of this so-called precipitate is in characteristic round granules like unstained fat, but some of it is in the characteristic scarlet red color, interpreted as a less successful staining manipulation.

Microscopic examination of the trunk dark muscle (slides N53–57).—These sections all show a relatively small amount of fat in the myocommata.

The muscle tissue as a whole shows much fat in the critical region at the surface of and between the fibers. Under the oil immersion it is apparent that a large amount of this fat is between the fibers in droplets from 2.5 to 3 μ in diameter. The muscle fibers are so compact in arrangement that it is difficult to identify the exact limits of the fiber. Certain unquestioned regions show this fat between the

fibers. In other regions one can as definitely say that there is fat beneath the sarcolemma but outside the substance of the fiber. At the border of the section a number of fibers have been slightly split apart and some turned in a horizontal position. These fibers confirm the above.

As a rule, through the section there is only a small amount of intramuscular fat in chains of liposomes through the bodies of the fibers. In the horizontal fibers the size of the liposomes is shown to be from 0.4 to 1.5 μ in diameter. The majority of the liposomes are of the larger sizes.

The striking characteristic of this tissue is the great differentiation as between the amount of fat in the body of the fiber and at the surface. There is apparently more fat in the dark muscle of this fish than in fish no. 128 and no. 129 from Celilo, undoubtedly more than in fish no. 131 of Cazadero, but the bodies of the fibers contain relatively less.

Microscopic examination of the caudal dark muscle.—The amount of intermuscular fat is greatly reduced over that of the trunk muscle, the droplets are smaller, and they are not so numerous as in that region. They are, however, sufficient to give a mosaic-like appearance to the section.

The intramuscular fat is extremely small except such as lies just under the sarcolemma. Certain of the sections show the ends of the fibrillæ clear and sharp. The pattern is the same as shown in figure 7, plate v, except that there are no fat spaces present. This certainly indicates that the muscle has not degenerated, yet nearly all the fat characteristic of the normal muscle is absent and there are only a very few liposomes in the fibers near the surface.

Spawning male salmon (no. 138), length 840 mm., weight 7,730 grams, from the spawning pens of the United States Fisheries Station, Cazadero, Oreg., on the Clackamas River, September 4, 1911.

This male salmon was one-half to three-fifths spawned. Color brassy, with black spots. Soft dorsal decayed and one fungus spot on dorsal fin. A fish in good condition but in late stage of exhaustion. It would probably have died in the course of 24 to 36 hours.

Microscopic examination of the trunk pink muscle.—The pink fibers of salmon no. 138 are not plump and round as in fish no. 118. On the other hand, their outlines form irregular polygons. Even the smaller fibers have this shape. The larger fibers bear histological evidence of great decrease in size (samples one day in formalin). A set of cross-sectional measurements show the following: 40 by 80, 60 by 70, 80 by 140, and 100 by 220 μ, outlines all very irregular. The cross sections show both the striations and the fibrillæ very nicely.

The sections are free of intermuscular fat except for a few of the finest droplets and liposomes. In a large section there is one such group of fat droplets in a large mass of connective tissue. There are a few thin strands of connective tissue between the fibers, and these carry occasional fat droplets not over 3 to 4 μ in diameter. The larger size is rare, though fat drops in the same locality in no. 118 from Ilwaco measure 100 μ and more. There are a good many tiny liposomes in this connective tissue, though the mass is extremely small.

In contrast to the dearth of fat between the fibers there is an unexpectedly large quantity of intramuscular fat within. As usual this fat is in liposomes. The size and number are both greater in the smallest fibers, yet the largest fibers have a pretty even sprinkling of liposomes of extremely minute size. I can not find a single fiber but that has some fat within its protoplasm. In the smaller fibers there is a great amount of fat around the surface in the region just under the sarcolemma. Taking the small fibers as a whole, it seems that the liposomic fat is present in a greater number of droplets and in slightly larger droplets than in the poorer specimens from Warrendale. There is decidedly more intramuscular fat than in fish no. 126 from Warrendale.

The teased fibers give a beautiful confirmation of the notes made on the cross sections. They show one variation from the typical arrangement of liposomes, namely, that the deep-lying liposomes are not in such regular rows as in the normal. The liposomes have a very irregular appearance, as if the smaller liposomes had disappeared. Those liposomes that are left are unusually large and uniform in size for pink fibers. The rows that are most definite and uninterrupted are located near the surface of the fiber. The larger liposomes are from 2 to 2.5 μ in diameter.

Microscopic examination of the caudal pink muscle.—The caudal pink muscle sections are free of all but traces of fat. The intermuscular fat is limited to a few droplets in the myocommata. Only a few irregular chains of very small liposomes are present in the fibers. These are more distinct in the smaller fibers. The striations are distinct and clear, but the fat is faint and scarcely distinguishable.

Microscopic examination of the trunk dark muscle.—The dark trunk fibers are very compactly arranged, so that it is difficult to distinguish between the intermuscular fat and subsarcolemmal fat. But it is apparent that there is a small amount of intermuscular fat. This is confirmed by the connective tissue of teased muscle. The drops are of liposomic size, rarely more than 2 to 2.5 μ in diameter.

The intramuscular fat is present in the usual localities. Under the sarcolemma the droplets are medium in size, 1.7, 2.8, 3, and 5.4 μ in typical droplets. The sarcoplasm of the fibers is rather evenly studded with unusually small liposomes for this type of muscle. The liposomes are even smaller than in the trunk pink muscle, are condensed about the surface of the fibers and are larger there, 0.4 to 1.2 μ. In the body of the fiber there are fewer liposomes and the size is from 0.2 to 0.8 μ.

The teased dark fibers confirm the above notes as to the fat disposal. The fibers have exceptionally clear striations which average 2.6 μ in length. The fat is irregular and the liposomes of the chains can not be followed in relation to the striations. The fat under the sarcolemma is in patches in these fibers. The whole appearance of the dark fibers closely approaches that of the pink fibers of this salmon.

Microscopic examination of the caudal dark muscle.—In the caudal dark muscle there is still enough fat between the fibers and under the sarcolemma to give a distinct mosaic-like marking of the outlines of the fibers. In some portions of the sections this surface fat is gone.

The intramuscular fat is still much less than in the trunk dark muscle. There are fibers that have the merest trace of liposomes in the muscle substance. Even in the fattest fibers only minute liposomes are present, the smallest amounts observed in this type of muscle, except in no. 140. There is the sharpest contrast between this tissue and the Ilwaco type.

Spawning male salmon (no. 140), length 980 mm., weight 8,070 grams.

This fish was selected as a type of spawning fish in good physiological condition but at the last stages before death. It was taken at the United States Fisheries station at Cazadero, Oreg., on the Clackamas River, September 6, 1911.

Microscopic examination of the trunk pink muscle.—There are traces only of intermuscular fat, which is in fine liposome-like droplets in the larger connective tissue strands and in the myocommata.

The small fibers still contain a sprinkling of liposomes, enough to give them a decidedly pink appearance under the low power. The 1/12 oil immersion shows that these liposomes are gathered chiefly around the superficial border of the fiber and that they are in groups in the neighborhood of intermuscular fat. The largest liposome observed in a small fiber measured 1.4 μ in diameter, but such are few in number. The average size of liposomes for these small fibers is only a fraction of a micron.

In a large fiber under the oil immersion there are a few areas of fat in irregular-sized droplets under the sarcolemma, traces in contrast with Ilwaco salmon. Through the body of the fiber there are chains of finest liposomes 0.1 to 0.2 μ in diameter, but the chains are short and irregular. In two other typical fibers in the field, one medium and the other rather small, the liposomes are present in about the same number of chains as in the large fiber, but are slightly larger in size. In all these fibers the particular characteristic feature is the irregularity in the chains of liposomes. The individual chains have not the usual arrangement of larger liposomes in the middle of the chain and the size tapering down to small ones at the end of the chain. They are irregular in size throughout the chain.

Microscopic examination of the caudal pink muscle.—There is practically no fat in this caudal muscle. The trace that is present (1/12 oil) is only enough to give a faint stain to the superficial border of occasional fibers. There are scattered and irregular groups of a few small liposomes just at the surface of the fibers. Several fibers that have been turned horizontally show no chains of liposomes.

Teased fibers show no liposomes in the body, but occasional traces of liposomes just at the surface of the fibers. These traces are definitely between the sarcolemma and the sarcoplasm, an arrangement most often noted in pink fibers poor in fat.

Microscopic examination of the trunk dark muscle.—The outlines of the trunk dark fibers are marked by rather heavy rings of fat droplets. These markings are least prominent in the neighborhood of vascular areas. It is not always possible to distinguish between intermuscular and subsarcolemmal fat. Both are present. Most of the fat observed is judged to be under the sarcolemma. The fat drops between the fibers range in size from 3 μ to 8 μ.

The fat under the sarcolemma seems to be in rather small but numerous droplets. This fat runs from 2 to 4 μ in diameter. Out in the body of the muscle fibers there is a variable arrangement of

liposomes. In some fibers they are uniformly distributed through the substance of the fibers; in others there is apparently no fat in the middle of the fiber. In general, liposomes are present in the superficial areas even in those fibers which have the least fat. The whole appearance suggests a nutritive balance in which it is just a question whether all the fat will be used or whether there will be an excess sufficient for deposit.

The teased muscle shows great variation in the quantity of fat in the individual fibers (1/12 oil). At the surface of the fiber is a good deal of fat in small droplets, the subsarcolemmal fat. The liposomes in the chains are small like those in normal pink muscle. The number of chains is also low. In the fibers carrying the least fat these chains are all but absent.

Microscopic examination of the cheek muscle.—The muscle fibers of the cheek muscle are very compactly arranged. In comparison with the Ilwaco type the fibers are less rotund in outline. The histological structure is indistinct. In many fibers the fibrillæ can not be seen because of a disintegration which marks the first stage of degeneration. Certain fibers scattered irregularly through the section show a definite protoplasmic degeneration with vacuoles and pigmentation. The pigment granules are small in size, 0.1 to 0.2 μ in diameter. They are unevenly distributed through the fibers and apparently somewhat greater near the surface. There is great variation in the quantity of pigment in different individual fibers.

The fat in the cheek muscle is limited to a very few small groups of intermuscular fat. No traces of fat could be distinguished within the fibers themselves.

SIGNIFICANCE OF THE OBSERVED CHANGES OF THE AMOUNT OF FAT.

It is obvious that the salmon fat furnishes the food during the migration fast. The revelations of the microscope are convincing on this point, even if there were no collateral supporting evidence.

My unpublished chemical analyses of the tissues have revealed a dearth of carbohydrates in the salmon tissues at all stages of the migration. This fact is of vital significance in connection with the fat problem. The lack of carbohydrates and the abundance of fats support Miescher's assumption that fats furnish the source of the muscular energy expended by the salmon during the migration. In connection with a series of salmon-feeding experiments [a] it was shown that the salmon liver exercises a distinct lipogenic [b] function during the feeding and growing stage. Noël Paton has found that the amount of fat in the liver of the frog is increased after fat feeding.[c] It seems to me that in animals like the salmon the lipogenic function of the liver becomes a primary function, taking a rôle quite comparable to that of the glycogenic function of the organ for many mammals. Fishes of this class are carnivorous. Their food is of a highly oily character, as is also that of certain birds, and is continuously so. The food is rich in proteins and fats and in inorganic constituents, but it is poor in carbohydrates. In the adaptations to such a diet, if for no other reason, the salmon has reached the point in its phylogenetic development where fats furnish a direct and primary source of foods for the energy

[a] Now in manuscript.

[b] It was Loevenhart (American Journal of Physiology, vol. 6, p. 331, 1901) who first advocated the idea that we might have a "lipogenesis" in the body comparable in character to the "glycogenesis" of Claude Bernard. He suggests that wherever there is fat storage there will be lipase, and proves it by investigations on a number of tissues that contain fat, for example, the liver, mammary gland, pancreas, brain, spleen, heart muscle, blood, adipose tissue, etc. He says: "In the case of fats the areolar tissue is the great primary store, secondary deposits being found in all the tissues. In some animals even this difference in the storing of fats and carbohydrates is not to be noted. In many fish, notably the cod, the liver, at certain seasons of the year, becomes the great depository for fat. The liver we have found to possess powerful lipolytic activity, and hence, under proper conditions, it should be capable of storing fat. Moreover, this is in accordance with the experiments of Noel Paton, who showed that the fat contained in the liver of frogs is increased after a fatty meal. It is believed that both phases of lipogenesis are induced by lipase, a fat-splitting and fat-forming enzyme." From my observations I am convinced that lipogenesis is a definite and specific function of the liver in certain carnivorous animals whose normal food consists of a high percentage of fat, as is the case in the king salmon.

[c] Paton, D. Noël: On the relationship of the liver to fats. Journal of Physiology, vol. XIX, 1896, p. 167.

liberating tissues. The tissues, in short, can utilize the energy of fats by direct oxidations. It remains to examine the facts submitted and to discover, if possible, the mechanism whereby this great store of salmon fat is rendered so labile and so wonderfully efficient in the execution of the activities of this last lap of the salmon life cycle.

Of all the facts presented it seems to me the most significant are:

I. The appearance of intramuscular fat in the pink muscle at the beginning of the spawning migration, and

II. The maintaining of a relatively uniform distribution of this fat in the fibers until the death of the animal.

Just as soon as the salmon ceases to feed, and the products of digestion no longer reach the active musculature, then, and not until then, there is thrown into the pink muscle fibers a supply of fat adequate to the energy needs of this most critical period in the salmon life cycle. The excess of fat is deposited in an extremely finely divided state and is brought into intimate contact with the fibrillæ, one can almost say with the sarcous elements. Certainly in many rows of fat droplets between the fibrillæ the individual liposomes are in close approximation with corresponding sarcous elements in the fibrils. One can not escape the inference that this microscopic emulsion of the fat, its general distribution throughout the muscle fiber, and the intimate relation with the elemental fibrillar structure, all point to an immediate utilization of the fats in the production of muscular energy.

This hypothesis is further supported by the observations on the cheek muscle and on the fin muscles, all muscles in much more uniform, though less intense, activity than the lateral muscle. These muscles carry a light but strikingly persistent load of minutely divided intramuscular fat during the entire migration. They never have a great excess of storage fat, either intermuscular, as in the pink muscle, or intramuscular, as in the dark muscle.

The great fat storehouses are the intermuscular fat of the great lateral pink muscle, the inter- and intra-muscular fat of the dark muscle, and the fat of the adipose connective tissues. With the cessation of feeding no further fats, proteins, etc., are brought in as foods. With the external food supply now shut off the physiological mechanism of the salmon must turn to the food materials on hand, to the internal food supplies of the salmon's own body. The internal supply is limited to body tissues as such, and to the fats. It is the fats that are immediately drawn upon. From the fat deposits the fat is gradually but regularly transported to the active muscles, where it is maintained in a uniform and favorable distribution, and in amount adequate to supply the energy expended by the salmon in the migration fight against the currents and rapids of the rivers on its way to the spawning beds.

TRANSPORTATION OF FATS IN THE FASTING SALMON.

HISTORICAL.

The histological observations on the king salmon have given every confirmation of Miescher's original assumption, based on his study of the Rhine salmon, that the fat of the salmon can be transported from one part of the body to another; i. e., from tissue to tissue. He laid special emphasis on the utilization of the muscle fats in the building up of the fats of the ovaries, but he also suggested that fat was the source of

the nourishment of the animal.[a] It is irrelevant for the present purpose that Miescher considered the source of the fat to be a fatty degeneration of the muscle tissue. The fact remains that he demonstrated the presence of intramuscular fat microscopically and for this he should receive full credit. He must also be given full credit for the conception that the salmon fat can be transported for purposes of tissue growth, ovaries, and for use in energy production, muscles. I can not find that he has offered any explanation of the detail of the processes involved in the fat transference or that he has discussed the matter, but this work is so important that the three statements he makes are quoted in full in their setting, and in his own words. On page 186 he says:

Dass wirklich der Seitenrumpfmuskel die wesentlichste Stoffquelle ist, sowohl für die Ernährung des Thieres, als für die Gesclechtsreifung, wird evident bestätigt durch das Mikroskop. Schon die Winter- und Frühjahrssalmen zeigen nämlich zwischen den feinen quergestreiften Elementarfäden (Fibrillen) der ungleich dicken Muskelfasern, besonders in den dünneren, bald mehr bald weniger ausgesprochene Fetttröpfchenreihen, wie man sie als Anzeichen sogenannter Entartung des Muskelgewebes kennt. Die Menge dieser Fetttröpfchen nimmt gerade im Hochsommer, wenn der Eierstock rascher zu wachsen beginnt, beträchtlich zu und kann bis zur Undurchsichtigkeit mancher Fasern führen. Am stärksten degenerirt eine gesonderte dünne Muskelplatte, die an der Seite des Körpers direct unter der Haut liegt (Hautmuskel). Dagegen bleiben sozusagen völlig intact und fettfrei alle übrigen Muskeln, Brustflosse, Bauchflosse, Rücken- und Afterflosse, Kiefer- und Zungenbeinmuskeln, der obere und untere Längsmuskel und die Schwanzmuskeln im engern Sinne. Nur die Bauchflosse zeigte an einigen Stellen schwache Anzeichen von Degeneration.

The one comparison as to the intramuscular loading of the fibers with liposomes as the migration time continues is given on page 213.

So findet man denn bei den Frühsalmen jene schwache, hauptsächlich die dünneren Muskelfasern in mässigem Grade betreffende Durchsetzung mit Reihen feiner Fettkörnchen, bis dann im Frühsommer das Wachsthum des Eierstocks in seiner geometrischen Progression zu einem absoluten monatlichen Stoffverbrauch führt, dessen Anforderungen neben der eigentlichen Selbstzehrung sich gebieterisch in den Vordergrund drängen und wirksamere Hilfsmittel verlangen.

When the Rhine salmon spawn they begin the return migration to the sea with the associated recuperative processes. Concerning this stage, Miescher makes the following final statement on page 215 of his monograph (page 171 of the reprint):

Wie ganz anders das Bild, wenn wir Gelegenheit haben, Thiere zu sehen, die auch nur um 10 Tage, besser um ein paar Wochen das Laichen hinter sich haben (leere Weibchen, zu Ende December oder im Januar gefangen, aber auch eines aus Herrn Glaser's Fishkästen, gewiss nicht mehr als 10 Tage von seinen Eiern befreit). Die Haut ist wieder bläulich glänzend und klar, die Geschwüre übernarbt oder in Heilung, das Fleisch durchscheinend, von Fettkörnchen völlig oder fast völlig befreit; auch die Herzfasern in Reinigung begriffen; im Darm keine Spur von Nahrung. Dagegen enthält der Eierstock bald mehr bald weniger Eier, die, in einen serösen oder auch etwas eitrigen Erguss der Follikelhaut eingebettet, sichtlich zusammenschrumpfen und aufgesogen werden.

Mahalanobis[b] in Noël Paton's report on the life history of the salmon also calls attention to the storage of fat in the muscular tissues, and to the use of this fat in the reproductive organs and in the production of energy. This author calls attention to two important things, viz, (1) he observes that the fat is present in largest amount in the muscles of fish entering the estuaries, and in least amount at spawning stage, and (2) he refutes Miescher's degeneration theory as a means of accounting for the presence of

[a] Miescher: Statistische und biologische Beiträge zur Kenntniss vom Leben des Rheinlachses im Süsswasser, s. 186, 1886; also reprinted in Die histochemischen und physiologischen Arbeiten, s. 145, 1897.

[b] Paton, D. Noël: The life history of the salmon. Article 9, by Mahalanobis, S. C., Microscopical observations on muscle fat in the salmon. Fishery Board for Scotland Report, 1898, p. 106.

the fat in the fibers. Mahalanobis considers the fat deposition as a "fatty infiltration due to increased accumulation of fat from diminished utilization in the tissues."[a] In the same paragraph he says:[b] "Figure 3 is from a fish fresh from the sea—one that had been actively feeding, and consequently its blood and lymph were rich in fat, whence, in all probability, the muscle cells absorbed fat and stored it between the fibrils." And "as already pointed out, the fat granules in fish leaving the sea are more crowded immediately under the sarcolemma (fig. 3, pl. III)." These quotations include all the remarks tending to show the author's views as to fat transferences in the salmon tissue. It is evident that he has given attention only to the mechanism whereby the fat is originally laid down in the muscle, and his conception is that the process is one of "infiltration" or absorption from the blood and lymph.

Mahalanobis is hopelessly confused in his studies by the fact that he has failed to recognize the dissimilarity of two strikingly different tissues. This is shown in the following quotation and by the figures referred to therein: "In the fish leaving the sea this accumulation of fat in the fibers sometimes reaches an enormous amount, and a thick layer occurs under the sarcolemma. This will be evident from a comparison between figure 2 and figure 3, the former being from a late fish, no. 69, and the latter from no. 79."[c] The figures given by him exhibit differences both in structure and in fat disposal which bear no relation either to the seasonal type or to the stage of fasting which Mahalanobis observed, as the studies presented here on the king salmon conclusively show. The basis of this matter is more fully discussed in this paper in connection with the description of the tissues in question. Mahalanobis compared the dark muscle fiber of his fish no. 79 with a pink fiber of no. 69. The former muscle is normally loaded with enormous fat droplets in the fiber, whereas the latter muscle never has fat in larger size than liposomes. His figure 3 from fish no. 69 is from the intermediate zone of pink fibers. Had he chosen a deeper group of fibers the dearth of fat would have been undoubtedly greater. The figures are illustrative of the two normal extremes, are from wholly different types of muscle, and are, therefore, not directly comparable. This fact he apparently fails to recognize, though his first quotation from Miescher should have helped in the identification of the muscle types he used.

On the comparative side of the question involved here the recent brilliant work of Bell should be presented.[d] Bell has studied the liposomes in the muscle fibers of the ox, dog, cat, rabbit, rat, and the frog. He has also examined the moth (*Phlegethontius*), and the fly (*Musca*). He presents a good historical statement of the work that has been done along this line, from the discovery of interstitial granules of muscle fibers by Henle in 1841, down to the publication of his own work in 1911. Bell calls the muscle interstitial granules that are of a fatty nature "liposomes," a term introduced by Albrecht.

Bell, in discussing the granules in the muscle fibers, says (p. 310), "All agree with Kölliker that the granules lie in the sarcoplasm between the fibrils," and later:

> In the skeletal muscles of vertebrates, when the cross markings are wide and distinct, it can usually be seen that the granules occupy the J-band. But when the striations are narrow the granules seem to extend the entire distance between adjacent Krause's membranes. Large granules nearly always lie

[a] Paton, op. cit., p. 110.
[b] Paton, op. cit., pl. I.
[c] Mahalanobis, op. cit., p. 108. His fish no. 79 was "a fish fresh from the sea."
[d] Bell, E. T.: The interstitial granules of striated muscle and their relation to nutrition. Internationalen Monatschrift für Anatomie und Physiologie, bd. XXVIII, s. 297, 1911.

partly at least in the Q-band. In many fibers the granules are arranged in distinct transverse rows, being apparently limited by Krause's membrane.

The nature of the muscle granules and particularly of the liposomes has been extensively studied by Bell. But as the question of the kind of fat has not been especially investigated in the salmon muscle the review of the discussion will be omitted at the present time. The contributions by Bell that are of special and far-reaching value in relation to the questions involved in this paper are two: First, the influence of starvation on the fat content of the muscle tissue; second, the influence of fat feeding on the number and size of the liposomes of the muscles. Under the subject of lack of food, Bell says:

In every animal there is a gradual disappearance of the liposomes during inanition. As the animal loses weight the liposomes gradually become smaller and less refractive; and they also stain with decreasing intensity. The muscle fibers of a well-nourished cat are usually full of coarse deeply stained droplets such as is shown in figure 1, from the frog.

Also:

In the rat there is a very rapid decrease in the number, size, refractive power, and staining intensity of the liposomes. A well-fed rat may contain a large number of strongly refractive liposomes in its muscle fibers, many of which may be stained with osmic acid. After a reduction in the body weight of 15 to 20 per cent only a few faintly refractive liposomes are usually left. After a reduction of 25 to 30 per cent, it is often found that no liposomes at all can be demonstrated. Every liposome has disappeared.

The remarkable sensitiveness of the liposomes in rat muscle to the food supply undoubtedly accounts to a considerable extent for the large variations one finds in animals gathered at random. It will be shown, however, later that the quality of the food is a factor of almost as much importance as the quantity. A rat whose body weight has been reduced 25 to 30 per cent may develop a large number of deeply staining liposomes in its muscle fibers (if fed on a diet largely composed of fat meat) though the body weight remains far below normal.

There is, as has been shown, a marked difference in the number and character of the liposomes of a well-nourished normal animal and those of an emaciated animal; but the liposomes of an animal in ordinary condition may not differ essentially from those of a very fat individual. No particular differences were noted between the muscle liposomes of steers, in which the subcutaneous fatty layer was 6 cm. thick, and those of steers in which this layer was only 5 mm. thick. It was also noted in rats and dogs that excessive amounts of connective tissue fat are not coordinated with excessive development of the liposomes.

It is however clear from the above-described disappearance of the liposomes during inanition that they consist of some form of reserve food substance. This conclusion is in accord with the view that they consist of true fats or fat-like substances. The gradual decrease in the refractive power and staining-intensity of the liposomes indicates that the fats are mixed in the liposome with some substance other than fat.

Under the topic of "The effect of special feeding on the liposomes" Bell says:

Some interesting results were obtained by feeding summer frogs on special rations. It has been pointed out above that in the summer months (June, July, and August) the muscle fibers contain very little fat. In a great many animals, in July and early August at least, no liposomes at all can be demonstrated in the light fibers, and those in the dark fibers are very small and faint and can only be stained with Herxheimer's solution. Some young frogs were found in which no liposomes at all could be shown. It was found that when frogs in this condition were fed heavily on olive oil or fat meat for a few days the fibers become loaded with liposomes, giving a picture similar to that found in winter animals.

Bell also tested the fat content of the muscles of frogs caught in the field showing that the muscles of leopard frogs before feeding had a "few faint liposomes in the dark fibers, none in the light fibers," but after feeding with fat meat and olive oil all the

fibers are loaded with liposomes. Those in the dark fibers are large and stain with considerable intensity.

After a series of experiments on leopard and bullfrogs he says: "It is apparent that if the frog be fed an excessive amount of fat the fat will be rapidly stored up in the muscle fibers." Similar experiments were performed on rats, which were kept on a low ration until the liposomes were removed, then were fed on a ration of fat meat. Under this diet the rats gained in weight and the muscle "fibers filled with liposomes."

By these brilliant experiments Bell has conclusively proved that the liposomes in the muscles of vertebrates, frogs, and mammals bear a distinct relation to the state of nutrition. The liposomes decrease in number and quantity under a low state of nutrition and they can be increased in size and number when the animals receive a favorable food. These experiments are of peculiar importance to the problem of the present paper, since they prove that the presence of the liposomes in muscle tissue is to a certain extent an index of the nutritive condition of the animal in question. It does not of necessity follow that the liposome content of the tissues of an animal in the fasting condition, as in the case of the salmon, will have the same significance. From my previous work, however, and from numerous field observations, I had arrived at the working hypothesis that this was the case in the salmon, a position strengthened by the conclusions of Prof. Bell, which he kindly communicated to me before his results were published.

The salmon muscle fat is a filtration fat, not a fatty degeneration. It may be stated here that the studies on the king salmon tend to disprove Miescher's theory that the intracellular fat of the salmon muscle, of whatever type the muscle, is a fatty degeneration, a "Fettentartung";[a] and support the observations of Mahalanobis that the process is an "infiltration." In short, the observations made on the king salmon have tended to confirm the view expressed above that the intracellular fat of the king salmon is an expression of the nutritive state of the muscle. It is a loading of fat by a process of infiltration, as will be explained more fully, and is not a degeneration of the muscle substance.

It seems surprising that the test of degeneration versus infiltration should not have been applied to the material under discussion by Miescher and by Mahalanobis. Any examination of histological sections ought to have shown that there was no appreciable and adequate conversion of cell proteins into fat, and this observation would have settled the matter. Transverse sections of dark muscle taken at a late stage in the migration journey show great regularity of structure, and this structure is of the normal type. If the muscle protoplasm had undergone fatty degeneration commensurate with the amount of fat found in this tissue at the time of its greatest load of fat, it is evident that there would be little normal protein left, and that this little would show pathological structure. This pathological condition I have never seen except in the extreme emaciated condition found at the time of death. Even then it was found to be extensive in only one tissue, the great masseter muscle, and this muscle contained no fat.

If argument were still lacking to establish an alibi for the "fatty degeneration" process of laying down fat in the salmon muscle, it ought to be supplied by the fact that the young and actively growing dark muscle fibers of the superficialis lateralis muscle bear a heavy load of intracellular fat. These fibers take on a rich deposit of intracel-

[a] Miescher, op. cit., p. 207.

lular fat, both when the muscles are small and immature and when they are larger, also at a time when they are undergoing longitudinal cleavage.

I have several stages of relatively young fish, from 7 to 16 cm. long, all of which show a rich deposit of fat in the fibers of the superficialis lateralis. In the older fish,[a] as measured by the standard of size, there is a heavy loading of fat in the dark muscle with corresponding separation of the bundles of fibrillæ. There is, however, no disappearance of fibrillæ or other unusual characteristic than the distortion that comes from the presence of such enormous quantities of fat. These remarks all apply, of course, to the dark type of muscle. In the pink muscle there is little or no intracellular fat in the muscle fibers during any phase of the growth cycle. This fat appears only after the feeding ceases.

It seems obvious that intracellular fat of the muscle can not, in the salmon, be attributed to "fatty degeneration" in any true sense as signifying a protein degeneration, or, for that matter, a protein cleavage. The amount of protein present does not justify such a conclusion.

MECHANISM OF FAT TRANSFERENCE IN THE SALMON BODY.

Fat metabolism in most animals, in the Mammalia for example, always involves the two intertwined problems of most nutrition experiments, namely, fat intake and fat mobilization. The former carries with it the detail of fat digestion, absorption, and the laying down of the fats in the fat-storing tissues. The latter involves the taking up of the fats from the storage tissues and their utilization in the production of new tissue or in the liberation of energy, as the case may be, and such transferences in the body as either method of utilization may entail. In most animals complete separation of these two groups of processes involves more or less abnormal conditions for the animal. But for the salmon the long fasting period is a perfectly normal process. We, therefore, can make observations under the grim assurance that the salmon will not, in fact, can not, eat. There is no added fat being absorbed during this fasting period, hence we have present at this period only the uncomplicated mobilization and utilization processes.

The discovery of a fat-splitting enzyme, or lipase, was made by Claude Bernard in 1846, and it was early suggested that the fats of absorption might be resynthesized in the intestinal epithelium. It was not, however, until 1900, when Kastle and Loevenhart [b] announced their brilliant discovery of the reversible action of lipase, that we have had an adequate and thorough comprehension of the mechanism whereby the animal body can transport fats from tissue to tissue. In light of the reversibility of lipase action it is easy to see how a fatty infiltration can make its appearance in a tissue so stable in structure as striated muscle, without assuming a disintegration of its protoplasm, as in the fatty degeneration theories.

In the problem before us I have already discussed at length the comparison with regard to the actual loading of fat in the two chief types of tissue, the lateral dark muscle and the lateral pink muscle. These are very different types of muscle, and, while the problem and the controlling factors are essentially similar, it will greatly simplify the

[a] I have during a number of years found salmon of various sizes entering the fresh waters, all of them exhibiting a great variation in maturity of sex organs. These two facts, i. e., size and maturity, are independent of each other.

[b] Kastle and Loevenhart: American Chemical Journal, vol. XXIV, p. 491, 1900.

presentation of the matter to consider the phenomena of fat mobilization in these tissues separately. I will take the simpler case first, i. e., the pink muscle tissue of the lateralis profundis muscle.

TRANSFERENCE OF FAT IN THE PINK MUSCLE.

Let the reader recall the characteristics of this muscle up to the time of the entrance of the salmon into fresh waters, viz: (1) The intermuscular fat of the pink muscle is slight in the early growing stage as represented by fish 10 to 15 cm. long which are migrating toward the sea. The intermuscular fat increases in quantity after they reach the sea, and reaches its maximum when the fish cease feeding—that is, when they begin the adaptation process preparatory to entering the estuaries. (2) The intramuscular fat is absent in the young salmon of fresh water, also in the voraciously feeding sea forms, up to the time the salmon cease to feed, except for traces of fat in the smaller fibers just at the end of this period. (3) The intramuscular fat after the beginning of the spawning migration makes its appearance throughout the substance of the pink muscle fibers of all sizes. It appears in short chains of very small liposomes that are quite evenly interspersed among the groups of fibrillæ of the muscle cells. This intracellular fat is present within the pink muscle fibers throughout the migration and at the time of death after the spawning.

The special contrast is in the distribution of muscular fat just before and just after the salmon cease to feed. The important change is in the relatively sudden appearance of the liposomes among the fibrillæ of the pink fibers. For this phenomenon the following explanation is offered:

Active feeding salmon are also rapidly growing salmon. While growth is taking place all excess of fat is laid down in the connective tissue or in the dark muscle and never in the muscle fibers of the pink muscle. The concentration of the fatty products never exceeds the oxidations in the fibers of the pink muscle, hence no intracellular deposit occurs.[a] The transition from a feeding to a fasting state is associated with numerous tissue changes in other parts of the body, changes which are accompanied by equally important functional readjustments. Among the functional changes the one that most concerns the present argument is the increased production of the fat-splitting enzyme, lipase. Assume for the moment that the products of the last digestion have been absorbed into the blood and have already been utilized by the tissues. Assume also that this state has reached a point where the expenditure of energy must be done by drawing on the body reserves. Then what can happen?

The salmon tissue glycogen is a negligible quantity. There is no adequate supply in either muscle or liver, as in the mammalia. Glycogenesis can not, therefore, come to the support of the body in this crisis.

There is an abundant store of fat in the intermuscular depot, great quantities of it, and a lipogenesis[b] comes to the support of the salmon in a way quite comparable to the glycogenesis of the mammal as conceived by Claude Bernard. Under these conditions the activity of the muscular tissue is directly dependent on the fat as a source of energy. The muscle oxidizes fatty bodies in the salmon, just as it oxidizes carbohydrate bodies in certain other well-known animals.

[a] An exception may be found in the border zone of fibers between the pink and the dark muscle.

[b] Loevenhart: On the relation of lipase to fat metabolism, lipogenesis. American Journal of Physiology, vol. VI, 1901, p. 331.

It is to be assumed that the muscle fibers absorb the fatty bodies from the lymph and blood, presumably as soluble fatty acids and glycerin. The fatty bodies of the blood and lymph are derived from the stored fat by a process of lipolysis. To that extent to which the store of intermuscular fat of the pink muscle is eroded by this process of lipolysis will the percentage concentration of the cleavage products of the pink muscle lymph be high. From the lymph the fat cleavage products dialyse directly into the pink fibers and become available for oxidation. In the early stages of the fast there are numerous tissues besides the muscle containing an excess of stored fat; the digestive tube, the pancreas, the liver, the skin, etc., as well as the connective tissue and the muscles. Loevenhart [a] has stated that the limits of lipogenesis are "nearly proportional to the amount of enzyme acting" and "nearly independent of an excess of ethyl butyrate" in the experiments of his series. Hence, with increasing production of lipase in the blood there is an ever-increasing percentage of fatty bodies thrown into solution in the blood and lymph.

Hand in hand with the increase of fatty bodies in the blood and lymph will go an increase in fatty products in the substance of the muscle fibers. Muscular oxidations are not rapid enough to keep down the increasing quantity of fatty bodies, hence they will diffuse through the muscle protoplasm in considerable excess. The lipase of the blood and lymph will also diffuse into and be present in the muscle fiber, a fact demonstrated for other muscle tissues. Under the law of reversible lipase action this excess of fatty cleavage bodies is bound to be reconverted into and deposited as neutral fat. Thus arise the chains of microscopic liposomes of the pink muscle at the beginning of the salmon fast.

The number and size of the chains and of the individual liposomes in the pink muscle, therefore, is a result of the interaction of a number of factors, chief of which are the following:

a. The relative abundance of the stored fat in the tissues of whatever source, i. e., the gross amount of fat available for lipolytic erosion in all parts of the body.

b. The relative abundance of lipase throughout the body, chiefly of the blood and lymph, but having origin in lipase producing tissues.

c. The structural and physical factors controlling the rapidity of the absorption from the blood and lymph into the muscle fibers; i. e., the sarcolemma, sarcoplasm, etc.

d. Especially the rapidity with which the fatty bodies are utilized, oxidized, by the muscle sarcoplasm.

The constants of lipase action have not yet been determined sufficiently to enable one to apply to this specific instance definite governing laws. It is hoped that something may be accomplished along this line as this work progresses. At present, however, one may say that in a general way *e*, the size and number of the liposomes in any given fish's pink muscle, will vary directly as *a*, the abundance of stored fat, *b*, the relative abundance of lipase, *c*, the structural and physical factors governing the diffusion of the lipolysed products, and inversely as *d*, the rate of oxidation of fats in the muscle fibers. The relation may be expressed as follows:

$$\frac{a \times b \times c}{d} \times k = e$$

where *k* is a complex constant representing the unknown facts and relations referred to above.

[a] Lovenhart, op. cit., p. 350.

TRANSFERENCE OF FAT IN THE DARK MUSCLE.

It is stated earlier in the paper that in the active feeding and growing salmon large quantities of fat are laid down in the dark muscle fibers. This deposit of fat begins in the earliest stage observed in the young salmon. It reaches its maximum somewhere near the time when the salmon begin their migration journey. At the Ilwaco station the amount of fat deposited in this type of muscle is astoundingly large. (See fig. 1, pl. III.) The amount is especially significant when it is remembered that the deposit has taken place as a storage process in a tissue that is supposed to be most active in the giving off of mechanical energy.

The variations noted in the dark muscle at the different stages in the migration journey are variations in the amount and character of the distribution of fat. Extensive discussion has been presented showing the facts as regards this picture at the different migration stages. Attention is here called especially to two points, first, the striking variation in the amount of fat of the dark muscle of different parts of one and the same animal as given in such fish as no. 125 and no. 126. The second factor is the relatively large amount of fat present as liposomes in the dark fibers at the death of the salmon.

As regards the first point, it is obvious that the diminished quantity of fat along the courses of certain of the smaller blood vessels, as shown in fish no. 120, also no. 126, represents a process of fat erosion. It would seem that the fat in the process of being removed is taken up first along the course of the blood vessels. Apparently we have to do here with the simple process of lipolysis. If this be the correct view then it is evident that the fat products of the dark muscle are handled in a way analogous to the fats in the pink muscle in so far as the process of solution and utilization goes. Therefore there is nothing peculiar about this tissue in this particular regard.

In an animal in which the fats have reached a certain stage of consumption and in which the processes of fat solution are going on rapidly we will have the greatest contrasts as between the highly vascular and the less vascular areas. The former favor in every way the rapid solution and removal of the fats. In a comparatively large section of dark muscle through, say, the trunk region of such an animal, one will notice a decided mottled or marbled appearance of the section viewed under comparatively low magnification. The less fat areas will be lighter, with less of the scarlet red stain, while the fatter regions will be relatively deeper red in appearance. Often the light areas form distinct patterns which conform to the smaller veins and arteries.

In the salmon at this stage the contrasts as between the trunk muscle and the caudal muscle are always sharp. The dark muscle, like the pink muscle, will contain relatively less fat in the caudal region than in the trunk region. The microscope will show that this caudal muscle fat is in smaller droplets which are fewer in number than in the trunk region. In the less vascular areas of the caudal region there will often appear fibers that have only traces, sometimes no fat. These factors are attributed to the more rapid using of fat for the production of energy in the caudal muscles as the more active tissues.

The utilization of the fats of the dark muscle does not present as acute a problem as regards the numerous smaller liposomes which we found in the case of the pink muscle. The salmon begins the fast with the dark muscle fully loaded with intracellular fat. Therefore, the first change that will occur in this muscle will be a process of using up the fat on hand in the cell. When at any time or for any reason this intracellular dark muscle fat is wholly consumed, then the dark muscle will be in the same category as the

pink muscle in so far as its source of material for the production of energy is concerned. Regions of dark muscle which have reached this stage are found with the arrangement of liposomes that is described as typical for the pink muscle. On the other hand, the dark muscle will have the chains of relatively small liposomes rather uniformly distributed throughout the muscle mass. These liposomes at this stage are relatively small, as for example in the fishes described from Cazadero. Rarely will fibers be found with no liposomes. It seems to me that should a certain area of dark muscle fibers through excessive activity consume all of its liposomes, then fat would be thrown into those fibers by the process of lipolysis and fat transference in exactly the same way that it is thrown into the pink fibers. This detail is fully described in connection with the discussion of the pink muscle.

As regards the second factor mentioned above, namely, the high percentage of fat still present in the dark muscle at the time of the death of the salmon, it seems to me the matter is more complicated. The operation of no ordinary factor would maintain a higher percentage of fat in the dark muscle at a time when the fats were almost consumed. One is led to suspect that there is some special factor operative in the dark muscle. In all probability this factor is the same in the late stage in the life cycle as that operating in the earlier stage in the salmon development which results in the loading of fats into this type of muscle. I have observed no special facts which of themselves explain this situation. There are, however, certain accessory facts which permit of an explanation which will be offered as a tentative hypothesis. Of these facts the most important is the fact of the loading of the dark muscle during the embryonic stage of its development.

Undoubtedly such deposits of fat as occur in no. 97, fig. 7, represent a perfectly normal process which is to be interpreted as a function of this muscle. Histologically the dark muscle differs slightly in its structure from the pink muscle. The dark fibers contain more sarcoplasm and somewhat larger and fewer fibrillæ. At an early embryonic stage this difference between the dark and pink muscle is rather more striking than it is later. This suggests that the dark muscle is a less highly differentiated type of muscle than the pink. One may assume, therefore, that it retains more primitive characteristics. In the sections which cut the borderland between dark and pink muscle a few of the pink fibers of the intermediate zone are found to be filled with liposomes. This loading of liposomes is greatest in the fibers nearest the surface of the great muscle mass and is totally absent in the deeper portions of the muscle. The fibers in question are of the pink fiber type. Their loading of fat must therefore be due to some special factor. These three facts, namely, (1) the excessive loading of fat in the growing dark muscle, (2) the more generalized type of dark muscle, and (3) the tendency of the neighboring zone of pink muscle to load intramuscular fat, all suggest that the dark muscle has still strongly developed one of its general functions. This function is the production of lipase. It may be anticipating a bit in the following discussion, but it is evident that the presence of a relatively high concentration of lipase results in the seizing of the fats during the growing stage and their concentration in the lipase-producing tissues. This view is borne out by the deposit of large amounts of fat in the pancreas as well as in the dark muscle. A relatively high concentration of lipase in the dark muscle would lead to a greater concentration of lipase in the tissue lymphs in those tissues which surround the dark muscle, namely, the connective tissue of the skin and the superficial layer of

the trunk pink muscle. The deposit of liposomes in the intermediate zone of pink muscle fibers is occasioned by this greater concentration of lipase.

If it can be granted that the dark muscle retains this assumed power of lipase production throughout its whole life, then it will follow that at the spawning grounds we will still have in this particular tissue more than the average amount of lipase in the muscle fluids. If so, there will be a tendency to hold fats and fatty acid in solution in this tissue, and, other things being equal, the tendency to maintain a somewhat higher content of fat in the form of liposomes.

The liposomes will form in those tissue spaces in which there is greater stagnation of the tissue fluids. In so far as the dark muscle is concerned, this point is immediately under the sarcolemma. Should the cleavage products of fatty acid and glycerin diffuse from the sarcoplasm through the sarcolemma, then it would be picked up ultimately by the blood stream and washed away. If, on the other hand, fats are coming into the pink muscle, then the diffusion will pass first through the sarcolemma and then the sarcoplasm, which is using fat in its oxidations. Therefore, the central portion of the muscle fiber will contain fewer liposomes and smaller ones while the superficial portion and the region immediately under the sarcolemma will contain relatively larger liposomes. This picture corresponds to the facts whether or not the theory offered in explanation be true. The factors discussed in previous pages which determine the number and size of the liposomes are therefore the same for dark muscle as for the pink, except for one, namely, the factor *b*, given on page 126, the relative abundance of lipase in the dark muscle. This factor *b* is greater than in the pink muscle, since the dark muscle itself is presumed to be producing lipase.

RELATIVE ABUNDANCE OF STORED FAT AND ITS DISPOSAL IN THE ORGANS.

My chemical analyses have shown that there is always a great variation in the percentage of fat taken from any standard region of different individual salmon from any particular station. No school of salmon contains individuals of uniform characteristics as regards either size or condition, for example, the specific loading of fat. Considering only the region under discussion here, namely, the mid-lateral portion of the body, I find that there is great variation in the total amount of fat present. In the histological examination of the variation of fats this will show itself in the number and especially in the relative size of the fat droplets. The loading of fat, as indicated by these facts, is most constant in the salmon obtained at the mouth of the Columbia River. It is to be assumed that if one could obtain salmon just in the region where and at the time when they cease feeding, then the loading of fat would be most nearly constant. Even then a great variation might be expected, since in salmon of the same size it can not be assumed that there has been uniformity of opportunity in obtaining food during the long period of development. Therefore, there are great variations present in the total loading of fat in the lateral pink muscle, even at the Ilwaco station, variations that are the expression of a multiplicity of factors.

As salmon pass up the rivers in their migration and since the stored fat forms the total source of the energy-giving material, it follows that there will be a diminution in the total fat directly proportional to the length of time and the relative expenditure of energy since the beginning of the fast.

Instances of variations in fat between the fibers of the lateral pink muscle at the first station, Ilwaco, are to be obtained by reference to the protocols of fishes nos. 111,

113, 115, and 118, representing very fat fish taken during early August, 1911. The commercial fishermen report that the very fattest specimens are obtained earlier in the season. Fish no. 114 and especially no. 117 represent the opposite extreme, i. e., the poorest fish for the August season. Undoubtedly no. 117 was at a stage in which there was considerable diminution of fat below its maximum. This diminution may have been due to any one of a number of factors, but one is led to suspect that the fish has been long without food, even at this low station on the river. The marking experiments of 1908 [a] give evidence that some at least of the August fish remain long in the waters of the lower river.

It is self-evident that where a fish has a low percentage of stored fat to begin with there will be a less abundant erosion by the action of the lipases; therefore, other things being constant, one can not expect in such an animal as high a percentage of concentration of the fatty products in the blood and tissue fluids as in fish that contain a greater fat content. This factor *a*, the variation of the storage fat, has its influence on the factor *e*, the number and size of the liposomes, as is indicated by the variations noted in fishes nos. 120 and 125.

SALMON LIPASES.

The rapidity with which the stored fat of the salmon tissues is eroded when the fasting begins will, of course, depend chiefly upon the second factor mentioned in the section on the transference of fat in the pink muscle, namely, the amount or concentration of the lipases.

The phenomena of fat mobilizations in the body belong strictly within the group of enzyme actions, for which the general law is so admirably stated by Wells: [b]

> All metabolism, then, may be considered as a continuous attempt at establishment of equilibrium by enzymes, perpetuated by prevention of attainment of actual equilibrium through destruction of some of the participating substances by oxidation or other chemical processes, or by removal from the cell or entrance into it of materials which overbalance one side of the equation.

The presence of lipase in various animal tissues has been demonstrated often enough. Hanriot [c] demonstrates the presence of lipase in the following fluids and organs: Blood, lymph, urine; liver, pancreas, testes, spleen, thyroid. The lipase was in greatest amount in the blood, liver, and pancreas. Kastle and Loevenhart [d] showed the presence of lipase in intestinal epithelium, which on account of its position as an absorbing tissue is of more than passing interest. The evidence as regards the presence of lipase in the blood plasma and in the lymph is somewhat contradictory, yet such lipase is not only demonstrated, as given above, but its quantity has been shown to vary under certain pathological conditions. Pathologists have followed the variations in the quantity of lipase in necrosis [e] with fat formation. The pancreatic cells are well-known lipase producers. It is to be expected, therefore, that there will be a variation in the quantity of lipase that will reach the body fluids from this tissue. In confirmation of this point lipase has been found in the urine of a clinical case of fatty necrosis associated with inflammation of the pancreas. [f]

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[a] Greene, C. W.: The migration of salmon in the Columbia River. Bulletin U. S. Bureau of Fisheries, vol. XXIX, 1909, p. 131.

[b] Wells, H. G.: Chemical pathology, p. 68. Philadelphia, 1907.

[c] Hanriot: Comptes rendus de la Société de biologie, 1896, p. 925.

[d] Kastle and Loevenhart: American Chemical Journal, vol. XXIV, p. 491, 1900.

[e] Flexner: Journal Experimental Medicine, vol. II, p. 194, 1904.

[f] Opie, Eugene L.: A case of hemorrhagic pancreatitis. The occurrence of a fat-splitting ferment in the urine. Johns Hopkins Hospital Bulletin, vol. 13, p. 117, 1902.

I have examined the pancreas of the king salmon for lipase, testing extracts of the fresh normal glands of salmon caught in active stages of digestion. The experiments were preliminary only, yet the tests were positive and the reactions vigorous. The salmon pancreas secretes an active lipase. In my fat-absorption experiments there was always a vigorous loading of the epithelial cells of the intestinal mucosa, especially in younger fish. There was a greater mass loading than I have ever seen in mammalian intestinal epithelium. This, by our current theories of fat absorption, is as strong circumstancial evidence of the presence of lipase in the salmon mucous epithelium as one could well expect to discover. Aside from these tests no studies have been made on the lipases of any of the Salmonidæ. It is, of course, highly desirable that such studies should be made. The amount and the variations of the lipase content of the salmon blood, and especially of the muscles, should be determined by a series of quantitative tests. These are the tissues of peculiar interest to the problem in hand. There are other tissues directly concerned in the fat metabolisms of the salmon—the liver, the divisions of the alimentary tract, the pancreas, etc.

However, on a priori ground, there is every reason for assuming that the salmon is well supplied with lipase in its fat metabolizing tissues, particularly in the alimentary mucous epithelium (the gastric epithelium is also fat absorbing), the muscles, the liver, etc. The blood and the lymph can not escape a lipase content in an animal in which so many of the tissues are concerned in the lipolytic processes.

In previous discussions the conclusion has been reached that there is a marked increase in lipolysis at the particular time the salmon cease feeding and begin the migration journey. This carries the assumption that there is at this time an increase in the amount, i. e., percentage, of lipase in the blood and the tissues, including the muscular tissues. Whence come these lipases?

SOURCE OF THE LIPASES.

The presence of a greater percentage of lipase when the cessation of feeding occurs may be explained on two physiological grounds, both of which are probably active; first, there may be an absolute increase in the lipase produced in a given time; and, second, it is possible that with the cessation of feeding the lipase that is usually consumed in the intestine and pyloric cæca in the processes of digestion and absorption is now thrown more fully into the blood stream. To this extent it raises the percentage content of lipase in the blood.

The pancreas.—The salmon pancreas is one proven source of lipase. The pancreas is morphologically of the type described by Legouis [a] as the diffuse pancreas. The gland filaments are quite separate from each other. They form an open meshwork running over the pyloric cæca, the blood vessels and mesentery of the stomach and intestine, the inner loop of the stomach, and the mesentery of the spleen.

These pancreatic filaments are richly supplied with blood vessels, and these vessels anastomose with the blood vessels of the cæca, intestine, etc., of the region. Pancreatic ducts have been described by Legouis as converging to a common duct that enters the intestine either with, or in the neighborhood of, the bile duct.

[a] Legouis, P.: Recherches sur les tubes de Weber et sur le pancreas des poissons osseau. Annales des Sciences Naturelles Zool., 5th ser., t. 17, 1873. See also t. 18, 2d article.

My histological observations, which will be given in detail in a later paper, show that the pancreatic cells are not all compactly arranged in acini, as is usually the case, but that they are more or less scattered. Every cross section of the pancreas reveals the fact that a large amount of adipose tissue is present in association with the pancreatic tissue. The cross section of a pancreatic lobe is somewhat triangular in shape. The central portion of the triangle in an adult organ always contains one or two blood vessels of relatively large size, together with a considerable amount of fatty tissue. The pancreatic cells are arranged around the surface of the gland and in the interstices among the fat cells and blood vessels. There are many acini where definite arrangement of pancreatic cells around a central lumen can be shown. This lumen rarely forms a space as large as one sees in the corresponding region of a mammalian pancreas. In these groups, in favorable preparations, that portion of the pancreatic cell bordering on the lumen is highly granular and the granules stain in a way characteristic of zymogen granules. The pancreatic cells around the surface of the gland and in many portions of the deeper region have a rather scattered arrangement in which it is extremely difficult to show definite relation to ductules. Such relation is questionable in a large proportion of the gland. The diminutive size of the pancreatic ducts, the presence of the large amount of fat in the gland, the rich vascular arrangements of the gland, and the diffuse arrangement of the gland cells of the salmon pancreas have led me to the conclusion that the secretion of this gland is largely internal, i. e., that the pancreatic secretion is largely thrown into the lymph and blood stream of the organ. In this statement it is not meant to minimize the importance of the gland in the production of the pancreatic juice. Rather, the intention is, to emphasize the importance of the internal lipase secreting function.

When digestion stops and the animal begins its fast, the pancreatic gland undoubtedly continues to function. This is shown especially by the histological appearance at different stations of the migration journey. There is obviously no digestive function to be accomplished by the secretion during the fast. Therefore, during this phase of the life cycle the internal secretive function becomes the main, in fact, the only one. The vascularity of the pancreas and of the pyloric cæca does not change in proportion to the amount of retrogressive change shown in the cæca and in the rest of the alimentary tract. Putting these facts and deductions together they may be summarized thus:

1. The pancreatic gland is abundantly active during the migration fast.

2. The activity consists in the production of an internal secretion which is not essentially different in character from the normal secretion produced at an earlier stage in the life cycle. It is therefore rich in lipases.

3. The pancreatic lipase produced during the fasting period is chiefly, if not wholly, discharged into the tissue spaces, from whence it reaches the blood stream.

4. The circulation carries the lipase to the tissues of the body, which includes both the fat-storing tissues and the active fat-using muscles now under especial consideration.

Lipase from the granule cell layer.—There is a second tissue filled with zymogen granules which I believe to be an active source of lipase, namely, the granule cell layer of the alimentary tract. I have already called attention to the presence of a layer of granule cells in the mucous coat of the alimentary tract. This layer is especially richly developed in the pyloric cæca. In every section of the retrogressing cæcum, one is strongly impressed with the continued large size of this granule cell layer. Even in

fish from the Ilwaco station, specimens which represent the first stage in the changes following the beginning of the fast, this layer is notably large. The granule cell layer is much thicker and more prominent than in the normal Monterey salmon tissue. I have made computations that indicate an actual increase of the mass of this granular layer.[a] The greater apparent increase in mass may be explained on the ground that the normal volume of the granule cell layer is retained while the volume of the various other structures of the alimentary tract is sharply diminishing, a question that is now under investigation. The structure of the cells in this layer does not materially change while the other tissues around it are sharply degenerating. The cells remain loaded with granules which have a strong affinity for the basic dyes. The loading of granules remains characteristic of the cells even in the most degenerated salmon examined. Here, again, one must interpret the granules as zymogen granules. Gulland [b] has given the name eosinophile leucocytes to cells of this region, an identification to which I can not subscribe. By implication he would ascribe to the granule cells a very different function from that which appears to me to be the true one. The granules, which do stain sharply with eosin as Gulland observes, are too large to be compared with the granules of eosinophiles. The granule cells themselves are larger, differently arranged, and have a very different type of nucleus from the typical eosinophile. Also one never finds granule cells of the type characteristic of the cells of this layer in the salmon blood vessels of either the cæca or of the intestine. The matter is more fully discussed in another paper, but the points are mentioned here in order to meet objections to the view which is offered for the function of these cells; namely, the granule cell layer of the pyloric cæca and of the alimentary tract of the salmon is an internal secreting organ which has for its probable function the production of lipase.

In the normal organ, when active digestion is going on, the lipase produced within the granule cells is thrown out into the tissue spaces. Some of this lipase quite probably reaches the epithelial layer and supplements the lipase produced by the epithelial cells. Such lipase will, of course, facilitate the absorption and the resynthesis of the fats in the epithelial cells, according to the laws of fat absorption. A considerable portion of this layer lies outside the stratum compactum. It seems to me improbable that the internal secretion of those cells will diffuse through the stratum compactum with the same facility that it will pass out into the tissue spaces of the adjacent muscle coats. The granule cell layer itself is almost free of blood vessels. The muscle coats, on the other hand, are richly supplied with blood vessels. It would follow that any secretion diffusing into the muscle coat would quickly be taken up by the blood, and would be carried throughout the body. During the fasting period this lipase-producing function of the granule cell layer is no longer of use in the absorption of fats but is of great supplementary aid to the pancreas in maintaining an adequate supply of lipase in the blood. This offers an explanation that would account for the persistence of the organ.

Increase in lipase from change in isotonicity of the tissues.—In the recent brilliant work of the Rockefeller Institute in isolating and growing tissues of pure culture, it has been shown that physiological activity, as measured by growth, is influenced by the degree of concentration of the nutritive fluids. Tissues growing in physiological solutions of

[a] These determinations were made by computing the cross-sectional area of the granule cell layer of cæca from Monterey and from Ilwaco salmon. The Ilwaco specimens exceed the Monterey by an average of 11 per cent. But the probable error is high.

[b] Paton, Noel: Life history of the salmon. Article 3. The minute structure of the digestive tract of the salmon, and the changes which occur in it in fresh water, p. 13, 1898.

slightly lower tonicity than that to which the tissues are adapted, undergo a more active growth than those in fluids of relatively high tonicity. These factors have been worked out on several tissues, but very strikingly on the rate of healing of wounds in the skin of frogs which had their body fluids decreased in tonicity by injections of water.[a]

I have no doubt that these principles are operative in the salmon during the migration. The salmon tissues in this stage of the life cycle are most of them long past the period where growth is the prominent physiological activity. There is, however, one marked exception, namely, the reproductive organs. These organs are quite immature at the time the migration begins. They suddenly take on an active stage of growth which proceeds readily till complete development at the time of spawning. Other tissues show their variations in physiological activity in ways other than growth, the muscular tissues in increased production of energy, the glandular tissues by variation in secretory activity, etc.

In work on the Sacramento River[b] I showed that there is a marked decrease in the depression of the freezing point of salmon serum as the fish migrate from the sea to fresh water. The depression of the freezing point is a direct measure of the tonicity of the blood. As this diminution begins with the process of migration from salt water to fresh water it is evident that it will have the same type of influence on the tissue activities of the salmon as that shown in the experiments on tissue growth. This is undoubtedly a factor in the stimulation of the reproductive organs to the sudden increased growth which takes place in the salmon at this time. It is a safe inference that this change in isotonicity of the blood and tissue fluids if exerted on other tissues of the bodies will cause variation in their physiological activity. Applying this principle to the pancreas and to other tissues where lipase is produced we have a factor accounting for an increase in metabolites, of which lipase is one, at the critical time of the beginning of the migration.

Lipase from tissue degenerations.—Another source of lipase may arise through tissue degenerations. Extensive degenerations are taking place in the alimentary tract. At Ilwaco the mucous membrane of the intestine and pyloric cæca has already reached a considerable degree of disintegration. On the current theories explaining degenerative change one may assume that these cells have already passed through a stage of physiological hypertrophy. Kastle and Loevenhart have shown that these cells are active lipase producers in the normal condition. Therefore, one may assume that the inflammatory condition preliminary to the actual necrosis is associated with an increase in lipase production. The disintegration of the cells and especially the chromatolysis is the final step in the process. These pathological changes accelerate the physiological production of lipase in the mucous epithelium of especially the pyloric cæca just at the time when such an increase in lipase is of most value to the salmon, namely, at the beginning of the fast. Lipase from this source would almost cease at a quite early stage. However, the epithelium is rarely wholly disintegrated.

Lipase from the liver and other tissues.—There is evidence that other tissues of the salmon are more important in producing lipase. Of these one may mention the liver, whose function in this regard will be presented in another paper.

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[a] Ruth, E. S.: The influence of distilled water on the healing of skin wounds in the frog. Journal of Experimental Medicine, vol. 13, p. 422, 1911.

[b] Greene, C. W.: Physiological studies of the Chinook salmon. Bulletin U. S. Bureau of Fisheries, vol. XXIV, 1904, pp. 446, 449.

Also the group of dark muscle fibers represented in the musculus superficialis lateralis has already been mentioned as lipase producing tissue. But when all the less important sources which have been discussed are left out of account there still remains an adequate lipase producing mechanism in the pancreas and in the granule cell layer of the alimentary tract to account for the presence of sufficient lipase in the blood and tissues to meet the need of the fat transference that we have under discussion. Histological evidence has been given to show that there is no diminution of the activity of the pancreas at the inauguration of the fasting period. If the pancreatic lipase production even remain constant then the amount of lipase which this gland will produce as an internal secretion will tend to raise the total lipase of the blood and tissues. The lipase that is consumed in the process of digestion during the feeding period will now be left to be thrown into the circulation. It follows that there will be an increase in the percentage of lipase in the blood, therefore, according to Loevenhart, an increased solution of the fats with which this lipase comes in contact. These fats are the stored fats. An increased solution of the stored fats will raise the fatty acid and glycerin content of the tissue fluid and the blood. The inevitable result will be an increased supply of these fat cleavage products to the active muscular tissues. This supply will diffuse through the muscle spaces, the sarcolemma, and throughout the sarcoplasm of the muscle fiber in an ever increasing quantity. Since the relative amount of activity of the muscles can not be assumed to change, i. e., is comparatively constant, it follows that the percentage amount of fat will increase within the active muscle fibers.

It is shown on page 81 that the pink muscle fibers contain no intramuscular fat during the feeding stage, or at most, only a trace of such fat at maturity. This is only another way of saying that the consumption of fatty substances in the muscle fibers of the feeding salmon is in balance with the fatty bodies penetrating the fibers. There is never a sufficient excess of fatty acid and glycerin within the fibers to produce resynthesis and deposition of the fat in visible form. But with the increasing percentage of these substances penetrating the fiber after the fast begins there will be a synthesis of neutral fats and these will be deposited and can be identified. The liposomes present in the lateral pink muscle of the salmon taken at Ilwaco represent such deposits that have taken place since the beginning of the migration. The amount of neutral fat present in the pink muscle fibers is a measure of the excess of fatty acids and glycerin brought into the fibers over those oxidized in the muscular activity. If oxidation diminishes, then fats will be deposited and the excess is expressed in the number and size of the liposomes (fig. 8, pl.VI).

The character of the liposomes, that is, their number, size, and arrangement in the pink muscle depends also on one other very different group of factors. This is the structure of the muscle (fig. 13, pl. VIII). That the fat is laid down in chains of liposomes of the minute sizes that have been described must depend largely upon the structural arrangement of the fibrillæ and of the interfibrillar sarcoplasm. It is not desired, however, to discuss this factor beyond merely calling attention to it.

RÉSUMÉ.

The points made in this investigation that call for special mention may be categorically stated as follows:

1. Fat is the prominent and immediate source of the energy of the salmon expended during the spawning migration.

2. The salmon fat is stored in the body during the stage of feeding and growth, and reaches a maximum at the time the feeding stage ends, i. e., at the beginning of the migration fast. This fat can not in any proper sense be looked upon as a fatty degeneration.

3. The fat storage tissues are primarily the muscles and intermuscular connective tissues. Storage tissues of minor importance are the cutaneous and other adipose tissues, the liver, the alimentary tract, and the skeleton.

4. There are two distinct and characteristically different types of muscle—the superficial lateral or dark and the deep lateral or pink muscle. The latter represents the major portion of the great lateral muscle mass.

5. The pink muscle is characterized (*a*) by the enormous load of fat between the fibers, intermuscular fat, and in the myocommata at the time of maturity; (*b*) by the great variation in the size of its fibers.

6. The pink muscle fibers have no intramuscular fat, or at most only traces of fat, during the feeding stage.

7. Immediately at the beginning of the spawning migration the pink fibers are loaded with numerous chains of very small liposomes. This loading of liposomes increases during the early stage in the journey, and then decreases somewhat up to the spawning time. The fat never wholly disappears even in dying salmon.

8. In the active caudal pink muscle the liposomes are much less constant and are often completely absent as advanced stages of exhaustion appear.

9. The pink muscle fibers are plump and cylindrical at the time the migration begins. But at the spawning time the larger fibers have the appearance of being shrunken by decrease in mass. They become polygonal in cross-sectional outline. The sides of the polygon are often concave to the exterior, as if compressed by the adjacent smaller fibers.

10. The dark muscle is characterized (*a*) by the enormous loading of intramuscular fat at all stages of the life cycle, but especially at the time the spawning migration begins; (*b*) by the relatively small and uniform size of the fibers.

11. The stored fat of the dark muscle is gradually eroded during the migration until the fat reaches a quantity and distribution comparable to but still greater than that in the pink fibers. The fat is never completely eroded and is present in considerable quantity at the death of the salmon after spawning.

12. The smaller muscles of the fins and of the head of the salmon take little part in the fat storing. The food supply of these muscles, however, is the same, namely, the fats.

13. Distinct degenerative changes were found in the adductor mandibulæ muscle of a spawned male at the dying stage. This degeneration is a simple atrophy with pigmentation.

EXPLANATION OF PLATES.

The drawings presented were all made from camera lucida outlines. Fat is represented in the characteristic red color obtained by the scarlet red method of staining fat: All the drawings and outlines were made for me by Mr. George T. Kline, biological artist of the University of Missouri.

PLATE III.

FIG. 1. The transparency of a segment of dark muscle fiber of salmon no. 115 from the mouth of the Columbia River, Ilwaco, Wash. The most superficial liposomes and fat droplets are represented somewhat darker, while the paler colored droplets are deeper in the fiber. Magnification, Leitz ocular 2, objective 7.

FIG. 2. A small segment of dark muscle fiber from a young salmon, from the Columbia River, Warrendale, Oregon. Magnification, Leitz ocular 3, objective 7.

FIG. 3. Section of trunk dark muscle of salmon no. 120, adult in prime condition from the Columbia River at Warrendale, Oreg. The amount of fat present is almost as great as in the Ilwaco fish no. 111 and no. 115. Magnification, Leitz ocular 2, objective 7.

PLATE IV.

FIG. 4. Trunk dark muscle of salmon no. 126 from the Columbia River at Warrendale, Oreg. This salmon is representative of a late stage in the fat removal from the tissues. Certain fibers near the large blood vessel to the right are free of all but the smallest liposomes. Other fibers are still well supplied with fat. Magnification, Leitz ocular 2, objective 7.

FIG. 5. Dark muscle from salmon no. 138, a spawning salmon from the Clackamas River, Cazadero, Oreg. This figure represents the latest stages in fat removal from the trunk dark muscle. Magnification. Leitz ocular 2, objective 7.

PLATE V.

FIG. 6. Transverse section of dark muscle from an exhausted, naturally spawned salmon no. 108, McCloud River, Baird, Cal. This figure represents the extreme exhaustion of fat from the dark muscle. The salmon was an enormous male which was taken just at the time of natural death. The fat is in finest liposomes condensed at the surface of the fiber but absent between the fibers. The representation of the size of the liposomes is somewhat strong. Magnification, Leitz ocular 2, objective 7.

FIG. 7. Dark muscle from young fish no. 97. The preparation is a paraffin section stained with Mallory's analine blue connective tissue stain. The figure presents well the excessive number of clear spaces which represent vacuoles produced by extracting the fat in the imbedding process. One fiber has recently divided longitudinally into two. This fiber shows no fat along the new portion of sarcolemma. Magnification, Leitz ocular 3, objective 1/12. (From American Journal of Anatomy, vol. 13, 1912, p. 175.)

PLATE VI.

FIG. 8. Trunk pink muscle of salmon no. 118 from the mouth of the Columbia River, Ilwaco, Oreg. Attention is called to the great variation in the size of the fibers, to their characteristic outlines, the great amount of fat between the fibers, and to the general distribution and extreme fineness of the liposomes in the fibers, which have come out rather too strong in the reproduction, many of them being actually just perceptible. This figure without the liposomes in the muscle fibers would represent the normal condition of the salmon pink muscle at the beginning of the migration fast. Magnification, Leitz ocular 2, objective 4.

FIG. 9. Segments of two trunk pink fibers with adherent intermuscular fat drops from salmon no. 118. Magnification, Leitz ocular 2, objective 4.

PLATE VII.

FIG. 10. Trunk pink muscle of salmon no. 126 from the Columbia River at Warrendale, Oreg. This salmon is the one presented as a typical poor condition fish. The intermuscular fat is reduced to groups of droplets in the stronger connective tissue septa. The intramuscular fat is extremely low, limited to the smallest and medium sized fibers. These fibers retain their normal histological structure as shown in figure 13. Magnification, Leitz ocular 2, objective 4.

FIG. 11. Trunk pink muscle from salmon no. 132, a spawning female from the Clackamas River, Cazadero, Oreg. The intermuscular fat is practically eliminated, yet all the fibers except the largest show a considerable sprinkling of liposomes. In the small fibers these droplets are quite uniformly distributed, in the medium fibers concentrated around the surface, and in the largest fibers present only in traces at the surface. The outline of the largest fiber to the upper right-hand side of the figure indicates that it is approaching a degeneration stage, though the microscopic fibrillar structure is still normal in appearance in this particular fiber. Magnification, Leitz ocular 2, objective 4.

PLATE VIII.

FIG. 12.—Cheek muscle of salmon no. 140, a spawned male from the Clackamas River, Cazadero, Oreg. Fat is present in a few groups of small droplets in the connective tissue septa. There is no intramuscular fat. One fiber in the center of this group is in an advanced stage of atrophy with pigmentation, shown in the granules of this fiber (not to be confused with similar appearance of liposomes in other figures). The three fibers to the right of this pigmented one show the first stages of degeneration represented by a swelling and blending of the fibrillæ. This detail of structure is not shown in the figure. Magnification, Leitz ocular 3, objective 4.

FIG. 13. A highly magnified portion of a trunk pink fiber from salmon no. 126, Columbia River, Warrendale, Oreg. This small segment of a medium-sized pink fiber shows the normal fibrillar arrangement. The amount of fat present is indicated in figure 10, plate VII. Traces of fat were present in this particular segment just under the sarcolemma and between the outer series of fibrillæ. This figure is offered in evidence as showing that the elimination of fat from the pink muscle is not accompanied by any immediate breaking down or degeneration of the finer structure of the tissue. Magnification, Leitz, ocular 3, objective 1/12 oil immersion. Camera lucida outlines.

PLATE IX.

FIG. 14. The trunk dark muscle of young salmon no. 97 from the McCloud River, Baird, Cal. The muscle fibers are drawn in outline to show the compact arrangement and relative size of the fibers as compared with the adult. One particular fiber in this figure showed an exceptionally large fat drop in the middle of the fiber. Magnification, Leitz ocular 4, objective 3.

FIG. 15. Dark trunk muscle of salmon no. 126 from the Columbia River at Warrendale, Oreg. Drawing to show the outlines of the fibers of the adult fish after the fat is largely removed. This figure should be compared with the preceding. Magnification, Leitz ocular 4, objective 3.

PLATE X.

FIG. 16. Outline of the trunk pink fibers of the young fish no. 97 from the McCloud River, Baird, Cal. The figure shows outlines of the fibers at a stage in which active growth is taking place. The large number of relatively small fibers have recently split off the larger in the process of fiber multiplication. Magnification, Leitz ocular 4, objective 3.

FIG. 17. Trunk pink muscle fibers from adult salmon no. 118 from the mouth of the Columbia River at Ilwaco, Wash. The outlines of the fibers show the relative symmetry of the adult prime condition muscle. The separation of the fibers is due to the loading of fat in the interstitial connective tissue. Should be compared with figure 8, plate VI. Magnification, Leitz ocular 4, objective 3.

PLATE XI.

FIG. 18. Trunk pink muscle from salmon no. 122 from the Columbia River, Warrendale, Oreg. This outline figure shows the more compact arrangement of the fibers of pink muscle that has lost most of its intermuscular fat. The fibers themselves are normal in outline. In this fish the pink fibers in general seem somewhat smaller in size than the average for adult fish of mature size. This point should be kept in mind in comparing the absolute size of the fibers shown in this figure and the preceding. Magnification, Leitz ocular 4, objective 3.

FIG. 19. Trunk pink muscle from fish no. 140, a spawning male from the Clackamas River, Cazadero, Oreg. The outlines of the fibers shown in this fish are typical of the stage just before natural death. The larger fibers do not show any unquestioned structural signs of degeneration, though they have the decrease in plumpness. Magnification, Leitz ocular 4, objective 3.

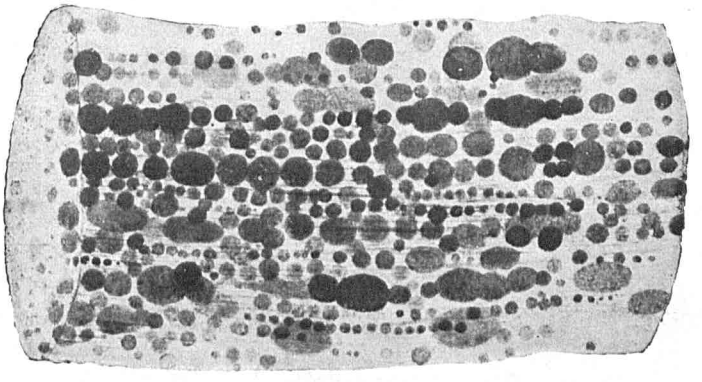
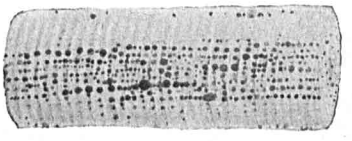


FIG. 1.



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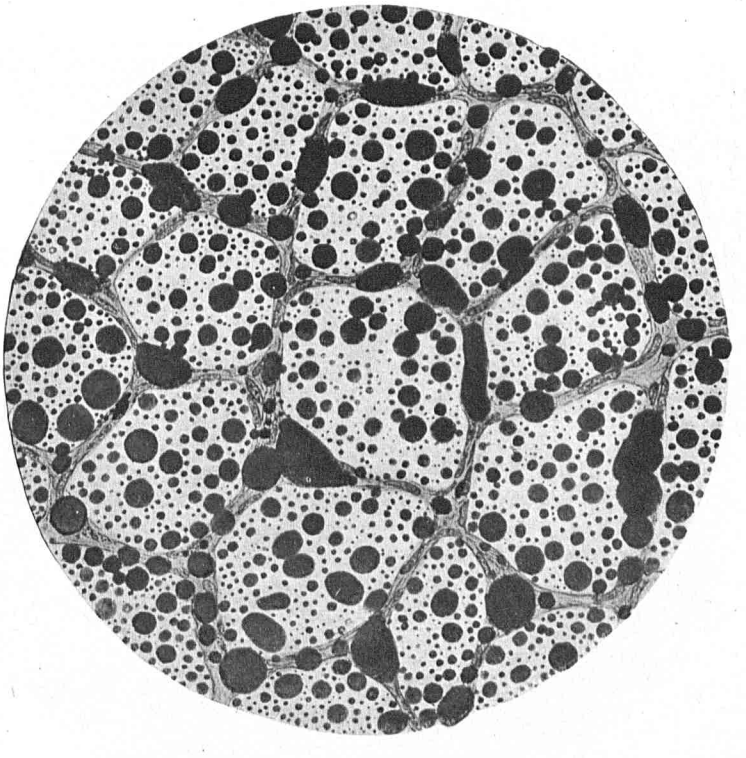

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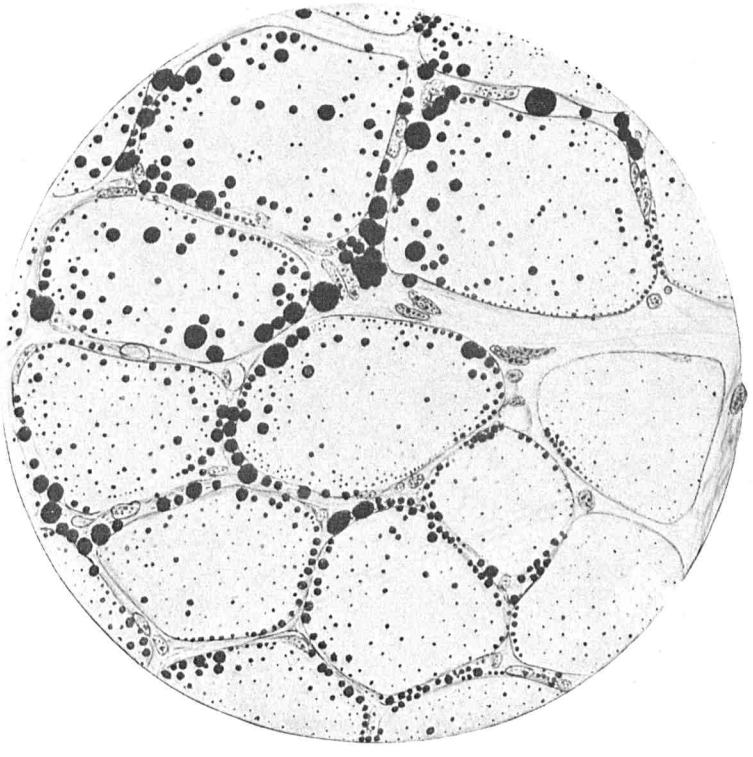
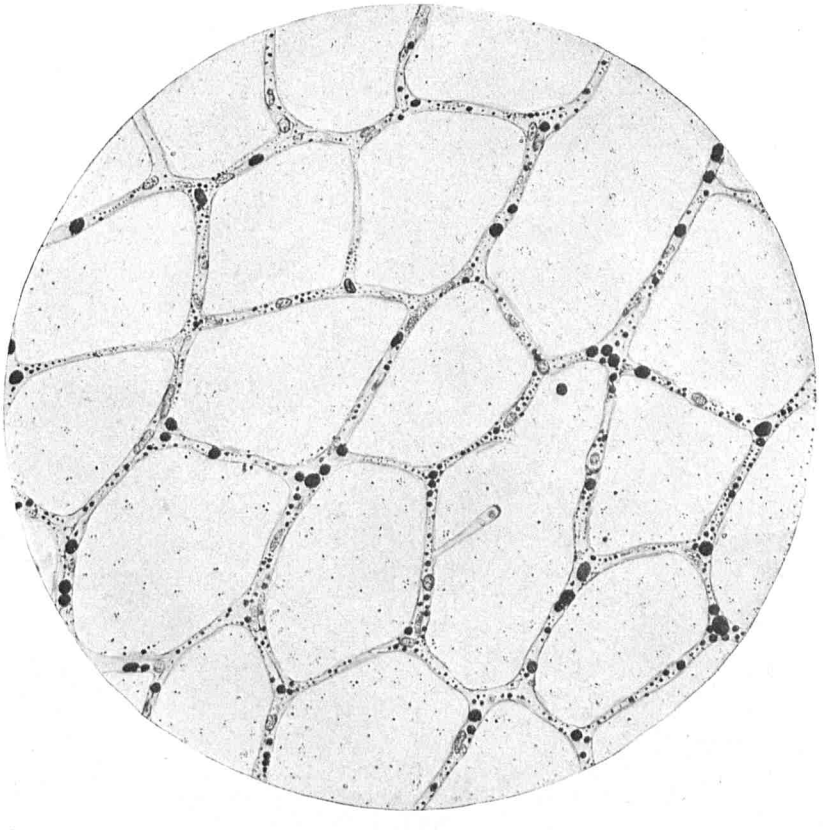


FIG. 4.



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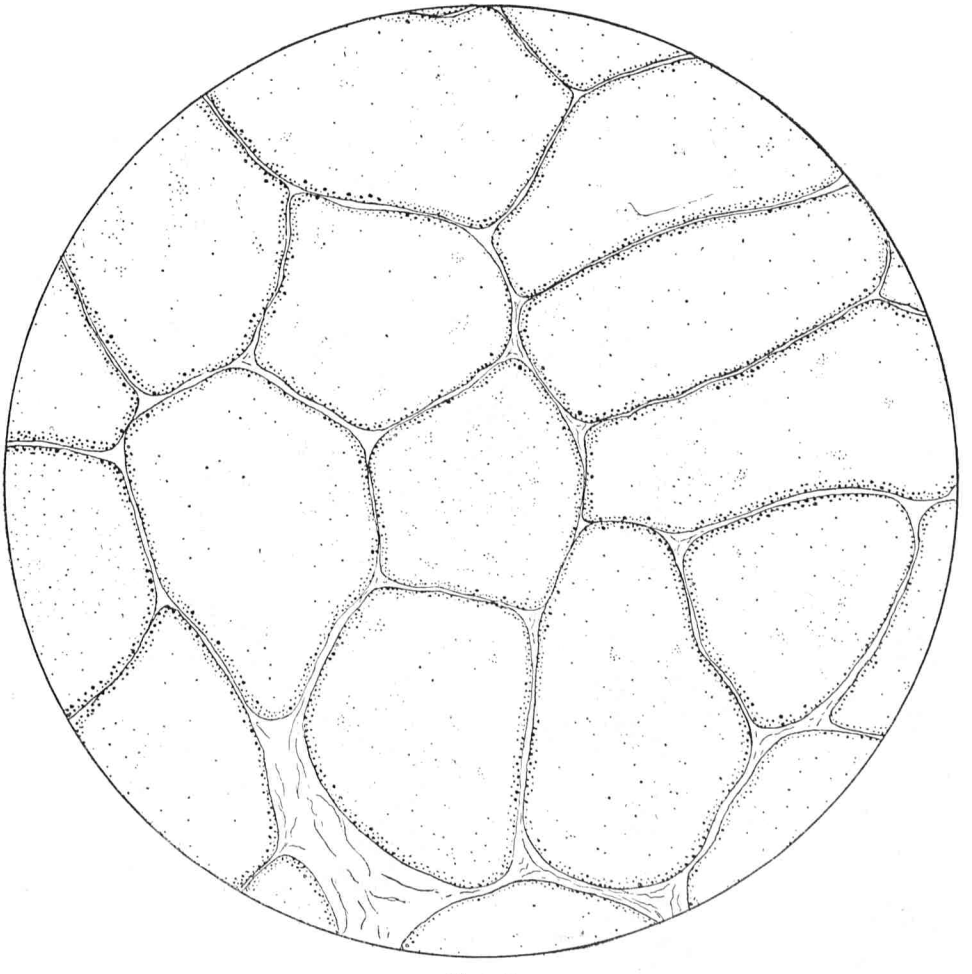

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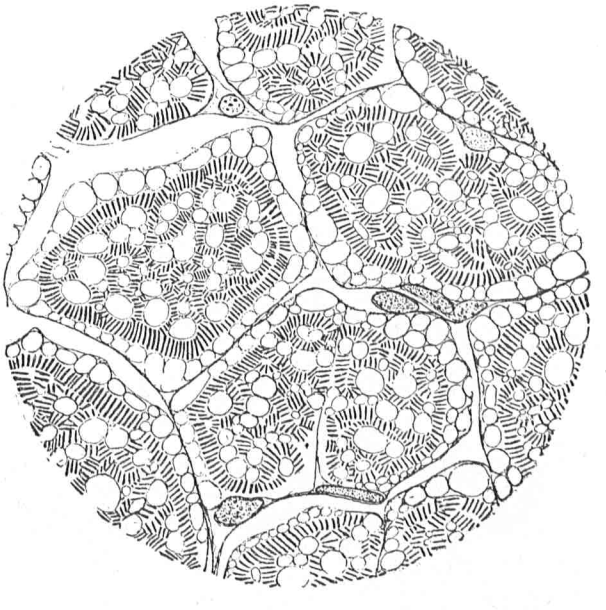

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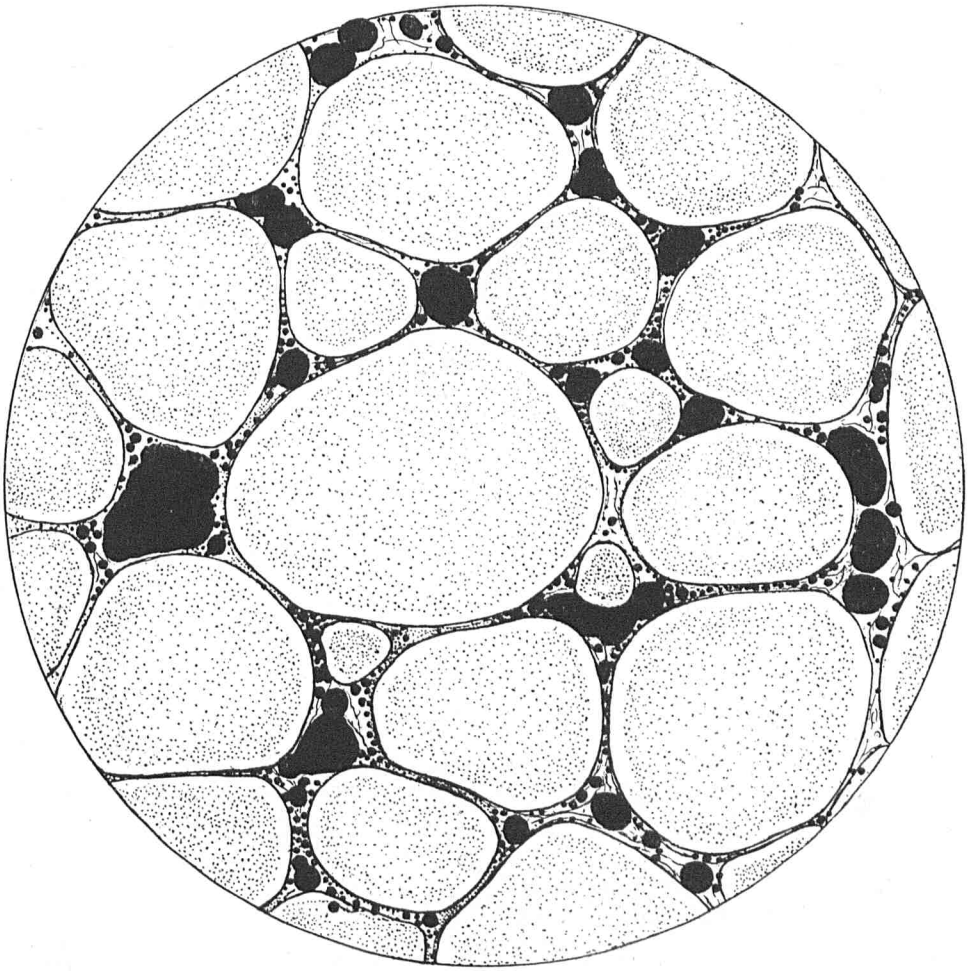

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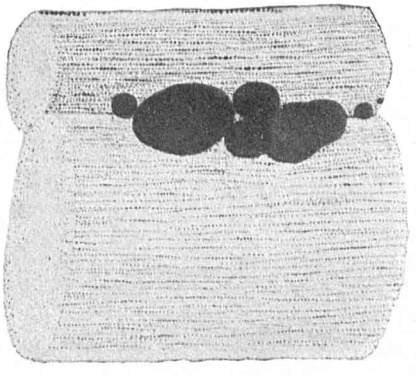

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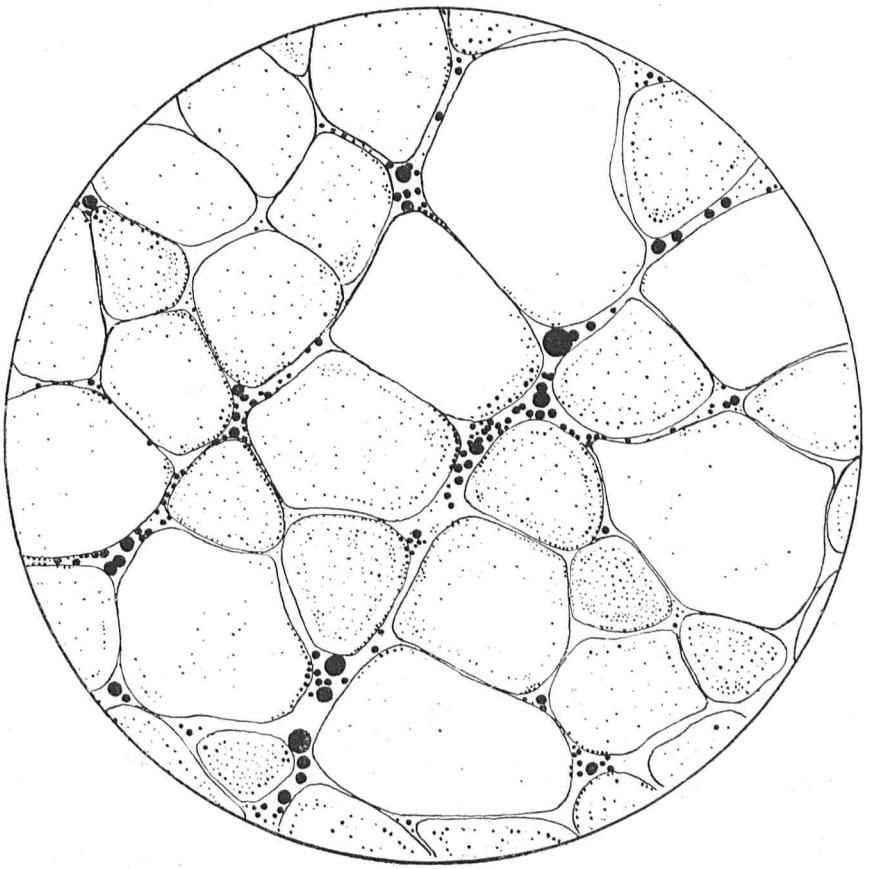

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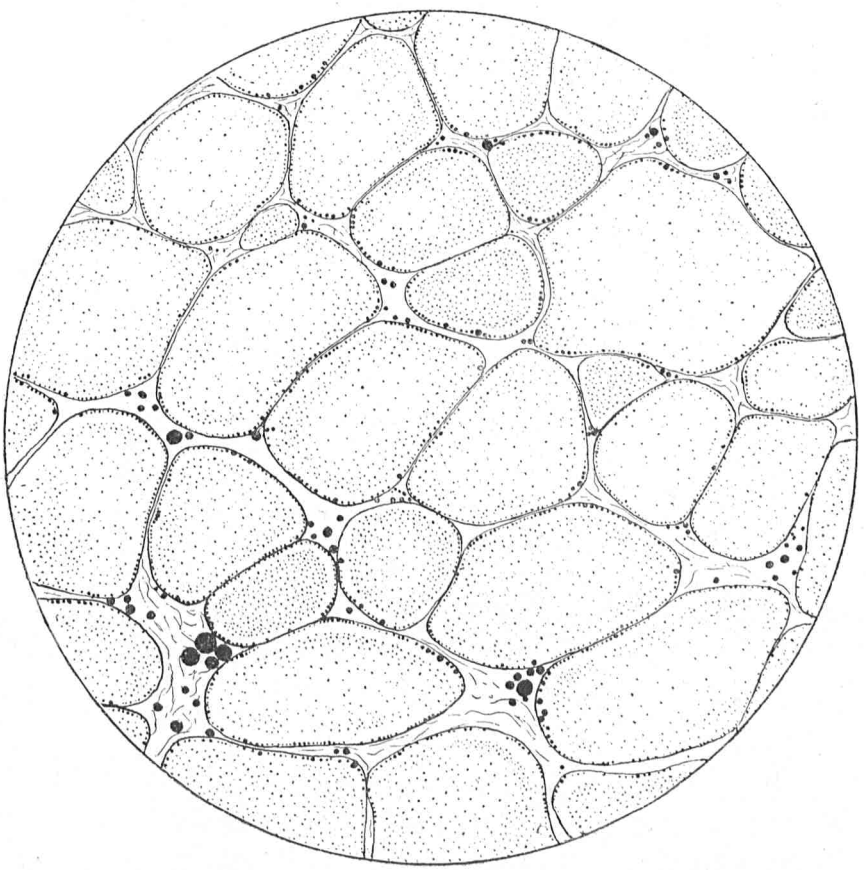

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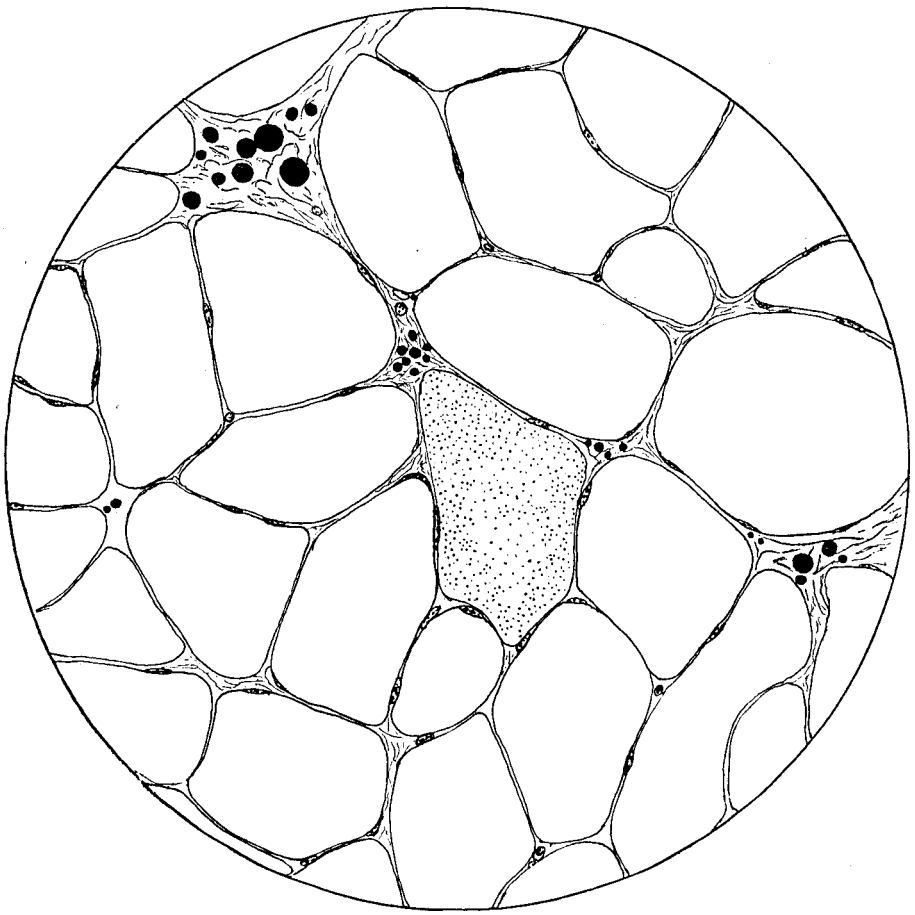

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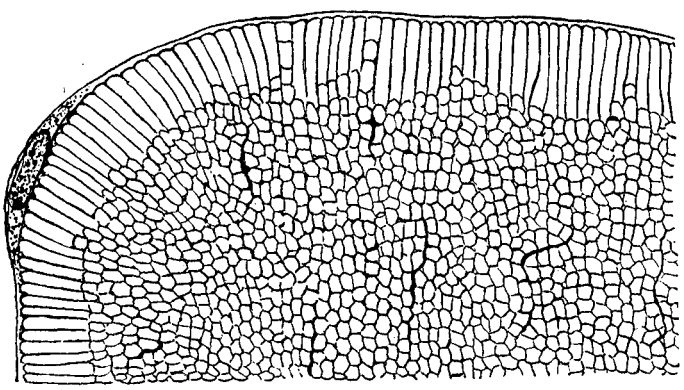

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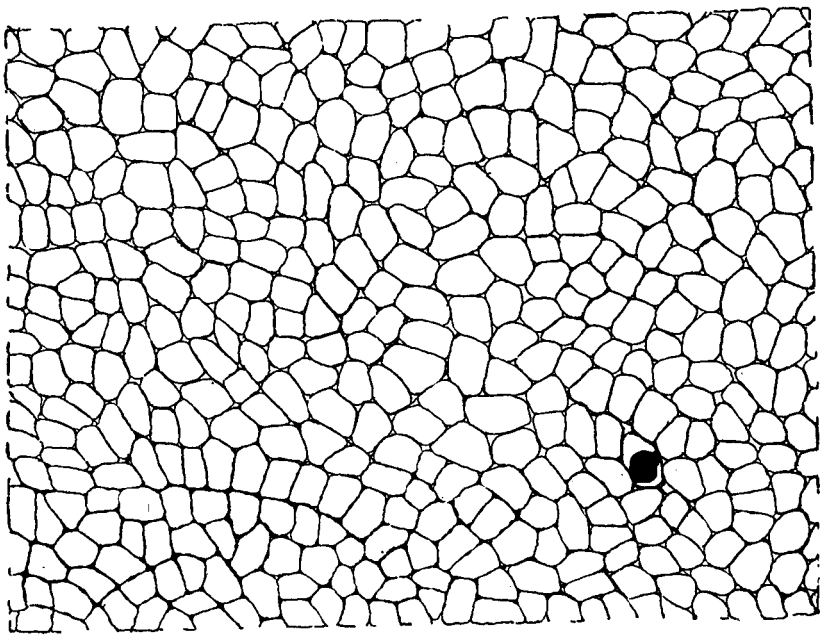

FIG. 14.

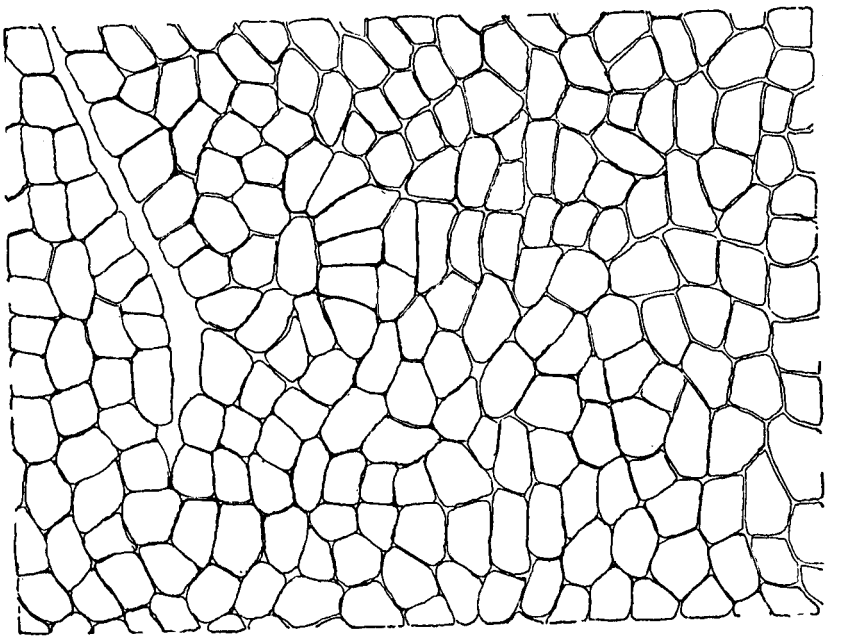

FIG. 15.

FIG. 16.

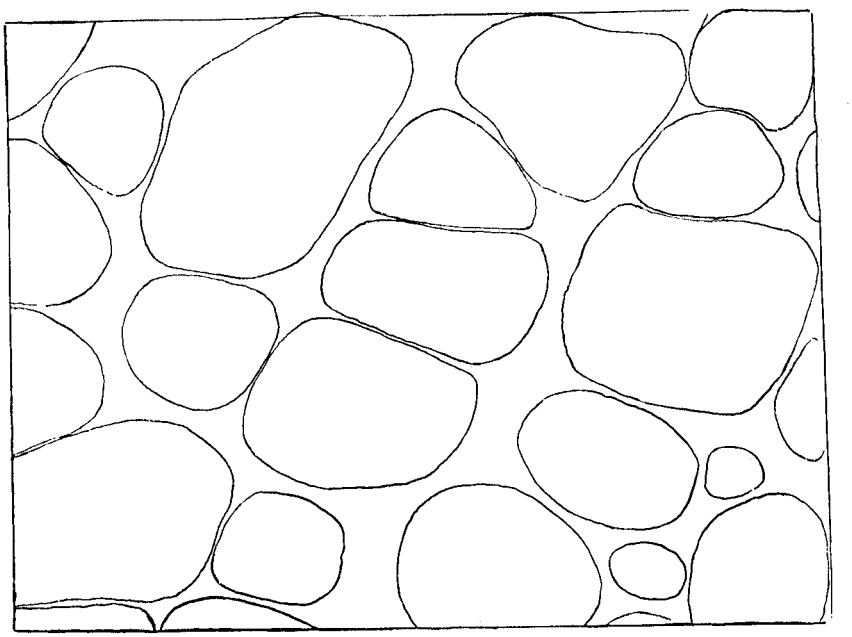

FIG. 17.

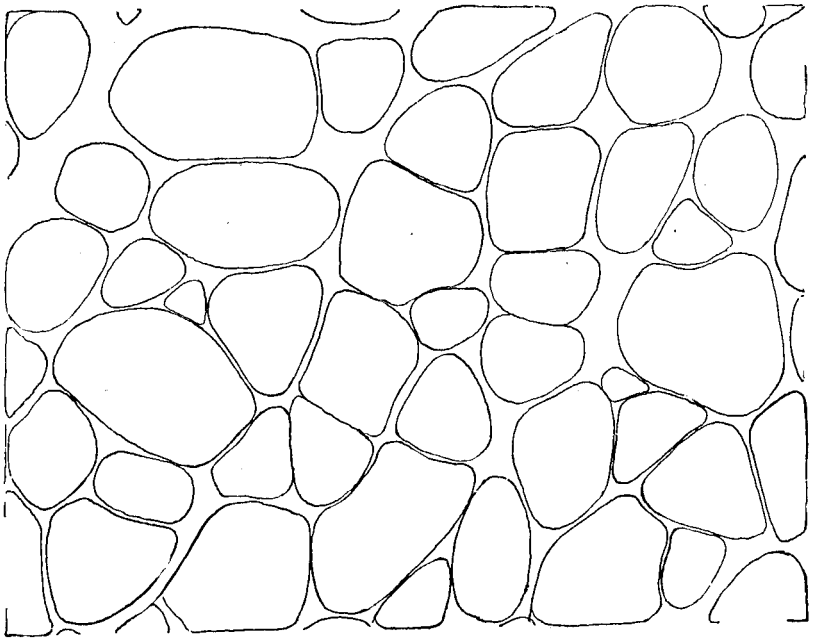

FIG. 18.

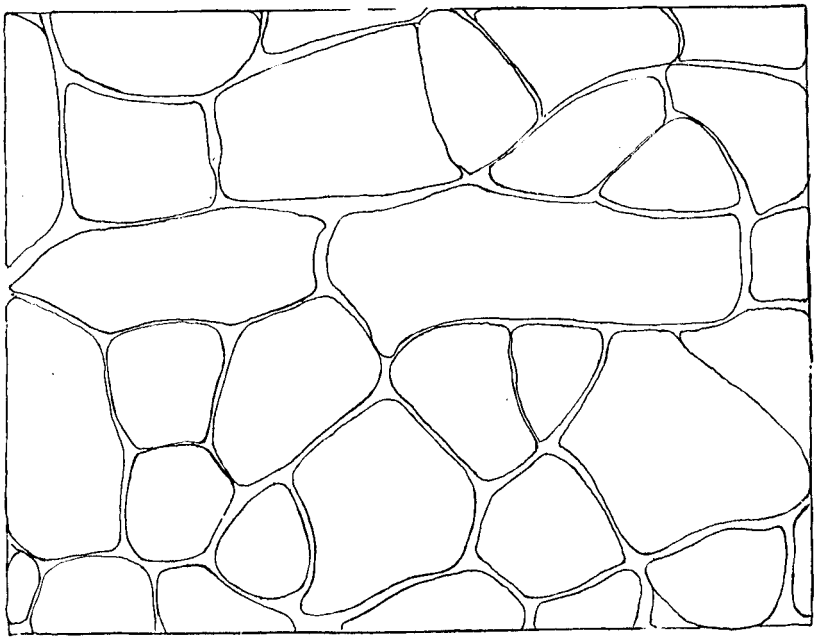

FIG. 19.

CORRELATIONS OF WEIGHT, LENGTH, AND OTHER BODY MEASUREMENTS IN THE WEAKFISH, CYNOSCION REGALIS

By William J. Crozier and Selig Hecht
College of the City of New York

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CORRELATIONS OF WEIGHT, LENGTH, AND OTHER BODY MEASUREMENTS IN THE WEAKFISH, CYNOSCION REGALIS.

By WILLIAM J. CROZIER and SELIG HECHT,
College of the City of New York.

INTRODUCTION.

During July and August, 1912, an opportunity was afforded at the United States Fisheries Laboratory, Beaufort, N. C., to make some studies on the correlation of external characters in the squeteague; the results are contained in the present paper.[a]

The weakfish, or squeteague (*Cynoscion regalis*) is common in Beaufort Harbor, and during July and August was taken almost every day from the pound net operated by the laboratory, in quantities up to 300. By far the greatest number of these fish were about 31 cm. long. The specimens used were therefore, to a certain degree, selected according to length, with a view to having a series covering as large a range as possible. Inasmuch as the squeteague is known to spawn in late spring, physiological disturbances due to spawning are negligible. All the fish examined (over 400) were either "spent" or unripe; so we are sure that none of the weights recorded are influenced by the ripening of the gonads.[b]

The material was brought from the pound in a live car and immediately removed to the laboratory. Measurements were made as rapidly as possible, the time for the complete measurement of a single fish rarely occupying more than five minutes. The possibility of shrinkage and of loss of weight through evaporation was carefully considered. To check this a number of fish were weighed and measured at 11 a. m., placed in a bucket, and covered (with a towel), our usual procedure, and four hours later no difference in measurements could be detected.

CORRELATION OF WEIGHT AND LENGTH.

For the determination of the relation of weight to length, 390 fish were examined. Of these 274 were females, 111 were males, and 5 were too immature for sex identification. By length is meant total length, from tip of mandible of the closed mouth to the extreme end of the caudal fin. This was taken by placing the fish on a board, its body perpendicular to and the tips of its tail just touching a raised end piece. The length was read by means of a centimeter scale along the line from the mandible to the base of this end piece. "Weight" means weight after the surface water and mucous have been removed with a towel, and is corrected for the weight of the stomach contents. The weighing was done on a platform balance sensitive to 0.1 gm.

[a] We wish to thank Dr. J. F. Abbott, of St. Louis, for his advice in the biometrical treatment of the data; we are also indebted to Dr. A. J. Goldfarb, of New York, for his suggestions during the course of the work.

[b] Paton, D. Noël (Report of the Investigations on the life history of the salmon in fresh water, Fisheries Board for Scotland, p. 1, 1898), for example, notes that in the European salmon, during April and May, the ovaries are 1.2 per cent and the testes 0.15 per cent of the total weight of the fish, whereas in November, near the spawning period, they represent 23.3 and 3.3 per cent of the total weight, respectively.

The results are shown graphically in figure 1, where length is abscissa, and weight ordinate. A large number of the points represent duplicates, triplicates, and even quadruplicates, and in many cases include both sexes. For example, the point (24.5,

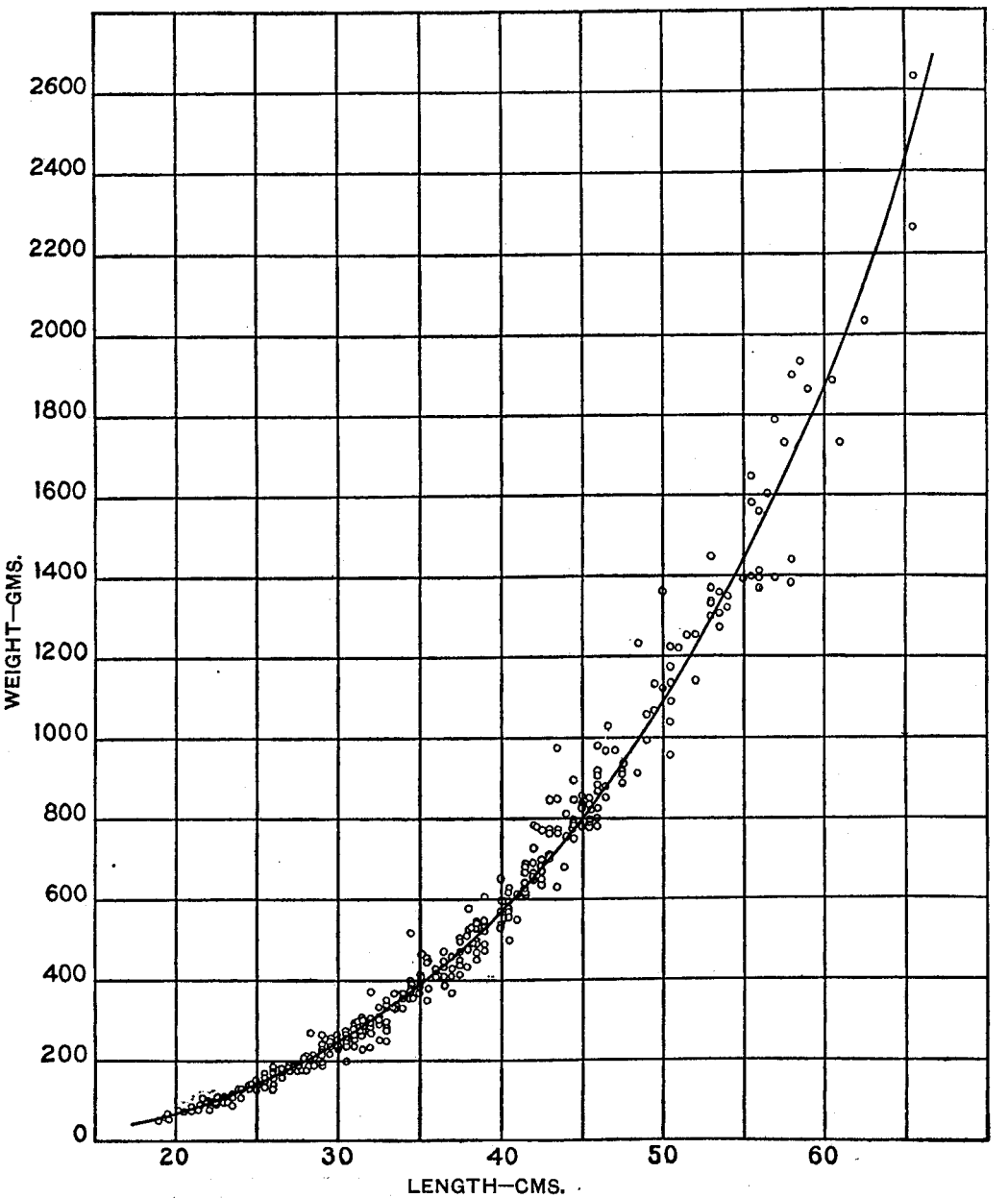

FIG. 1.—Showing relation of weight to length in 390 fish.

135) represents 2 females and 1 male; the point (41.5, 680) represents 2 males and 1 female; the point (27.0, 170) represents 2 males and 1 female; and the point (29.0, 220) represents 2 males and 2 females. From the distribution of the points about the smoothed

curve, it is clear that sex does not influence the relation between weight and length.[a] This does not mean that there is no difference in the weight and length of *Cynoscion regalis* of different sexes for the same age; it means that for a given length or weight of fish sex does not affect the correlation.

The regularity of the curve shown in figure 1 enables its mathematical equation to be calculated with considerable accuracy. Comparing the abscissas and ordinates of any two points on the curve, we find that the weight varies as the third power of the length. The equation therefore will be of the form $y = a\,x^3$, in which y represents weight, x length, and a is a constant, the value of which depends on the units used. When length

WEIGHT—GMS.

LENGTH—CMS.	0 to 150	150 to 300	300 to 450	450 to 600	600 to 750	750 to 900	900 to 1050	1050 to 1200	1200 to 1350	1350 to 1500	1500 to 1650	1650 to 1800	1800 to 1950	1950 to 2100	2100 to 2250	2250 to 2400	2400 to 2550	2550 to 2700	
15 to 20	4																		4
20 to 25	37																		37
25 to 30	11	77																	88
30 to 35		51	24	1															76
35 to 40			25	25	1														51
40 to 45				12	27	12	1												52
45 to 50						22	11	3	1										37
50 to 55							2	6	10	5									23
55 to 60										8	4	2	3						17
60 to 65												1	1	1					3
65 to 70																1		1	2
	52	128	49	38	28	34	14	9	11	13	4	3	4	1	0	1	0	1	390

FIG. 2.—Correlation table showing closeness of relation between weight and length of fish represented in figure 1.

is measured in centimeters, and weight in grams, a has the value 0.008771 ± 0.000117, and the equation becomes, weight $= (0.008771 \pm 0.000117)$ (length)^3.

From this it is apparent that the weight in grams of a specimen of *Cynoscion regalis* may be obtained by multiplying the cube of its length, in centimeters, by approximately 0.009.

Paton, Fulton, and other investigators[b] refer to the approximation with which the weight of fishes vary as the cube of their lengths, but they present no comparable evidence from which such conclusions can be derived with any degree of accuracy.

[a] Kellicott, William E. (The growth of the brain and viscera in the smooth dogfish (*Mustelus canis*), American Journal of Anatomy, vol. 8, p. 319, 1908), has shown that in the smooth dogfish, the sexes can not be distinguished with respect to either absolute or relative weights of internal parts, except the gonads.

[b] Fulton, T. Wemyss: On the rate of growth of fishes, 24th Annual Report of the Fishery Board for Scotland, 1905, Pt. III, and in other reports. Paton, D. Noel: loc. cit., p. 6. Williamson, Charles H.: On the herrings of the Clyde and other districts, 27th Annual Report of the Fishery Board for Scotland, 1908, Pt. III.

As a measure of the closeness of the relation between weight and length, we have determined [a] the coefficient of correlation r, which is the index of relation between two variables, such that the amount of variation in one is a measure of the amount of variation in the other. Using length as the *type* and weight as the *array*, the correlation table (fig. 2) was constructed. In each square is given the number of specimens which fall within the weight group and length group indicated. From this arrangement of the data the coefficient of correlation is found to be $r = 0.952$, with a probable error of $\pm$ 0.0032. Remembering that unity represents a theoretically perfect correlation, it is apparent that this coefficient indicates an extremely high correlated variability.

RELATION OF BODY MEASUREMENTS TO TOTAL LENGTH.

With a view to discovering the relation between the dimensions of the external parts of the fish and its total length, a series of measurements was taken on 123 of the 390 specimens used in the work discussed above. Of these, 80 were females and 43 males.

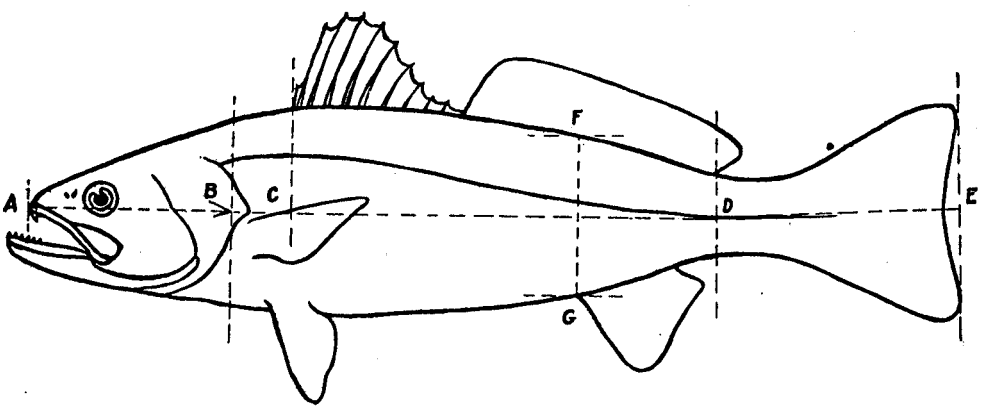

FIG. 3.—Showing parts of fish measured for comparisons plotted in figure 4.

Referring to the diagram, figure 3, the measurements, in addition to total length and weight, were:

1. Standard length, from tip of snout to end of last caudal vertebra.

2. Head length, AB, from tip of snout to end of opercular bone, i. e., excluding the opercular flap.

3. Body length, BD, from the end of the opercular bone to a point on the lateral line immediately below the posterior limit of the base of the soft dorsal fin.

4. Tail length, DE.

5. Body width, taken at the point C on the line AE, immediately below the origin of the spinous dorsal.

6. Depth, GF, from the origin of the anal fin, G, to F, on a line perpendicular to the long axis of the fish.

For the depth measurement, 73 specimens were examined; of these 49 were females and 24 males. [b]

[a] Davenport, Charles B.: Statistical methods, with special reference to biological variation, ch. 4, New York, 1904.

[b] As shown above, and also by the plots in figure 4, sex is a negligible factor in a discussion of this data.

The lengths were measured by means of a centimeter scale placed on the fish; width and depth were taken with the aid of spring calipers, using the same scale. For the measurement of width and depth it was necessary to secure points that would not be influenced by the amount of food material in the stomach. The abdomen of the squeteague is extremely elastic, and its volume varies considerably with the stomach contents. Significant measurements in this region of maximum depth are likewise impossible after the removal of the contents of the stomach. The places selected fulfilled the requirements suggested and were found to be sufficiently near the maxima for our purposes.

The curves shown in figure 4 were derived from the data obtained. For every specimen total length was plotted as abscissa and the other measurements detailed above as ordinates.[a] From the resulting straight lines it is at once apparent that there is a simple relation between the dimensions of the external parts of the fish and its total length. It is clear that with increasing length there is a constant, directly proportional increase in all the body measurements taken.

From the slopes of the lines the rates of growth of the corresponding parts relative to the growth of the total length may be calculated. Using the units shown on the plot, the "tangent" of any line is determined by dividing the vertical distance between two points on this line by the horizontal distance. These tangents are as follows:

Standard length	0.840
Body	.530
Tail	.273
Head	.215
Depth	.135
Width	.115

From this it is obvious that, of the body parts, the body has by far the greatest rate of growth, while the width has the least. It is also clear that the head and tail have approximately the same rates of growth, and that the depth and width also grow at about the same rate. It is, of course, to be understood that when the "rate of growth" is mentioned, we do not mean "rate" with regard to time, but relative growth per unit increase in total length. Thus, for every 10 cm. increase in total length the standard length will increase 8.40 cm., the body 5.30 cm., the tail 2.73 cm., the head 2.15 cm., the depth 1.3 cm., and the width 1.15 cm.

RELATION OF BODY MEASUREMENTS TO WEIGHT.

From the regularities shown in the previous section we may conclude that there exists a relation between any body measurement and weight similar to that which exists between total length and weight. Yet another relation, however, may be demonstrated. Since depth and width are each equal to a constant multiplied by the total length, we may substitute in the formula for the derivation of weight,[b] depth, and width divided by their respective "tangents," and thus secure a formula for the weight in terms of length, width, and depth. This formula is $W = k.\ l.\ w.\ d.$ By direct calculation from figures 4 and 1, $k = 0.5513 \pm 0.0088$, and the equation becomes weight = (0.5513 ± 0.0088) (length) (width) (depth).

[a] Here also many of the points represent duplicates and triplicates.

[b] See p. 143.

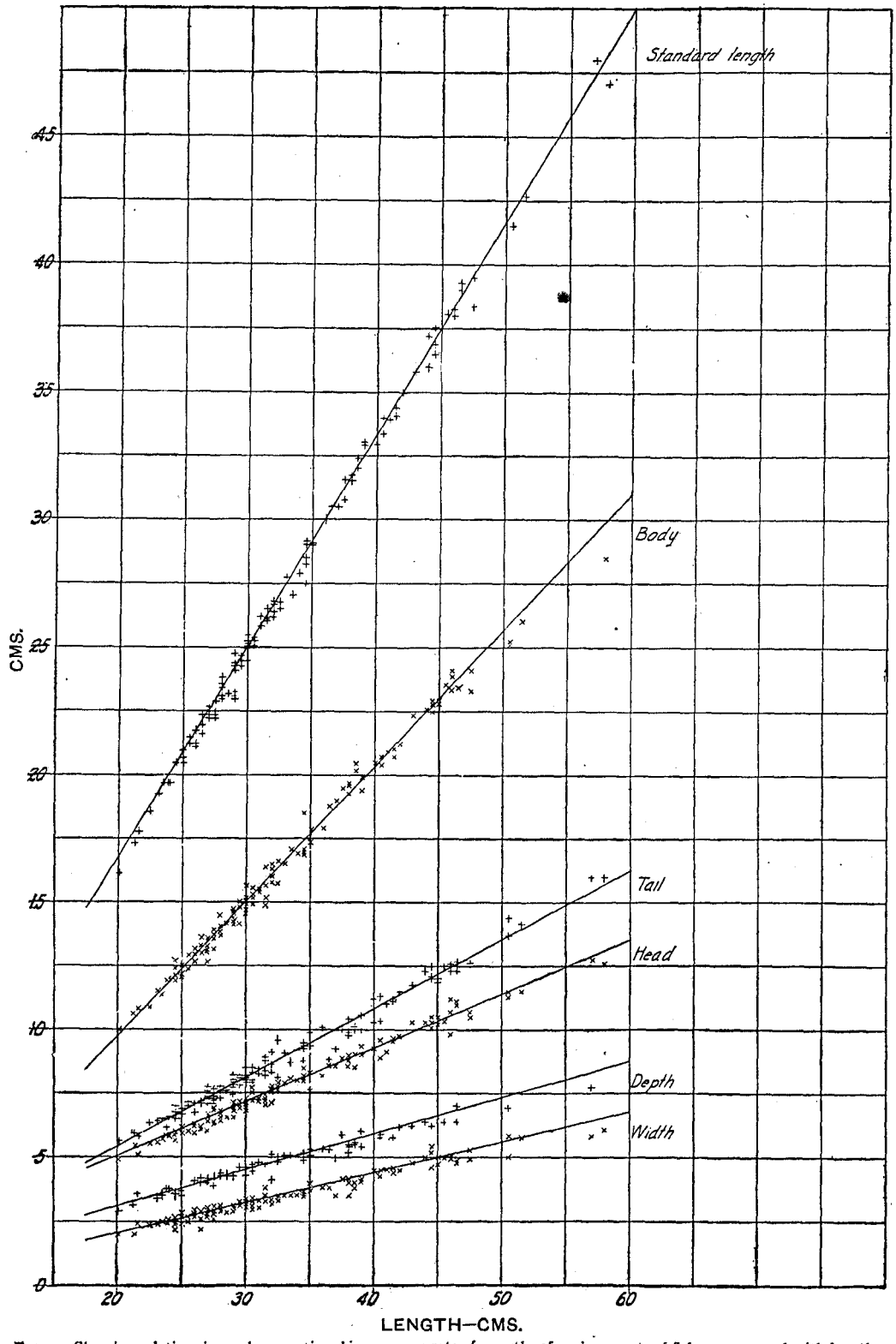

FIG. 4.—Showing relative size and proportional increase, or rate of growth, of various parts of fish as compared with length.

SUMMARY.

1. In squeteague of both sexes, between the length of 15 and 70 cm., the correlation of weight and length is extremely close, as expressed by the coefficient of correlation, $r = 0.952$.

2. Weight may be accurately expressed by the equation: Weight in gm. = (0.00877) × (length in cm.)^3.

3. Standard length, head length, body length, tail length, width, and depth, are directly proportional to total length. (See statements of tangent measurements, p. 145, and fig. 4.)

4. From the curves in figure 4 the growth of these parts relative to total length is readily calculated.

5. Weight, as a function of total length, width, and depth, is expressed by the equation: Weight = (0.5513) (length) (width) (depth).

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THE FAT-ABSORBING FUNCTION OF THE ALIMENTARY TRACT OF THE KING SALMON

By Charles W. Greene, Ph. D.

Department of Physiology and Pharmacology, Laboratory of Physiology
University of Missouri

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THE FAT-ABSORBING FUNCTION OF THE ALIMENTARY TRACT OF THE KING SALMON.

By CHARLES W. GREENE, Ph. D.,

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REVIEW OF THE LITERATURE.

The absorption of fats by the alimentary tract of man and animals has been a subject for discussion and investigation for many decades. Our present views concerning the topic have been arrived at almost exclusively by the study of the higher mammals. Few observations along this line, especially of an experimental nature, have been made on fishes. The setting of the problem which has led to the investigations here presented is found in our current views of fat absorption. These views have been concisely and admirably summarized by Wells.[a]

> In the intestines fat is split into a mixture of fat, fatty acid, and gylcerin; but as the fatty acid and glycerin are diffusible, while the fat is not, they are separated from the fat by absorption into the wall of the intestine. Hence an equilibrium is not reached in the intestine, so the splitting continues until practically all the fat has been decomposed and the products absorbed. When this mixture of fatty acid and glycerin first enters the epithelial cells lining the intestines there is no equilibrium, for there is no fat absorbed with them as such. Therefore the lipase, which Kastle and Lœvenhart showed was present in these cells, sets about to establish equilibrium by combining them. As a result we have in the cell a mixture of fat, fatty acid, and glycerin, which will attain equilibrium only when new additions of the two last substances cease to enter the cell. Now another factor also appears, for on the other side of the cell is the tissue fluid, containing relatively little fatty acid and glycerin. Into this the diffusible contents of the cell will tend to pass to establish an osmotic equilibrium, which is quite independent of the chemical equilibrium. This abstraction of part of the cell contents tends to again overthrow chemical equilibrium, there now being an excess of fat in the cell. Of course, the lipase will, under this condition, reverse its action and split the fat it has just built into fatty acid and glycerin. It is evident that these processes are all going on together, and that, as the composition of the contents of the intestines and of the blood vessels varies, the direction of the enzyme action will also vary. In the blood serum, and also in the lymphatic fluid, there is more lipase, which will unite part of the fatty acid and glycerin, and by removing them from the fluid about the cells favor osmotic diffusion from the intestinal epithelium, thus facilitating absorption.
>
> Quite similar must be the process that takes place in the tissue cells throughout the body. In the blood-serum bathing the cells is a mixture of fat and its constituents, probably nearly in equilibrium, since lipase accompanies them. If the diffusible substances enter a cell containing lipase, e. g., a liver cell, the process of building and splitting will be quite the same as in the intestinal epithelium. The only difference is that here the fatty acid may be removed from the cell by being utilized by oxidation or some other chemical transformation.

[a] Wells, H. G.: Chemical Pathology, p. 67, Philadelphia, 1907.

This point of view has been arrived at through certain classic researches which will be reviewed briefly and which for the present purpose may begin with the work of E. H. Weber.

In 1847 Weber[a] demonstrated that during fat absorption the superficial epithelium of the duodenal villi was filled with fat granules. He says that the cylindrical epithelial cells "swell up and contain chyle granules" and that "the cylindrical cells are no longer cylindrical or prismatic, but are round, and many become whitish-opaque while others are filled with transparent oily fluid." This he explains as due to the power of the cells to absorb the food materials. Weber showed that some of the parenchymal cells also became opaque, containing oil-like fluids. This seems to have been the first microscopic observation of the absorption of fats by the intestinal epithelium.

Kölliker[b] in 1857 made similar observations, showing that fat was absorbed in the stomach. He also used the histological method. Kölliker found highly refractive fat granules in the fresh tissue. On page 175 is the statement, "I have never failed to find fat in the stomach epithelium in the dog, cat, or mouse from the second day after birth on. The mass of fat was indeed very variable. The cells may contain only slight masses of fine granules or they may be gorged with fat in which not only the finer but also larger fat drops are present." It is significant that he found the fat only in the cylindrical cells, i. e., not in the pavement epithelium of the mouse's stomach. This definite work of Kölliker on the ability of the gastric mucosa to absorb fat has apparently been overlooked by physiologists until the last few years.

The question of the method by which fat is absorbed has been inseparably associated with the observations of the actual physical processes of absorption. Our current view, that fat must be dissociated before absorption, received its first experimental support, so far as the stomach is concerned, by the work of Marcet[c] in 1858. This author delivered a course of lectures in London, discussing in the last of the series the evidence of the digestion of fat in the stomach. The keynote to his work is explained in the following quotation: "The experiments were undertaken upon dogs, and repeated four times with the same result. The animals were made to take a meal, consisting of cooked meat and sheep's fat, and were killed from one to five hours later; the contents of the stomach being at once submitted to examination yielded in every case fatty acids."

This chemical work of Marcet gave an admirable support to the histological observations of Kölliker, showing that the gastric mucosa is capable of producing fat-splitting ferments, or as we now call them, lipolytic enzymes. In the light of our present knowledge, these two pieces of work, that of Kölliker and of Marcet, should have established the fact of the fat digestion and absorption in the stomach. But no further reference along this line seems to be available from the literature until the work of Cash[d] in 1880, who, without reference to the previous work of Marcet, again investigated the fat-splitting properties of the gastric juice and of the extracts of the gastric mucosa.

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[a] Weber, E. H.: Ueber den Mechanismus der Einsaugung des Speisesaftes beim Menschen and bei einigen Thieren. Müller. Archiv für Anatomie und Physiologie, bd. 6, 1847, p. 400–402.

[b] Kölliker, A. von: Einige Bemerkungen ueber die Resorption des Fettes im Darme, ueber das Vorkommen einer physiologischen Fettleber bei jungen Saugethieren und ueber die Function der Milz. Verhandlungen der physikalisch-Medicinischen Gesellsch. Bd. 7, 1857, p. 174–193.

[c] Marcet: A course of lectures on the chemistry, physiology, and pathology of human excrements. Lecture v. The Medical Times and Gazette, vol. 17, 1858, p. 209.

[d] Cash, Th.: Ueber den Antheil des Magens und Pankreas an der Verdauung des Fettes. Archiv für Anatomie und Physiologie (Phys. Abth.) 1880, p. 323–333.

In 1890 Krehl [a] made a restudy of the question of fat absorption from the intestinal tract. His drawings showing different stages in the microscopic loading of the epithelial cells with fat granules have become classic in the literature. The most significant fact on which Krehl lays emphasis is "the fat is not taken up from the intestine in globular form, but is absorbed in solution, and is resynthesized" giving rise to the droplets observed in the pyloric epithelial cells which the author presents in his figures. The conclusion that fat is absorbed in the dissolved state was later advocated by Pflüger (1900), after which it received general acceptance.

In 1901 Schilling [b] again observed fat in the gastric epithelium of the calf, in this case after a meal of milk. Schilling noted that the epithelial cells were thickly studded with microscopic fat droplets and that fat deposits appeared in the connective tissue of the tunica propria and parenchyma. He also noted fat in the lymphatic glands during absorption. He apparently did not investigate the presence of fat in the lymphatic radicles from the stomach.

In 1908 Van Herwerden [c] published the results of extensive and valuable studies on the gastric digestion in fishes. This subject he investigated under two heads, the second of which, namely, "Enzymes in the gastric mucosa," concerns us here. Van Herwerden made his observation chiefly on sharks, but also on bony fishes. These fishes he fed with olive oil or egg-yolk emulsion, the food being introduced into the stomach by way of the mouth. Having previously determined that fasting animals were relatively free of fat granules, he states that upon killing animals after a certain number of hours following feeding, "one finds fat drops in great numbers in the superficial epithelium." He states further that "the fishes contained fat granules everywhere in the submucosa between the musculature and especially in the lymph vessels which accompany the blood vessels. In hungering fishes I have never found this to be the case."

Van Herwerden also tested the activity of glycerin extracts of the gastric mucosa. He found in *Scyllium* a decided increase in the formation of fatty acids; also, in teleosts, his tables show the presence of an active lipolytic enzyme. Extracts previously boiled gave always negative results, as did also extracts from the muscle walls of the alimentary canal.

These interesting observations of Van Herwerden seem to be the first that have been made along this line upon the fishes. This splendid article had escaped my search in the literature until after the publication of the preliminary report of the present work.

Three previous communications have been made with reference to the present work; the first relating briefly the observations on fat absorption from the pyloric cœca of the king salmon,[d] and the last two, one a preliminary [e] and the other a brief statement of the facts of fat absorption from the stomach of the king salmon.[f]

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[a] Krehl, Ludolf: Ein Beitrag zur Fettresorption. Archiv für Anatomie und Physiologie (Anat. Abth.) 1890, p. 97.

[b] Schilling, F.: Die Fettresorption im Magen. Fortschritte der Medicin, bd. 19, 1901, p. 613.

[c] Van Herwerden, M.: Zur Magenverdauung der Fische. Zeitschrift für Physiologische Chemie, bd. 56, 1908, p. 453–494.

[d] Greene, Charles W.: The absorption of fats by the alimentary tract with special reference to the function of the pyloric cœca in the king salmon, *Oncorhynchus tschawytscha*. Read before the St. Louis meeting of the American Fisheries Society, 1912. Transactions American Fisheries Society, 1912, p. 261.

[e] Greene, Charles W.: The absorption of fat by the salmon stomach. Preliminary notice. Proceedings American Physiological Society, American Journal of Physiology, vol. 29, 1912, no. 4, p. xxxvi.

[f] Greene, Charles W.: Absorption of fat by the salmon stomach. American Journal of Physiology, vol. 30, p. 278, 1912.

During the progress of this work a preliminary notice and final paper have been published by Greene and Skaer[a] reinvestigating the fat absorption from the stomach in mammals; also a paper by Weiss[b] briefly presenting the fact of fat absorption by the gastric mucosa in the snake and in mammals.

EXPERIMENTS DEMONSTRATING THE ABSORPTION OF FATS.

METHOD.

The method of determining the character and degree of fat absorption from different portions of the alimentary tract of the king salmon has been that of microscopic examination. Tissues were examined fresh and after formalin fixation followed by the newer fat stains, Sudan III, scarlet red, etc. The chief reliance for staining the fat in the cells has been on the alkaline scarlet red. These methods of observation have been confirmed by more careful tissue fixation in Flemming's osmic acid mixture and by the corrosive bichromate method of Bensley. Flemming's solution not only fixes the tissues but gives the characteristic osmic acid staining of the fats. The Bensley fixation, when followed up by paraffin sections and differential staining, gives a negative picture, since the fats are dissolved out by the clearing fluids, leaving only fat vacuoles.

The detail of procedure for staining with scarlet red is as follows: The perfectly fresh material, living tissue if possible, was dropped into a 10 per cent formalin for two hours or more. Precautions were taken to insure penetration and proper fixation. The material fixed in formalin was then frozen in a freezing microtome and cut as thin as possible. The frozen sections were cut directly into 70 per cent alcohol, and stained in alcoholic solutions of scarlet red. The stain was made by heating an excess of scarlet red in 70 per cent alcohol containing 2 per cent sodium hydroxide to a temperature of about 80° C. This procedure, which is recommended by Bell,[c] gives a stain which on cooling leaves a saturated solution of greater staining powers than the ordinary alcoholic scarlet red. The stain was always filtered into shallow dishes just before using. Shallow oval bottom salt cellars were used, and these immediately covered to prevent evaporation. The sections were lifted from the 70 per cent alcohol, the excess of fluid quickly removed, and then they were dropped into the stain. Staining is comparatively rapid and requires only from 5 to 15 minutes for a successful impregnation.

Sections were taken from the stain, the excess of adherent stain being removed by a momentary immersion in 70 per cent alcohol, and then were immediately plunged into a large dish of water. When the particular tissues were delicate, an intermediate grade of 35 per cent alcohol was used. In this case the sections must be in contact with the alcohol only long enough to remove the adherent stain, otherwise the stain in the tissue itself will be drawn. As a matter of routine practice it was found desirable to add to the wash water bath a couple of drops of hydrochloric acid. The faint acidity was found favorable to the more rapid removal of the traces of alkali. This step contributes decidedly to the keeping powers and clearness of the sections after they are mounted

[a] Greene, Charles W., and Skaer, William F.: Absorption of fat by the mammalian stomach, Proceedings American Physiological Society, American Journal of Physiology, vol. 29, no. 4, 1912, p. xxxvii. Evidences of fat absorption by the mucosa of the mammalian stomach, American Journal of Physiology, vol. 32, 1913, p. 358.

[b] Weiss, Otto: Die Resorption des Fettes im Magen. Pflüger's Archiv für die gesamte Physiologie, bd. 144, 1912, p. 540–543.

[c] Bell, E. T.: The staining of fats in epithelium and muscle fibers. Anatomical Record, vol. 4, 1910, p. 199–212.

in glycerin. Pure glycerin was used to make the mounts. Sealing with a mixture of paraffin and beeswax around the cover glass was the final step in the mounting and preservation of the sections.

The more permanent sections of the tissues fixed as described were made by the paraffin method, in which no special features in technique were introduced.

The previous fixation in formalin was found to be decidedly advantageous in the preparation of frozen sections. The brief time of immersion in the formalin does not introduce a change in the character and distribution of the fats. On the other hand the tissues are coagulated, hence firmer, and can be carried through the technique with a much more satisfactory result. When the frozen sections were made directly from fresh living tissues, then at the moment the frozen section was immersed in the alcohol preliminary to the scarlet red staining, considerable contraction and sometimes tearing took place. It was found that the distortion of the sections by this step was detrimental to the securing of normal pictures of the structure and relations of the contained fat.

SELECTION OF SPECIMENS.

Two types of fish were used for the determination of the points detailed in this report. First, salmon of various sizes and presumably of different ages collected from the markets in the city of Monterey. The fish selected were those delivered directly from the fishing boats, which had made their catch by trawling on the ocean fishing banks in the vicinity. These fish came into the market with living tissues, a fact that could easily be determined. The alimentary tracts were taken from the salmon at the slaughtering tables of the fish-packing establishments of the Booth Packing Co.[a] If the tissues were proved to be alive in material chosen then histological samples were selected and placed in fixative immediately, so that the question of prefixation changes does not enter into consideration.

The second class of material is that derived from young salmon collected from two stations. The first collecting ground was that of the Brookdale hatchery maintained in the town of that name on the San Lorenzo River in the Santa Cruz Mountains. Young salmon were also obtained from the McCloud River in the Shasta Mountains in northern California. Both these groups of young salmon had never been in salt water. The ages of the young salmon varied from one to two years, the latter being those obtained at the ponds from Brookdale.

NORMAL-FEEDING SALMON.

The class of adult salmon mentioned above, which were secured at Monterey, were in an active aggressive stage of ocean feeding. These salmon come into the markets often with the stomach and intestinal tract gorged with food. The natural food is of a varied class, but at Monterey consists mainly of three kinds: First, the squid; second, the local species of herring; and, third, marine Crustacea, chiefly a rather large amphipod.[b] The king salmon is a voracious feeder and his ability to capture a great variety of food

[a] For the courtesy extended by this company I am indebted to Mr. Frank E. Booth, the president.

[b] In July, 1911, quite a number of salmon were noted with large numbers of these Crustacea in their stomachs. One salmon stomach in particular contained 4 or 5 (estimated) ounces of such food. It would have been interesting to have counted the actual number of crustaceans present, but the content of the stomach was partly lost before the thought occurred to make such an enumeration.

material besides the forms mentioned above is shown by the various species of fishes occasionally noted in the food at Monterey. These natural foods are all relatively oily, the point which particularly concerns us here. As digestion proceeds and the protein framework is dissolved away these oils are liberated in the alimentary canal and form no inconsiderable portion of the food of the king salmon. When one remembers the characteristics of the salmon flesh, charged with oil as it is, and evidently storing great quantities of oil, the interest which attaches to the question of the source of the oil in the food and the method of digesting and absorbing oils is obvious.

As a matter of fact it was in the course of a study of the character and microscopic distribution of the fats in the salmon tissue that I instituted observations on the alimentary tract of the king salmon which made it obvious that large quantities of oils were absorbed from the foods in these normal feeding salmon.

FAT-FED SALMON.

The inability to control the relation between the time of taking food and the chance of securing the fish and making observations of the stage of absorption in the normal feeding salmon renders it extremely difficult to settle the question of the characteristics of fat absorption in such. As a matter of fact, my observations made it very clear that much absorption of fat was taking place in salmon feeding under natural conditions, yet it was found next to impossible to determine the nature and details of the process from the specimens available. For this reason the idea of feeding salmon in the aquaria was conceived and its immediate execution was made possible through the courtesy of the directors and superintendent of the Brookdale hatchery. Young salmon were transported in live cans from Brookdale to the Hopkins' Seaside Laboratory at Pacific Grove, Cal. Two sizes of salmon were available, one group of yearlings from 6 to 7 centimeters long, and a group of small 2-year-olds from 14 to 16 centimeters long.

These young salmon were fed olive oil by rectal injection. This was found to be an extremely reliable and easy way of introducing the oil into the alimentary tract in such a way as to give one confidence in the accuracy of the results. A medicine dropper was drawn out in the flame to a slight cone of proper size. A desirable quantity of oil was then taken into the dropper, the tip inserted into the anal aperture and gentle pressure maintained until the oil was emptied into the alimentary tract. It is comparatively easy to hold the young salmon by a firm grip of a lobe of the caudal fin rays, the fish resting in the palm of the hand in such a way that the head and gills remain under water to prevent asphyxiation. Under these conditions the fish does not struggle as much as might be expected. The slight contractions of the muscles of the anal sphincter occurring when the pipette is first introduced soon relax, but one has always to maintain a gentle pressure on the pipette for a moment before oil begins to flow into the tract. The alimentary canal of the salmon is a simple S-shaped tube, as has been described and figured in a previous paper.[a] When the oil is injected into the posterior end of the canal in sufficient quantity it flows into the different limbs of the intestine and into the stomach, and from the stomach will be discharged from the esophagus into the mouth if an excess of oil is used. In my later experiments this fact was adopted as an index of when the proper quantity of oil was administered.

[a] Greene, Charles W.: The anatomy and histology of the alimentary tract of the king salmon, *Oncorhynchus tschawytscha*. Bulletin, Bureau of Fisheries, vol. XXXII, 1912, p. 73–100, pl. XXV–XXVIII.

A series of artificial feeding experiments was executed at the Hopkins' Seaside Laboratory, followed by a more extensive series at the Federal salmon hatchery at Baird, on the McCloud River in northern California. In this later series the question of absorption in relation to the time following the administration of oil was especially investigated. Furthermore, in the Baird series it was possible to maintain the young fish without food an adequate time to insure the complete elimination of the fat from the alimentary canal which might previously have been derived from natural foods.

The Monterey series consisted of two salmon of the 2-year-old group with confirmations on two salmon of the small 1-year-old group, with different time allotments for absorption, ranging from 20 to 70 hours. Careful examinations were made extending over the stomach, intestine, and various pyloric cœca of each of these series.

GENERAL RELATIONS OF THE ORGANS OF ABSORPTION.

The critical regions for the study of the absorption of fat in the salmon are three, namely, the stomach with its two divisions, the cardiac and the pyloric ends; the intestine with its two great divisions, the pyloric and post pyloric; and the numerous pyloric cœca which have their origin from the pyloric intestine.

These great divisions are of necessity to be described separately. Logically, one might take them in the order, stomach, intestine, cœca; but because of the way in which the evidence was accumulated and other questions attached to the subject it is more convenient to discuss the details in the reverse order, i. e., absorption in the pyloric cœca, in the intestine, and in the stomach.

ABSORPTION OF FATS BY THE PYLORIC CŒCA.

The gross anatomy and the normal histological structure of the alimentary tract of the king salmon have both been presented in a previous paper.[a] Figure 1 of that paper is an illustration showing the general relations of the cœca to the pyloric end of the intestine from which they arise in such profuse numbers. Those cœca which originate from the beginning of the intestine, that is, in the neighborhood of the pyloric valve, are much longer than those that arise from the posterior end of the series. These cœca often reach a length of from 10 to 15 centimeters and even more in the adult feeding salmon. They have a normal diameter of 5 to 8 millimeters. In the sea salmon taken at a time when food is abundant and digestion has been going on actively for some time the cœca are always gorged with material and distended to their full length and diameter.

The content of the pyloric cœca under these conditions is peculiar in appearance. One never finds solid particles of food. Instead, there is only, as Gulland and others have mentioned, a creamy, yellowish, puslike mass which has a viscid adhesive consistency. This content is never very fluid, i. e., of limpid character. The exact color of the contents varies with the class of food material which the salmon is digesting at the time. If the food is made up of Crustacea then the content of the cœca has a darker color, often of a deep orange red. It is apparent that the viscidity of the mass is due to the secretion of mucous by the epithelial lining of the cœca themselves.

In the younger salmon the pyloric cœca have the same relative size, but of course are smaller in proportion to the gross size of the fish. In no instance have I observed

[a] Greene, Charles W., op. cit.

any extensive mucous content of these young cœca. In the specimens that were fed fat there was an occasional increase in the transparency, which was interpreted as due to the presence of oil. In the intestine of such fish the excess of oil was easily and often shown.

EVIDENCES FROM SALMON FEEDING NORMALLY.

Fat droplets were always observed in the epithelial lining cells of the pyloric cœca of the Monterey salmon. However, the fat was not present in all cells. Certain portions of the epithelium were filled with fat droplets, while other portions were relatively free. In almost every animal observed, and in different regions of the same animal, certain extended portions of the epithelium were observed to contain no fat droplets, while in the neighboring regions, often in the same section or perhaps in the next mucous fold, fat would be present. These facts could not readily be explained by the assumption that fat was loaded into these cells by way of storage, being brought in from other portions of the body. On the other hand, such observations strongly suggest a process of fat absorption. Previous observations on fat absorption in fishes are apparently very limited; at any rate the search in the literature has thus far revealed to the writer only the observations of Van Herwerden[a] "On Gastric Digestion in Fishes." This splendid paper deals largely with digestion and the digestive enzymes. But it definitely demonstrates fat absorption in *Scyllium*. It follows that the chief guide in the interpretation of the present results is that to be found in the comparative literature on fat absorption in other animals, a portion of which has been referred to and reviewed in a previous chapter.

The mucous epithelium of the salmon cœca is very extensive, considered in proportion to the size of the tubes. The measurements of the superficial extent of the mucous coat show that it is from 6 to 8 times the extent of the external surface of the cœcum itself. These folds are very complex in arrangement, though the epithelial coat itself is of uniform and simple type, a matter that is discussed in the paper presenting the normal structure of these organs. It is this complex folding, and therefore the relative variation in the contact of the epithelium to portions of the contained food mass, that explains the fact of unequal loading of fat in the epithelial cells. Hence there is no doubt that the fat observed was absorption fat.

HISTOLOGICAL APPEARANCE OF FAT IN THE EPITHELIAL CELLS.

A cœcum containing fatty food material in an advanced stage of digestion and absorption will almost always present epithelial cells in all the stages of fat loading. The appearance of the cells loaded with fat is characteristic and changes progressively as absorption proceeds. In a general way, though some allowance must be made for the comparison, the histological character of the cells would suggest three stages.

Fat absorption, stage 1.—The earliest stage of absorption is that of the passage of fat into and through the superficial border of the epithelial cells. The methods of staining, whether they be direct staining of the tissues with scarlet red or fixation and staining of the fat by the osmic acid mixtures, show a large number of very fine granules in the most superficial layer of the protoplasm of the cell. These fat granules are extremely

[a] Van Herwerden, M., op. cit.

small, the largest being less than 1 μ in diameter. In some instances they appear in such minute size that they are only just distinguishable under the oil immersion. As absorption proceeds the fat granules make their appearance deeper and deeper in the cell, loading up the zone between the free surface and the nucleus. Here the fat droplets are relatively large, ofttimes being 4.5 to 6 μ in diameter. In the intermediate area and between the superficial zone and the extra-nuclear zone are all sizes of fat droplets from the extremely minute ones just described to the large ones in the extra-nuclear zone. This picture is shown very clearly in figures 6 and 12.

Fat absorption, stage 2.—The second characteristic cellular appearance, which is designated as stage 2, consists in the filling of the inner or basal end of the cell with fat droplets. Not only that portion external to the nucleus will be loaded with fat, but the portion between the nucleus and base of the cell will also contain an excess of fat droplets.

The sizes of the droplets in the end of the cell are similar to those just external to the nucleus, but the number of droplets is rarely so great. When the cell is fully loaded it generally happens that fat will be found in the connective tissue of the tunica propria beneath. If fat absorption is continuous at this stage, as one might legitimately assume from the histological appearance, it is obvious that as the fat is entering the outer zone it will at the same time be discharging from the inner zone and passing into the channels which distribute fat through the body. Knoll [a] has recently reported experiments on fat absorption in the mammalia in which this condition is shown to hold.

Fat absorption, stage 3.—When absorption from the lumen of the cœcum ceases, the outer margin of the cell begins to clear of fat. This disappearance of fat apparently slowly and gradually extends over the whole area of the cell external to the nucleus. In favorable material in this stage epithelial areas will be found in which the outer or extra-nuclear zone of the mucous epithelium is almost, sometimes entirely, free of fat droplets. Still, fat droplets will be present in considerable quantity in the inner or basal zone. As a rule the basal portion of the cell will contain relatively large droplets in this stage and the connective tissue supporting the cell will be similarly loaded with fat droplets. However, some groups of cells are found in which the fat droplets in the basal portion of the cell are extremely minute, as shown in figure 13. In this particular figure the basal areas are heavily loaded with fat in extremely fine subdivision. The adjacent connective tissue of the tunica propria contains a similar distribution and size of fat droplets.

These three stages of course are only phases of an orderly and progressive process in which the fat enters the outer zone of the cell, is disposed within the substance of the cell in droplets, and is ultimately distributed from the cell to the basal pole, the opposite from which it entered. The variations in the size of the droplets in different zones of the epithelial cells, especially the extremely small droplets in the outer portion of the cells and the fine droplets in the bases of the cells at the time the discharging is almost complete, are very interesting when considered in relation to the theories of fat absorption. But the discussion of these theoretical points will be taken up again in a later section of the paper.

[a] Knoll, A.: Chemische und mikrosopische Untersuchungen über den Fetttransport durch die Darmwand bei der Resorption. Pflüger's Archiv, bd. 136, 1910, p. 208–247.

EVIDENCES IN ARTIFICIALLY FED SALMON.

A brief report on these experiments has been presented.[a] The first series of experiments carried on by the method of fat feeding described on the preceding page contains two young salmon, one 14 and the other 16 centimeters long.

Figures 6 and 7 present the histological picture of the amount of fat in the superficial epithelium of frozen sections of the cœca from salmon 45. Absorption proved to be extremely rapid and vigorous in this young salmon, not only in the cœca, but in the intestine, as will appear later. The epithelial cells, especially of those mucous folds which extended out into the lumen, were simply gorged with fat. The fat droplets were extremely large and filled not only the superficial portion of the cells but the basal portion as well. If the adjacent membranes of a deep fold were in contact with each other, thus preventing a free contact with the fat of the cœcal content, such places would show a relatively small amount of absorption fat in the cells. On the free loops of the mucous folds this situation did not exist, hence these portions were gorged with fat in all the sections examined. This fact is shown especially well in the high magnification of figure 11. In some portions of the tissue in the neighborhood of the areas drawn in this figure the fat was present in so great a quantity as to burst the cell membrane. It was believed at the time of the preparation that the fat absorption continued until the quantity within the cells produced a pressure greater than the cell surface could stand, hence the break, though one can not exclude the possibility of mechanical pressure during manipulation. Drops often reach a diameter of from 8 to 10 μ, or even more in the young, which is greater than the normal diameter of the epithelial cell, even at its largest end.

In figure 6, showing the fat in salmon No. 45 stained with osmic acid, a number of cells are shown in which the fat droplets of the outer portion of the cell are large enough to take up the entire diameter of the cell. In different regions of this particular histological preparation other than shown in the figure there are numerous confirmations of the above statement.

Both the positive staining and fixation of fat by osmic acid and the arrangement of fat vacuoles in corrosive fixed and paraffin sectioned material give confirmation of the direct observations of the fresh material stained with scarlet red. *The series of studies show that fat absorption takes place abundantly in the pyloric cœca.* Whatever else these organs accomplish, it is perfectly clear that the absorption of fat is one of their chief functions.

FAT IN THE TUNICA PROPRIA OF FAT-FED SALMON.

One of the most interesting confirmatory lines of observation which is largely cleared up by the fat-feeding experiments is the fact of the presence of fat in the tunica propria. In fasting salmon, especially in those used for control in the Baird series, practically no fat is present in the tunica propria. One must be guarded in such statements because this tissue holds on to its fat with great persistence. Fat will persist in the tunica propria when one can demonstrate absolutely no fat in the epithelial cells. But when absorption begins, as judged by the amount of fat in the epithelial cells, then

[a] Greene, Charles W.: The absorption of fats by the alimentary tract, with special reference to the function of the pyloric cœca in the king salmon, *Oncorhynchus tschawytscha*. Transactions of the American Fisheries Society, 1911, p. 261.

fat begins to appear in the tunica propria in an increased quantity. After 18 hours or more (see figs. 6, 7, and 8), the connective tissue layer supporting the epithelial cells becomes extremely full of fat droplets. The fat appears first in the vicinity of the bases of the epithelial cells, then is distributed through the substance of the tunica propria. The stratum compactum always forms a definite and striking boundary to the fat containing tissue of the tunica propria. This is shown especially well in the figures, particularly figure 6.

The tunica propria acts as a sort of reservoir for the fat immediately following periods of active absorption. The matter has not been sufficiently studied yet, but it seems obvious that the tissue building up this stratum of the cœcal wall seizes and holds fat with unexpected persistence. Fat will be found here in a relatively considerable number of droplets at a time when the epithelial cells are completely discharged.

The stratum compactum, as described in the discussion of the normal structure, forms a continuous sheath around the tunica propria. It is a continuous membrane with no discernible openings other than at the points where blood vessels enter. Any fat passing through the stratum compactum would have to pass through in solution or else be carried within in the lumen of the blood vessel. In either case no definite fat globules as such get by this membrane from the tunica propria.

PROTOCOLS.

BROOKDALE SALMON, FIELD SERIES NO. 45, LENGTH 14 CENTIMETERS, TAKEN JULY 6, 1911.

This young salmon was a 2-year-old reared by the Brookdale hatchery, California. It was taken from an aquarium and transported to the Hopkins Seaside Laboratory, Pacific Grove, Cal. This salmon was fed fat. In this instance it received first a fat emulsion consisting of 20 per cent olive oil in coagulated milk injected into the stomach through the mouth. This feeding did not seem to be very successful and was followed later by an injection of olive oil into the rectum. This method of feeding proved to be very successful, convenient, and satisfactory. The salmon was killed after allowing 18 hours for absorption of the olive oil (22 hours, counting time from the first attempt to feed by way of the mouth). Frozen sections were made of the fresh tissues, and certain portions of the tissues were fixed by different histological methods for later examination.

Fat in the pyloric cæca.—The epithelial cells are simply gorged with fat droplets. Especially are the outer ends of the cells so filled that the cell boundaries are obscured. The diameter of the droplets varies widely. The larger drops distort the cells. The basal ends of the cells contain a much smaller amount of fat.

The tunica propria is also well filled with fat, but not so great an amount as in fish 46. The drops are more uniform in size. This fat extends deep into the folds of the stratum compactum, but is never present in its substance.

Fat in the intestine.—The epithelial cells are as much crowded with fat as in the cœca, as shown in figures 2 and 4. The deeper folds of the intestinal mucosa are not always filled with fat, at any rate the amount of fat is not nearly so great as in the outer folds.

There is less fat in the intestinal tunica propria than in the cœca of the same animal.

BROOKDALE SALMON, FIELD SERIES NO. 46, LENGTH 16 CENTIMETERS, TAKEN JULY 6, 1911.

This young salmon was a mate to no. 45 and was transported at the same time. It was fed fat by rectal injection only, and was killed after 42 hours of absorption. Frozen sections were prepared and tissues were also fixed for permanent histological mounts.

Fat in the pyloric cæca.—The amount of fat in the epithelium of the pyloric cœca varied in different preparations. Those cells on the tips of the folds were crowded with fat, while those in the grooves between folds were relatively free. Figure 8 and figure 11 are from this specimen.

The tunica propria, as shown in figure 8, was more crowded with fat than in no. 45.

Fat in the stomach.—Sections of the gastric division of the stomach showed the outer ends of the epithelial cells medium full of fine fat droplets, shown in figure 1. These droplets are in the extreme outer ends of the cells just within the striated border. They are strikingly smaller than the droplets present in the intestinal and cœcal epithelium of the same specimen. A sprinkling of fat droplets is present in the inner limbs of the gastric epithelial cells.

McCLOUD RIVER SALMON, FIELD SERIES NO. 88, FEMALE, LENGTH 84 MILLIMETERS, TAKEN JULY 23, 1911.

This young salmon was seined from the McCloud River and was 1 year old as verified by scales. It was fed fat by the method of rectal injection and killed after 20 hours.

Fat in the pyloric cæca.—Frozen sections were made of the pyloric cœca and these stained with scarlet red and counterstained with hæmatoxylin. Absorption fat was present in moderate quantity, see figure 9. The greater portion of the fat is limited to the outer ends of the cells, but a few droplets were present in the inner ends of the cells and a small amount in the tunica propria.

Fat in the stomach.—This specimen showed an unusual amount of absorption in the gastric epithelium. Particularly was the pyloric epithelium loaded with fat. (See fig. 1.) Many of the deep folds of the pyloric epithelium were practically free of fat, but those cells dipping deepest into the cavity of the stomach were unexpectedly filled.

Two sections of cardiac stomach were fat-stained only. The slender cylindrical epithelial cells of the mucous ridges bordering on the lumen of the stomach and those cells extending down into the crypts of this somewhat contracted stomach all show numbers of droplets. The fat is greatest in amount in the cells of the free folds. The fat is finely divided in appearance; that is, in minute droplets. It is greatest in quantity in the outer thirds of the cells. There is a transparent superficial border of the epithelial coat in which the fat is in the form of finest liposomes, requiring the oil immersion lens for resolution. (See fig. 1 of osmic acid staining of no. 46, Greene, American Journal of Physiology, vol. 30, p. 280.) There is also fat in the inner limbs of the cells down to their bases, and this is more or less continuous with small amounts of fat in the tunica propria.

The gland cells of the secreting portion of the stomach in this section are granular in appearance and slightly pink with scarlet red. In several regions very small fat droplets, the size of which varies around 0.5 μ, are found in the basal portion of many of the gland cells. In connection with the large majority of the gland tubes in this slide there are cell areas over the surface of the tubes which seem quite thickly studded with finest fat droplets. This section is not counterstained, so it is difficult to determine to exactly what tissue these cells belong. In some instances they undoubtedly belong to the connective tissue of the tunica propria surrounding the gland tubes.

Several of the submucous areas just within the circular muscle and through which blood vessels run are finely punctate (oil immersion lens) with liposomes. Vascular areas in the longitudinal muscle coat are also stippled with liposomes, the droplets being located in the endothelial cells and in the walls of the blood vessels.

McCLOUD RIVER SALMON, FIELD SERIES NO. 91, MALE, LENGTH 83 MILLIMETERS, TAKEN JULY 25, 1911.

A young salmon seined from the McCloud River, fed olive oil by the method of rectal injection and killed after 70 hours.

Fat in the pyloric cæca.—Transverse sections of the pyloric cœca were made by the freezing method and stained for fat. Figure 10 shows a characteristic section from this fish. The amount of fat in the outer portion of the epithelial tissue is unusually great, though a very small amount had penetrated the inner limbs of the epithelial cells, and practically none to the tunica propria. The length of time that had been allowed for absorption would justify the expectation that the tunica propria would be loaded as shown in figure 8, from no. 46. Such was not the case. Possibly fat was late in entering the particular group of cœca examined.

The amount of fat in this material; as in the cœca of all of the fat-fed salmon, is unquestionably from fat absorption. The control materials, salmon no. 82 to 86, presented no fat in the epithelium of the cœca and only traces in the tunica propria.

FAT-ABSORBING POWER OF THE SALMON INTESTINE.

In the paper on the normal structure of the alimentary canal it has been shown that the salmon intestine has a histological structure relatively simple. It possesses the same epithelial lining coat, the tunica propria, the stratum compactum, and muscular membranes which are found in its diverticula the cœca. The mucous membrane itself is shown to be somewhat more complexly folded than in the cœca, a complexity that increases with the size of the fish. No differentiations are found in the different portions of the epithelial coat of the mucosa. Even in the deepest grooves or pits of epithelium the cells have the same general form and structural characteristics as in the most superficial folds.

The intestinal epithelium of the salmon is also a fat-absorbing tissue. Fat is taken from the lumen of the intestine with the greatest avidity by these cells. The judgment in this case is based on the histological showing made by the epithelial folds after fat-absorbing experiments. Unfortunately, no observations were made on the normal-feeding salmon and no opportunity has arisen to repair the deficiency. The facts presented here are wholly those derived from the studies of the young salmon which had been fed fat by the methods described above.

Figure 2, plate XII, from young salmon no. 45 presents a general view of the relations of the fat under a low magnification. Figure 3, plate XIII, is a highly magnified drawing showing the fat of one of the loops of one of the mucous folds of the section shown in figure 2. These figures show the epithelial cells gorged with fat for their whole extent external to the mucosa. In some instances the fat drops are large and have a diameter equal to or even greater than that of the normal cells. Often this fat appears in chains of drops extending from the surface of the cell to the nucleus. In other instances the droplets are somewhat smaller, but nevertheless crowd the cell body to the margin These statements are made on the basis of observations in both the pyloric and the post-pyloric loops of the intestine and on sections of the pyloric portion of the intestine. The section from which figure 6 is drawn, representing the fat stained with osmic acid in a fold of the cœcal epithelium, also contains a section through the pyloric intestine. The intestinal epithelium, too, is crowded with fat. In these epithelial cells the beaded arrangement of fat droplets is especially prominent. Where the section is accurately longitudinal through the epithelial cell, the rows of droplets of the larger size are shown filling up the whole body of the cell and to extend from the free surface to the region of the nucleus. It is comparatively seldom that fat is present in the basal portion of the cells, i. e., within the nuclear zone, in any such massive quantity as is so often found in the external limb of the cell. Here the fat is scattered along in fewer droplets, generally of fairly large size. In figure 5, plate XIII, the amount of fat in the inner nuclear zone is comparatively small.

It is to be emphasized that no fat droplets are present in the nuclei themselves. We have not observed any nuclear fat either in the epithelial or in the connective tissue nuclei supporting the epithelium of any portion of the alimentary canal. We are inclined to think that the nucleus does not for some reason ever receive a deposit of fat.

The intestinal epithelium discharges its fat into the connective tissue of the tunica propria just as observed in the pyloric cœca. In the material from which figure 3 is drawn this fact is very patent. Here the tunica propria contains a comparatively

heavy loading of fat. Considering the whole of figure 2, the showing of fat in the loop chosen for figure 3, plate XIII, is if anything too low for the tunica propria. In the intestine also the fat that makes its appearance in the tunica propria is not distributed over the whole of that structure down to the stratum compactum. The stratum compactum forms a very definite and limiting boundary to the fat-containing tissue. However, it is believed that this fat present in the tunica propria is not a true storage fat. No characteristic areolar fat cells are present such as are found in such numbers in the pancreas and in certain other definitely adipose tissues of the salmon. The tunica propria fat of the intestine is in comparatively small drops, rather evenly distributed over the structure, and bears all the histological evidences characteristic of the fat in the epithelial cells which is so obviously transient in its character. The fat in the tunica propria of the intestine is also retained with greater persistence, or at least for a longer time following periods of fat absorption, than is the fat of the epithelial cells. This characteristic has already been mentioned in discussing the cœca.

Further studies ought to be made before advancing the point, yet one must mention here that no obvious lymph channels through which the fat is being removed have been observed. That is, no structures comparable to the mammalian lymphatic radicles of the mammalian intestine have been observed during these studies. This is not to be interpreted as an assumption that there are none, because the observations are insufficient in number to establish a point of this character. The fact must also be mentioned that no evidence of accumulation of stainable fat in the cavities of the blood vessels has been secured. In fact, fat droplets do not appear in any of the coagulated plasma nor in any of the free blood cells in so far as yet observed either in the intestinal blood vessels or those of other parts of the body.

Minute liposomes have been found in the endothelial linings of blood vessels and in the blood vessel walls. Such findings are shown in figures 3 and 6, plate XIII. The quantity of fat disposed in such places is small, but it was found to be present in fishes in which fat absorption was at its maximum, a fact that suggests but does not prove a relation to fat absorption.

ABSORPTION OF FAT BY THE SALMON STOMACH.

The fact of the absorption of fat by the epithelial lining of the stomach was first observed on the young salmon which had been experimentally fed with fat. Observations were not made on the adult feeding salmon in a way to determine whether or not gastric fat absorption occurred. The absorption of fat in the young was observed in both series, i. e., the specimens from Brookdale, Cal., and from the McCloud River at Baird, Cal. The young salmon in the McCloud River are feeding, but evidently on a source of food which is not particularly rich in fats. At any rate the specimens seined directly from the river and examined without further feeding showed only small amounts of fat in the epithelium of the stomach. In the series of four young fish no fat could be identified in one, a trace of fat only in one, and two contained obvious and easily identified fat droplets. These specimens were taken as typical of the average of those secured from the McCloud River, and were therefore considered as normals. The specimens that received fat as food by the method previously presented were examined in comparison with the normal series just given.

ABSORPTION AFTER FAT FEEDING.

The amount of fat taken up by the mucous lining membrane of the stomach is not anywhere near so great in amount as that shown by the mucosa of other portions of the alimentary tract in one and the same animal. However, this fat is in amount quite sufficient to form a very striking picture.

The microscopic evidence of fat absorption is largely limited to the superficial epithelium. At any rate, this tissue is most distinctly loaded with fat droplets, and the loading apparently occurs earlier than in deeper portions of the gastric mucosa. An examination of the epithelium of the stomach showed fat droplets present in practically every portion of that organ. The absorption takes place not only in the cardiac division, but also in the pyloric stomach.

The earliest indication of fat absorption is found in the appearance of fat droplets in the more superficial epithelium and in the distal ends of the cells. As time is allowed for the digestive and absorptive processes these cells become more fully loaded—in fact gorged—with fat, first in the outer limbs, then later the droplets appear nearer the bases of the cells. The glandular tissue of the gastric mucosa also shows the presence of fat droplets in the later stages of fat absorption. Apparently not only the superficial epithelium and the crypts even down to the neck cells, but also the glandular cells themselves are capable of taking up fat in quantities sufficient to produce the numerous droplets which the microscopic examination reveals. Since the structure of the gastric mucosa is characteristic and strikingly different in the two divisions of the salmon stomach, these regions will be discussed separately.

ABSORPTION IN THE CARDIAC STOMACH.

In the series of fish fed at Baird one had little or no fat in the stomach coat, while three showed the presence of fat in decided quantities. In those fish in which fat was present in the stomach it was in relatively large amounts as compared with the normals. That is to say, the amount of fat in the epithelium of the stomach in the fat-fed fish was larger in amount than in the fish coming directly from the river.

The amount of fat in process of absorption by the epithelium was greatest in fat-fed fishes nos. 88 and 91 of the McCloud River series. The fat was present in superficial epithelial cells of both the cardiac and the pyloric divisions of the stomach, but the amount in the cardiac division was very obviously less than in the pyloric division.

The fat in the cardiac stomach is distributed chiefly in the cylindrical cells of the superficial epithelium. It is in greatest quantity in those cells bordering freely on the cavity of the stomach. In a typical section through the cardiac region all that portion of the epithelium outside the nuclear zone and within the extreme outer clear zone will be studded with minute fat droplets. The fat droplets here vary much in size, but seldom reach more than 3 μ in diameter. The most of the fat is in such small divisions as to require an oil immersion lens to distinguish the individual droplets. (See fig. 4, pl. XIII.) In the outer clear zone or border I found fat in only one fish, and in this instance the droplets were extremely minute, i. e., liposomes. [a]

[a] The size of the fat droplets in the stomach shows every gradation from the larger size of 2 to 3 μ diameter down to a size that is discernable only with the highest magnification. In the salmon stomach, indeed in the salmon tissues in general, I am quite unable to distinguish any constant differences in appearance among these fats. There is no line to be drawn either as regards color, size, or contour. It is true that the color shade and the size vary greatly, but not in any way that does not admit of explanation without assuming any characteristic difference in the composition of the fat bodies stained. Under these conditions I use the term liposomes without reference to the kind of fat, only to designate the extremely small size of the droplets.

The basal parts of the cells have fat droplets, but rather smaller in size and not so numerous as in the outer limbs of the cells.

In certain regions the fat is present in the cylindrical cells of the lining walls down in the deeper folds of the crypts, but in other regions it is entirely absent. I have seen the fat in these cells down as deep into the crypts as the region into which the deepest gastric gland tubes open. In every case there is a very noticeable difference in the amount of fat present in the deep-lying epithelium and the more superficial—always in favor of the greater quantity in the superficial.

The tubes of the gastric glands open into the sides and bottoms of the crypts. There is a quick transition from the superficial epithelium to the gland cell type at the point where the mouth of the gland opens. It is not often that a section passes longitudinally through the mouth of a gland. This is largely due to the fact that the glands are somewhat convoluted in shape, rarely straight and tubular as in the gastric glands of most mammals. A number of gland tubes usually open into each crypt. Some of these are very short, and are only a few cells in length, while others extend quite down to the basement membrane. Occasionally a single tube may be as straight and direct as in the mammalia, but the majority are irregular. The transition in the epithelium from the superficial to the glandular type is sudden and sharp. In medium magnification the superficial epithelium looks darker because of the intense stain (i. e., hæmatoxylin), while the gland cells are more clear and granular.

The gastric glands proper, the differentiated cells of the secreting tubules, seem never to carry fat in other than the finest division. The gland tubes often show a distinct reddish shade of color when stained with scarlet red. In the gastric glands of at least one fish definite droplets were present quite large enough to be conclusively identified as fat of the usual kind and appearance. These droplets appeared to lie near the bases of the cells, and, taken with the numerous finest liposomes present, formed a delicate net-like mosaic. The liposomes were present in greatest numbers in this specimen, no. 88. It seems to me that in this instance the liposomes bear a definite relation to the increased amount of fat present in the cylindrical cells and are to be regarded as absorption fat.

In preparations of the gastric mucosa of young salmon no. 46, fixed in Flemming's solution, the osmic acid has stained the fat droplets a brownish black, which brings them in sharp contrast with the surrounding tissues. Figure 1 of a previous brief publication concerning these facts [a] shows the superficial epithelial cells of the gastric stomach containing the absorption fat. This black stain in the ends of the cells forms a dense black mass, but it is granular in character. At any rate, where the black masses are broken up granules are seen when examined under the oil immersion. Cross sections of the necks of the crypts present rings of black granular masses around the lumen. These masses are the blackened ends of the cells. Where the section cuts the crypt through the opening of the gastric gland it is noted that the black masses become progressively smaller in the deeper portion of the crypt and are absent from the surface of the secreting gland cells. The cell bodies of the superficial epithelium are stained the dark brown of the osmic fixative. Sections across the cell just beneath the blackened ends present numerous clear areas. These areas are spherical and very small, though

[a] Greene, op. cit.

they vary in size. The cells have all the appearance of cells in sections cut through the pyloric cœca of this fish where large quantities of fat are known to have been present, but is of course now dissolved out by the oils used in imbedding the material in paraffin. In the cells of the superficial epithelium of the stomach the clear areas are smaller and do not form so large a proportion of the body of the cell as in the epithelium of the pyloric cœca.

Also, through the superficial epithelium one finds black round globules of relatively small size in the middle of the body of the cell. These black dots correspond to the areas above the nucleus which scarlet red shows to contain fat. (Fig. 4.) In the base of the cells, especially in the cells of the outer folds of the epithelium, the same black granules are present. Undoubtedly all these black granules are due to fat stained with osmic acid. The neck cells of the crypts do not contain the black granules in the main body. The black staining in salmon no. 45 is limited to the ends of the cells.

ABSORPTION IN THE PYLORIC STOMACH.

The difference in structure of the pyloric stomach mucosa has already been described in a previous paper presenting the normal structure of the alimentary tract.[a] This division of the stomach is a much more active region for fat absorption than is the cardiac division. The main portion of the pyloric division shows more numerous fat droplets in the cylindrical cells than is shown by the cardiac epithelium in an experimentally fed animal.

In the region near the pyloric valve the fat fills the more superficial epithelial cells to a maximum. The amount more nearly approaches that in the intestinal mucosa, although the fat droplets never reach the relatively large size of those of the cells of the latter region. A reference to figures 1, plate XII, and 4, plate XIII, will reveal the comparative amounts of fat in different gastric epithelial regions. The crypts of this portion of the pyloric stomach are more open and the fat is more often found in the lining cells of their walls, even down to the bottoms of the crypts. Yet, in the most heavily fat-loaded preparations there are always some crypts that show no fat while others may be quite red with the stained droplets.

In the pyloric stomach, where the epithelial cells are morphologically intermediate in character between the gastric type and that of the intestine, one can not but make the inference that the absorptive power is also intermediate in degree. Yet the epithelial cells of the free surface of the mucous fold are distinctly gastric in character, as has already been described. The cells of the free surfaces are most loaded with fat, as figure 1, plate XII, shows. Another point of comparison is found in the fact that the fat droplets in the epithelium of the extreme caudal end of the pyloric stomach are very much smaller in size than the droplets of the cells of the intestinal epithelium just on the other or intestinal side of the pyloric valve. Sections of the pyloric stomach have been prepared in which the first whorl of cœca lying close around the wall of this part of the stomach were also cut. The epithelial coats of the cœca were in every instance filled with very large fat droplets. The fat droplets were four or five times larger in diameter than the droplets in the neighboring pyloric gastric epithelium.

[a] Greene, op. cit.

ABSORPTION FAT IN THE TUNICA PROPRIA OF THE STOMACH.

The tunica propria of the stomach is very complex in its convolutions because of the fact that its net supports the irregularly shaped gastric glands. Varying quantities of fat droplets are found in the tunica propria of the young salmon during the time of absorption of fat. Often it happens that the connective tissue immediately beneath the superficial epithelium is perfectly free of fat droplets, in fact, is always relatively free of fat droplets. But during the active stage of absorption in the fat-fed specimens occasional minute fat droplets are to be found, as shown in figure 1, plate XII. In the later stages the fat seems to accumulate in the tunica propria and is removed only after long periods of time. In certain of the younger specimens observed the fat was still present at a time at which the epithelial cells were approximately free of fat droplets. In these late absorption stages the tunica propria fat is chiefly limited to that portion which lies just within the stratum compactum. The droplets are small in size and greater in number between the bases of the deep gastric glands and the inner border of the stratum compactum.

It would seem that the connective tissue of the tunica propria, like that in the intestine and pyloric cœca, holds on to its fat with great persistence. Stating the fact in other words, the lipolytic process whereby the fat is removed from this region to other parts of the body must proceed very, very slowly. It has seemed to the writer that this connective tissue region serves as a temporary storage of absorption fat, also that the process of dissociation and removal from the region is markedly influenced by the presence of the stratum compactum. In the paper on the normal structure of the alimentary tract emphasis was placed on the observation that the stratum compactum is a continuous membrane. Only at points where it is punctured by blood vessels entering into the deeper structures within the stratum is it punctured by other tissues. This mechanical structural feature would throw upon the organs concerned the physiological necessity of disposing of the fat by two possible channels. The first of these is the vascular channel. In order that the fat may be taken up by the capillaries within the tunica it must first be dissociated and diffused into the vascular channels. The second possibility is that the fat may pass through the substance of the stratum compactum. Here dissociation must also take place and be followed by diffusion through the relatively thick and dense substance of the stratum. Since blood vessels of the stomach do not form capillary nets in the statum granulosum immediately external to the stratum compactum it follows that the fat diffusion must be carried through this coat, i. e., the stratum granulosum, into the submucosa and muscular coats before it could be taken up by the circulatory system and washed away into the general regions of the body. In both instances the fat distributing process is comparatively slow, hence one may expect the removal of the absorbed fat from the tunica propria to be sharply retarded. These points of view coincide with the facts of observation as measured against the time which has elapsed from the moment of feeding and absorption to the time of the preparation of the tissue for examination.

THEORETICAL CONSIDERATIONS.

The observations detailed in the preceding pages made on adult normal feeding salmon and on younger specimens under artificial and experimental feeding of fats show beyond doubt that fat is absorbed by all portions of the alimentary tract. The food of the salmon, which is representative of the carnivorous fishes, is made up of living organisms. These are wholly marine forms during adult life and are represented by the crustacean, molluscan, and piscatorial forms. All these classes of animals possess a high percentage of fat in their tissues, particularly the fishes, which form so large a portion of the salmon foods. Fats, therefore, form a large percentage of the normal food substance for the king salmon. The importance of this food material needs no further emphasis. The question at issue in this paper, therefore, is that of the ability of the salmon to digest and absorb the fatty elements so rich in quantity in its foods.

It is of vital significance that the fats are digested and absorbed in all the great divisions of the alimentary canal. It is true that fat digestion as such has not been followed in this series of experiments, but much collateral evidence has been obtained, and certain experiments not reported have shown something of the digestive process. Of all the observations the most important would seem to be the establishment of the fat-absorbing function of the pyloric cœca on the one hand and, on the other, the fact that fat is absorbed in the stomach.

As regards the pyloric cœca, the function of these organs has previously been deduced rather than proven by scientific experiment. Cuvier, at the beginning of the nineteenth century, considered the cœca as pancreas. At a still earlier date the general theoretical view was advanced that the cœca had to do with absorption. In more recent times statements have been advanced that the cœca are concerned with digestion and absorption. Of course, in any division of the alimentary tract it is a safe assumption that the function has to do either with digestion or absorption of some one or more of the food principles.

So far as I can find, no one has, previous to my experiments, attempted to demonstrate the relation of the pyloric cœca to fat digestion and fat absorption. The preceding observations establish beyond further doubt that the pyloric cœca are primarily fat absorbers. Incidental observations indicate that fat digestion may and does take place in these organs as shown further in my first publication of facts from this investigation.[a]

The second important observation, that of the fat-absorbing power of the stomach, is also of great physiological significance. As was indicated in the introductory discussion of the literature, the fact that fats are digested and absorbed in the stomach has been established previously by work on mammals. Strange to say, this work has been largely overlooked or for one reason or another questioned, so that the full acceptance of fat digestion and absorption by the stomach has not even yet been granted. Van Herwerden first showed fat absorption by the stomach in fishes. Following the publication of my preliminary report,[b] Weiss[c] published a brief report on experiments showing the absorption of fats by the stomach of the snake. Emphasis was laid on the fact that the fat absorption takes place more readily in the young than in the adults.

[a] Greene, op. cit. [b] Idem, op. cit. [c] Weiss, op. cit.

In fact, Weiss states that in the young cat the stomach has the power to absorb fat, but this power is lost after a few months. Experiments carried out in this laboratory[a] indicate that the ordinary laboratory mammals—the rat, the cat, and the dog—possess the power to absorb fats not only in the young but in the adult.

It would seem, therefore, that the process of fat absorption in the stomach does take place with somewhat greater ease and facility in the young than in adults, but we are convinced that it is a function of the stomach which is retained throughout life and not lost at an early stage of development, as claimed by Weiss.

The process by which fats are taken up by the mucosa of the alimentary canal is quite naturally brought in question. The histological method used here does not follow the digestive processes. But there are certain facts under constant observation which indicate the nature of the absorptive process. The introductory quotation from Wells sets forth in terse and concise terms our current views of the mechanism by which fats are absorbed. Not only that, these views apply to the mechanism of fat transference in the body in general. Our general conception is that lipases are produced in the body and that through a process of dissociation the fats are split into easily diffusible forms. This dissociation takes place in digestion. In the resulting diffusible form the fats can readily enter the superficial border of the epithelial coat. The laws of lipolysis, as formulated by Kastle and Loewenhart,[b] readily account for the resynthesis of fats when once the fat cleavage products are present and in sufficient concentration within the walls of the cells. That this is the process in the salmon is indicated by two proven facts—first, the fact that fat droplets are never found exactly in the striated borders of the superficial epithelial cells of any portion of the alimentary tract of the salmon; the second fact is that these cells in the height of absorption are loaded with fat droplets of such size and numbers as to gorge the bodies of the cells. In fact, numerous histological pictures indicate that the cell boundaries are under internal tension or pressure. Figure 11, plate XV, as also a number of the other figures presented here, gives one a conception of the physical condition of the cell when loaded with fat. This condition can be explained by two links in the chain of evidence assumed by our present theories of fat mobilization. The first of these is the fact that during rapid digestion of fats, say in the cavity of a pyloric cœcum, the fatty acids and the glycerin will diffuse through the free wall of the columnar epithelial cells at a very rapid rate. Synthesis within the cell will convert these fat cleavage products into the relatively inert fat molecules which accumulate in ever increasing quantities. This removal of the cleavage products maintains an osmotic condition favorable for further and continued diffusion into the cell, thus producing a distinct pressure in an already mechanically overdistended tissue.

Emphasis can be laid on this process as an explanation of the enormous loading of the fats, as shown especially in figures 4, 5, and 8; also in lesser degree in a majority of the figures presented. In many instances the fat droplets within the cells are so large as to occupy the full diameter of the cell, and so numerous as to load the entire outer end of the cell from surface border to nucleus. When an epithelial cell is thus loaded with fat the fat is of mechanical necessity arranged in the regular beaded rows that give the diagrammatic appearance which is often presented by the figures.

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[a] Greene and Skaer, op. cit.

[b] Kastle and Loewenhart: Concerning lipase, the fat-splitting enzyme, and the reversibility of its action. American Chemical Journal, vol. XXIV, 1900, p. 491.

SUMMARY.

In summarizing the results presented in the preceding pages the salient facts may be mentioned as follows:

1. Fats are absorbed through the columnar epithelium of all portions of the alimentary tract of the king salmon.

2. The primary function of the numerous pyloric cœca is that of fat absorption. Probably the larger portion of the fats of the food of the salmon are absorbed by way of these organs.

3. The intestine is a region of active fat absorption. The power of the intestinal epithelium to take up fat is similar to that of the pyloric cœca.

4. The salmon stomach is also a fat-absorbing organ. Fat is absorbed by both the cardiac and the pyloric types of columnar epithelium.

5. The microscopic indications are that the fats pass through the outer portions of the columnar epithelial cells in a dissociated form and that resynthesis takes place within the cell, thus accounting for the numerous large fat droplets present in the cells during active fat absorption.

DESCRIPTION OF FIGURES.

The following list of figures was drawn for me by Mr. George T. Kline, biological artist of the University of Missouri. It is difficult, especially in low magnification figures, to represent the exact amount of fat in the plain of a cross section. But the relative amount is represented and by the aid of a camera lucida. In the figures of high magnification the exact size and location of every droplet has been followed with the greatest care. Figures 5 and 6 represent preparations in which the fat was stained by osmic acid. All other figures are from sections prepared from frozen section stained with scarlet red.

PLATE XII.

FIG. 1.—Showing fat absorption by the epithelium of the pyloric portion of the salmon stomach. This fish was a young specimen from the McCloud River, Baird, Cal. It was fed olive oil 20 hours before preparation. The superficial epithelium is crowded with fat. Other portions of the same section show even a greater loading, extending down to the cells of the bottoms of the crypts. Traces of fat liposomes are noticed in the lymph vessels in the folds. Fat-fed salmon no. 88. Camera lucida outlines. Magnification, Leitz ocular 1, objective 7.

FIG. 2.—Transverse section showing fat absorption in the posterior loop of the intestine in a fat-fed salmon from Brookdale, Cal. This young specimen had been fed 18 hours previous to killing. Fat is crowded into the cylindrical epithelial cells, and has passed in considerable quantity into the spaces of the tunica propria. The folding of the intestinal mucous epithelium is relatively simple in young salmon of this age. Brookdale salmon no. 45. Camera lucida outlines. Magnification, Leitz ocular 3, objective 3 with the lower lens removed.

PLATE XIII.

FIG. 3.—Showing fat absorption in a transverse section through the intestine of fat-fed salmon no. 45. This figure represents with larger magnification one of the folds shown in figure 2. The general outlines of the figure are drawn with camera lucida. The fat of the epithelial cells was laid in primarily from this section, but in part from a comparative study of other sections. The fat-bearing portion of the epithelium between the two goblet cells to the left was torn in the section, and this portion is all recon-

structed from the study of similar folds. The effort was made to present an accurate picture of the relative amount and distribution of the fat in the cells. The rather regular beaded arrangement of fat is shown in sections of the same fish, fixed in Flemming, in which the fat droplets are stained black in figures 5 and 6. It is also shown in material fixed in corrosive sublimate, where the fat has been dissolved out, leaving fat vacuoles. The inner ends of the epithelial cells contain only slight quantities of fat. No fat is ever found in the outer borders of the mucous cells.

The tunica propria is filled with a medium load of fat, the fat being caught in the spaces of the tissue and in the connective tissue cells. This fat is all laid in with the camera lucida. No fat is present in the stratum granulosum, either in the cells or in the supporting connective tissue. Traces of fat are present in the connective tissue surrounding the blood vessels, and also in the vessel endothelial cells. A few fat droplets are also present in the cells of the muscular coats, especially in the muscularis longitudinalis. Magnification, Leitz ocular 1, objective 7.

FIG. 4.—A high magnification of a section through the superficial fold of cylindrical epithelium of the cardiac portion of the stomach showing fat absorption in an early stage of the process. The fat is largely limited to the outer or most superficial zone of the cylindrical cells, but small amounts are present in the basal zone. This salmon had been fed olive oil by the method of rectal injection, the oil passing through the intestine and forward into the stomach. Brookdale young salmon, no. 46. Camera lucida outlines. Magnification, Leitz ocular 1, objective 7.

FIG. 5.—A section of a group of epithelial cells of the pyloric intestine fixed in Flemming's solution to show fat absorption. Young salmon no. 45 from Brookdale, Cal., which had been fed fat artificially. The amount of fat is not so great as present in the section of the caudal length of the intestine shown in figure 4. The same beaded arrangement of fat droplets is shown, but more smaller droplets are present in the ends of the cells—a fact showing either an earlier stage or a slower rate of absorption. Camera lucida drawing. Magnification, Leitz ocular 1, objective 7.

FIG. 6.—Showing fat in the transverse section of a fold of the pyloric cœcum of young salmon no. 45, the same fish as in figure 2. The salmon was previously fed olive oil by rectal injection and the tissue fixed in Flemming's solution. This section presents a typical picture of fat absorption in the pyloric cœca when the process is at its height. It is splendidly fixed, sharply stained, and is reproduced under camera lucida with the greatest possible care. Note the fine division of the fat droplets shown in the outer margin of the cylindrical epithelial cells, also the relatively small amount of fat of the zone within the nucleus. The tunica propria contains an excessive quantity of fat, the boundary limit of which is sharply marked by the broad band of the stratum compactum. In this specimen an occasional minute liposome is present in the connective tissue of the stratum granulosum as well as in the muscular coats, a fact that is very seldom shown. Camera lucida outlines. Magnification, Leitz ocular 1, objective 7.

PLATE XIV.

FIG. 7.—Showing fat absorption in the pyloric cœcum 18 hours after fat feeding. Young salmon no. 45. In this specimen fat is crowded in the superficial epithelium, also in the tunica propria. The details of histological structure are largely omitted in order the better to emphasize the great amount of fat present. The droplets in the tunica propria are especially numerous in this particular fish. The stratum compactum forms a sharp outer limit to the fat-bearing zone of the tunica propria. Fat-fed young salmon no. 45. Magnification, Leitz ocular 2, objective 3. ×45.

FIG. 8.—Showing fat absorption in the pyloric cœcum 42 hours after fat feeding. Young salmon no. 46, the same fish from which figure 1 was taken. The fat is largely removed from the superficial epithelium, except in the tips of the folds, but is supercrowded in the tunica propria. Camera lucida outlines. Magnification, Leitz ocular 2, objective 3.

FIG. 9.—Showing fat absorption in the pyloric cœcum of fat-fed young salmon no. 88, from the McCloud River, Baird, Cal. The structural detail is shown in only one-half the figure. Fat is rather evenly distributed throughout all portions of the cylindrical epithelium and is present in medium amount in the tunica propria. There are a few small droplets in the outer muscular coat. Time of absorption, 20 hours. Magnification, Leitz ocular 1, objective 4.

FIG. 10.—Showing fat absorption in the pyloric cœcum of a young McCloud River salmon no. 91, after 70 hours of absorption. This specimen shows the epithelial cells unusually crowded with fat. The fat has not yet reached the tunica propria, although the time for possible absorption is longer than in no. 88 of the same experimental series. Camera lucida outlines. Magnification, Leitz ocular 2, objective 4.

PLATE XV.

FIG. 11.—Showing fat absorption in the superficial epithelium of the free margin of a mucous fold of the pyloric cœcum of young salmon no. 46. This figure represents the maximal loading of fat. Many of the cells are so gorged with fat that their surface outlines are projecting as though under a high internal osmotic pressure. Camera lucida outlines. Magnification, Leitz ocular 2, objective 1/12.

FIG. 12.—Showing fat absorption in a portion of two adjacent folds of pyloric cylindrical epithelium from a normally feeding adult salmon from the fishing banks of Monterey Bay. The clear marginal zone is well shown in this figure, also the characteristic finely divided liposomic fat immediately beneath it. This zone shades off into one of larger droplets lying just external to the nuclear layer. Note the comparatively small amount of fat in the inner zone of the epithelium and in the thin layer of the tunica propria. The fat droplets are most carefully laid in from camera lucida outlines. Salmon number 22. Magnification, Leitz ocular 1, objective 1/12.

FIG. 13.—Showing fat absorption in a normally feeding adult salmon, no. 28, Monterey Bay, Cal. This figure represents a later stage of absorption than the proceeding. It shows a loading of the inner ends of the cells with finely divided liposomes and a similar charge of fat in the adjacent tunica propria. The fat has passed the outer zone. This stage of fat absorption was rather rarely observed. Camera lucida outlines. Magnification, Leitz ocular 1, objective 1/12.

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FIG. 1.

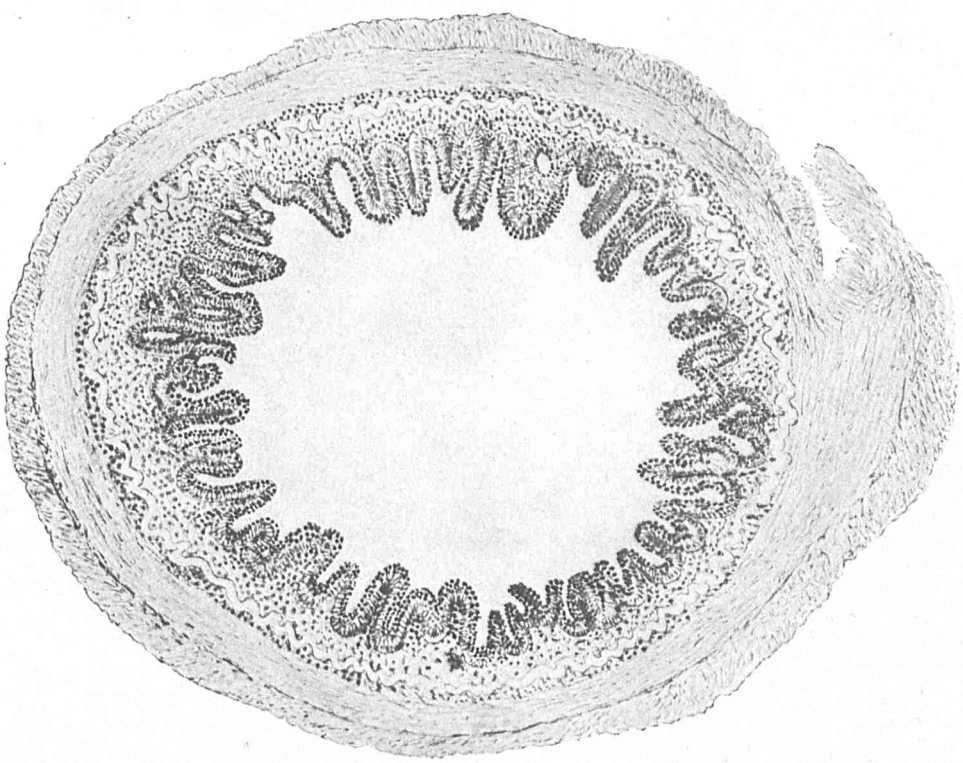

FIG. 2.

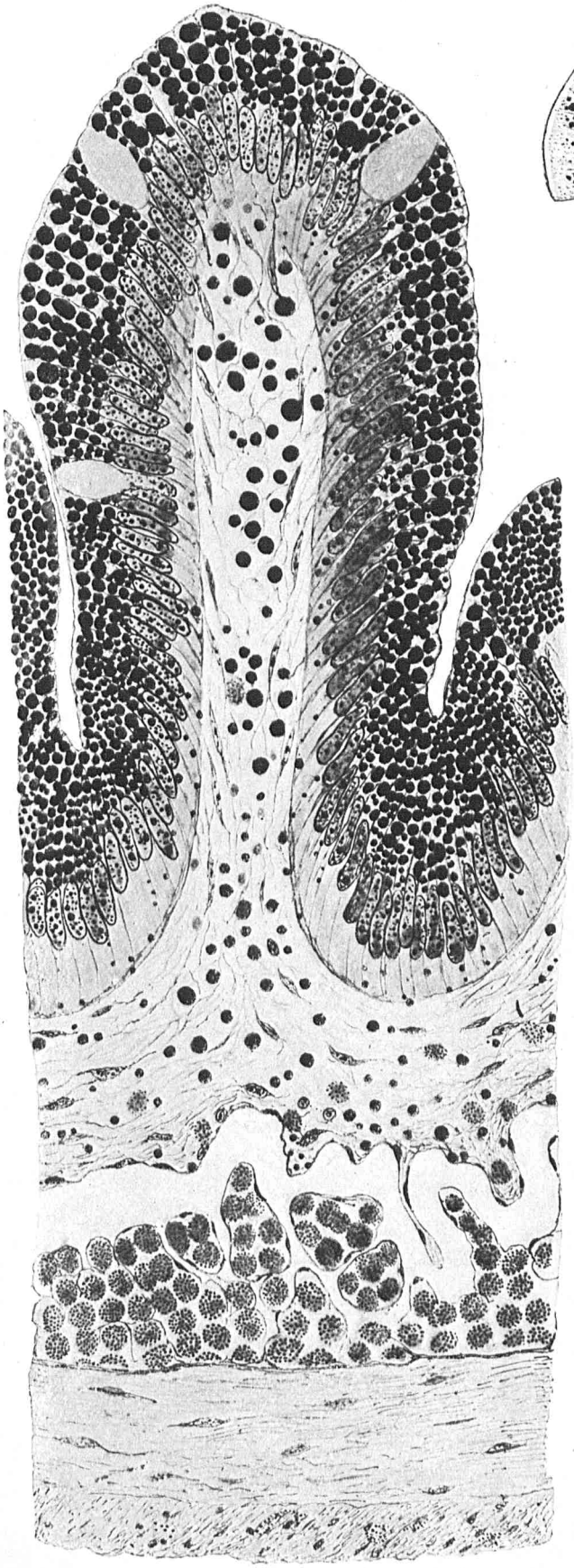

FIG. 3.

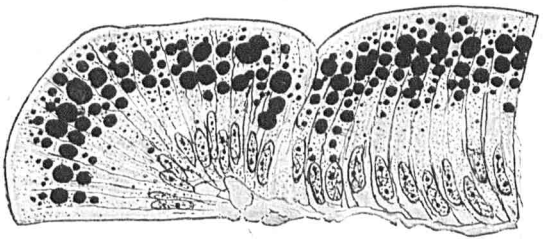

FIG. 5.

FIG. 4.

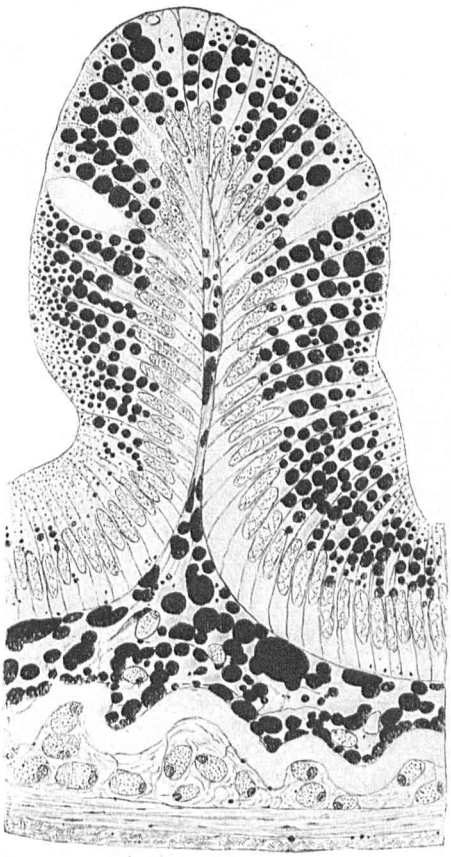

FIG. 6.

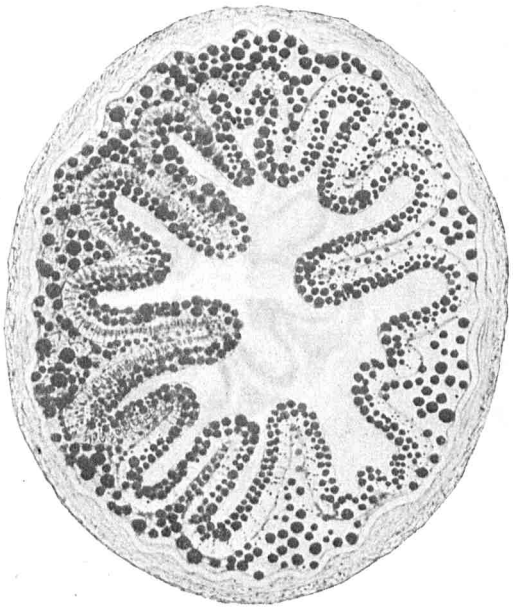

FIG. 7.

FIG. 8.

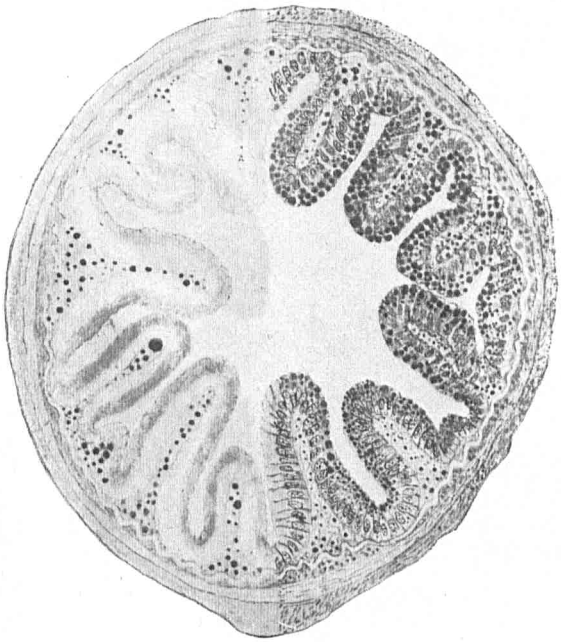

FIG. 9.

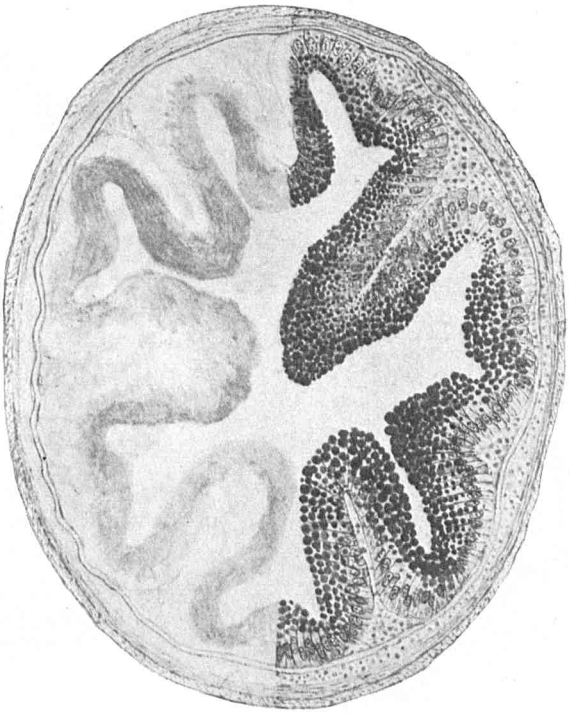

FIG. 10.

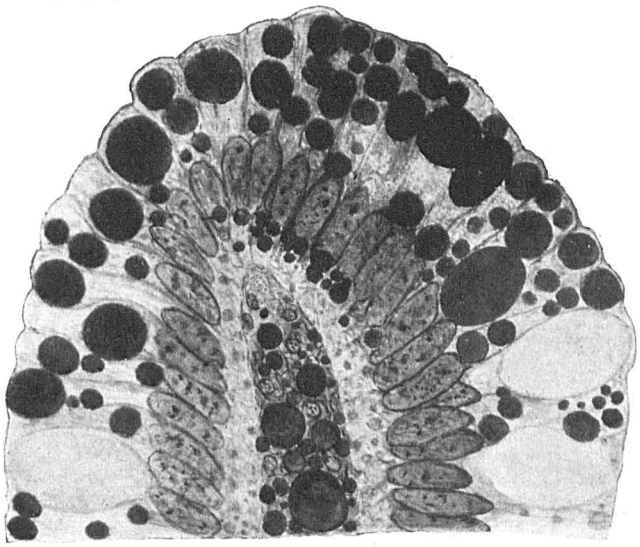

FIG. 11.

FIG. 12.

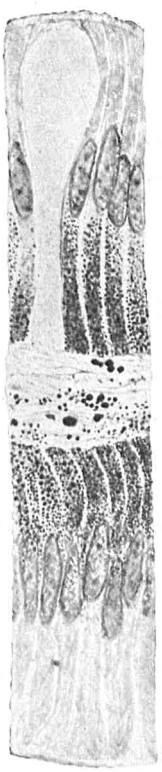

FIG. 13.

NOTES ON THE HABITS, MORPHOLOGY OF THE REPRODUCTIVE ORGANS, AND EMBRYOLOGY OF THE VIVIPAROUS FISH GAMBUSIA AFFINIS

By Albert Kuntz, Ph. D.

St. Louis University School of Medicine

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NOTES ON THE HABITS, MORPHOLOGY OF THE REPRODUCTIVE ORGANS, AND EMBRYOLOGY OF THE VIVIPAROUS FISH GAMBUSIA AFFINIS.

By ALBERT KUNTZ, PH. D.,
St. Louis University School of Medicine.

INTRODUCTION.

Gambusia affinis (Baird and Girard), according to Smith (1907), "is found along the coast from Delaware to Mexico and reaches inland as far as Illinois. In North Carolina it is excessively abundant in the lowlands, in swamps, ditches, creeks, and also in the open waters of the rivers." [a] It is known primarily as a fresh-water species, but occurs also in brackish water. Early in July and again on August 1, 1912, a considerable number of these fish were taken along the swampy borders of Mullet Pond, Shackleford Banks. The specific gravity of the water in which they were taken on August 1 was 1.0081. This reading, however, does not represent the normal specific gravity of the water along these swampy borders, as considerable rain had fallen during the preceding 12 hours. At the time the above reading was taken, water from the central part of Mullet Pond showed a specific gravity of 1.0106. On July 24, 1912, a single specimen was caught in the seine in the terrapin pens at the Beaufort Laboratory. This was a large female, bearing mature, unfertilized eggs. The water in these pens is salt, only a very little fresh water entering through small pipes from an artesian well.

Twenty-four of the fish taken in Mullet Pond on August 1 were transferred to sea water in a small aquarium, where they remained for a period of 10 days. At the end of this time 1 was found dead. The remaining 23 were apparently in a normal condition; they had, however, lost much of their pigment and their tissues had become slightly transparent.

During the entire month of July, 1912, these fish were present in abundance in a brooklet emptying into Beaufort Harbor just east of Beaufort. The water in this brooklet is supplied largely by springs. It was reddish brown with organic matter and contained considerable débris. Most of the fish used in this study were taken in this brooklet.

The generic name *Gambusia* is derived from the name "Gambusina," commonly used in Cuba, which means "small" or "of no importance." While of no commercial value, this species has an important economic worth. It feeds largely on insects and insect larvæ. Wherever it inhabits waters in which mosquitoes breed the mosquito larvæ constitute its principal food. The introduction of these fish into the natural

[a] Smith, H. M.: Fishes of North Carolina. North Carolina Geological and Economic Survey, vol. II, p. 153.

waters as well as into artificial ponds, aquatic gardens, etc., in mosquito-infested regions may, therefore, play an important rôle in the extermination of these pests. Experimental work of this kind already undertaken in New Jersey suggests that the plan of combating mosquitoes by the introduction of *Gambusia* and other fishes with similar habits is entirely feasible.

As was pointed out by Seal [a] (1908), *Gambusia* and the related genus *Heterandria* possess certain habits and characters which render them superior to all other fishes as mosquito destroyers. As suggested by their common name, top minnows, they feed at the surface. Being of small size they readily find their way into shallow waters which are inaccessible to larger fishes. *Gambusia affinis* is often found in large numbers in water less than an inch in depth. Furthermore, it habitually searches for food among the vegetation and débris along the borders of pond or stream. In Mullet Pond it is rarely found in the open water, but is present in abundance among the marsh grasses along the swampy borders, where it not only finds food but is also protected from larger fishes.

The small size of this species, its viviparous habits, and its hardy nature ought to render its introduction and maintenance in new waters comparatively easy. It thrives under a wide range of conditions. Furthermore, the young, being brought forth in an advanced stage of development, are not subjected to many of the dangers which beset the young of oviparous fishes.

The breeding season continues during the spring and summer, several broods being produced during the season. Seal [b] (1911), observing these fish in captivity, has demonstrated that two or more generations may be born in a summer.

The adult females vary greatly in size, ranging from 3 to 6.5 centimeters in length. The males are relatively fewer in number and smaller than the females. The adult males range from 1.8 to 3 centimeters in length. Nearly all of the adult females taken by the writer during July, 1912, carried either mature ova or embryos.

The present investigation was carried on at the United States Fisheries Laboratory at Beaufort, N. C., during the summer of 1912.

REPRODUCTIVE ORGANS.

FEMALE.

Ovary.—The ovary is located in the abdominal cavity just beneath the air bladder and dorsal to the posterior portion of the intestine. It opens directly into the urogenital sinus, which communicates with the exterior through the urogenital aperture just posterior to the anal opening. It is a paired tubular organ, but, unlike the ovary of many teleosts, it is not bifurcated and has no distinct median wall. The left side of the ovary is always shorter than the right. (Pl. XVI, fig. 7.) This disparity in the length of the two sides of the ovary is due to the position of the stomach, which is located in the left side of the abdominal cavity. When distended with mature ova or embryos, the ovary fills the greater part of the abdominal cavity beneath the air bladder and causes considerable distension of the abdominal walls. At the left the ovary in this distended

[a] Seal, William P.: Fishes in their relation to the mosquito problem. Bulletin Bureau Fisheries, vol. XXVII, 1908, p. 831–838.

[b] Seal, William P.: Breeding habits of the viviparous fishes *Gambusia holbrookii* and *Heterandria formosa*. Proceedings of the Biological Society of Washington, vol. XXIV, p. 91–96.

condition presses forward against the stomach, while at the right it extends anteriorly alongside the latter organ. (Pl. XVI, fig. 8.)

Unlike the ovary of many oviparous teleosts, the ovary of *Gambusia* is not lobulated and contains relatively few ova. In the same ovary may be found ova in various stages of development, ranging from almost microscopic dimensions to a diameter of 1.8 millimeters attained at maturity. A considerable number of ova apparently reach maturity at the same time. These being fertilized give rise to a brood of young. After the birth of this brood, another lot of ova reach maturity, and, being fertilized, give rise to a second brood. Thus, perhaps, all the ova required to produce the several broods which are born during a spring and summer may be present in the ovary at the beginning of the season.

The larger females usually give rise to a larger brood of young than do the smaller ones. The average number of embryos contained in the ovaries of females 5 to 6 centimeters in length, based on a limited number of counts, was found to be 33. The maximum number removed by the writer from a single female was 76. The number of embryos contained in the ovaries of the smaller females ranges from 2 or 3 to about 20.

In females of this species taken in the Potomac River early in June, 1912, Smith [a] found the average number of embryos contained in the ovary to be 100. This average is considerably greater than the maximum number observed by the present writer. This difference is probably due to the fact that the broods observed by Smith were the first broods produced during the season, while those observed by the present writer were the second or later broods. As suggested by Smith, the first brood of the season is probably considerably larger than the later broods.

The ova of *Gambusia* have no investment of their own save a delicate vitelline membrane. Each ovum is inclosed in a separate cellular follicle which is attached to a central rachis (Ryder) by a slender stalk. Running longitudinally in the central rachis are a pair of vascular trunks from which smaller blood vessels arise and pass out along the stalks of the follicles. These smaller blood vessels break up into capillaries which radiate in all directions over the follicular walls. These follicles were described by Ryder (1885) as follows: "The ovarian follicles of *Gambusia* containing mature ova or fœtuses are built up internally of flat or squamous polygonal cells of pavement epithelium, and externally of a network of multipolar fibrous connective tissue cells and minute capillary blood vessels with cellular walls, which radiate in all directions over the follicle. From the point at which the main arterial vessel enters it, this vessel, together with its accompanying vein and investment of fibrous tissue, constitutes the stalk by which the follicle and its contained naked ovum is suspended to the main arterial trunk and vein." [b]

In an earlier paper, Ryder [c] (1882) has furthermore described a minute aperture in the follicular membrane near the stalk of the follicle which he has designated "the follicle pore." Through this pore, he believes, the spermatozoa enters the follicle.

I was able satisfactorily to observe such a pore in the follicular membrane in only a few instances. I have no reason, however, to doubt the presence of a follicular pore

[a] Smith, H. M.: The prolificness of *Gambusia*. Science, vol. XXXVI, n. s., no. 920, 1912, p. 224.

[b] Ryder, John A.: On the development of viviparous osseous fishes. Proceedings of the U. S. National Museum, vol. VIII, p. 147.

[c] Ryder, John A.: A contribution to the embryography of osseous fishes, with special reference to the development of the cod (*Gadus morrhua*). Report of the U. S. Fish Commission, 1882, p. 461.

in all the ovarian follicles. Without assuming the presence of an aperture in the follicular walls, it would be difficult to understand how the spermatozoa could come in contact with the ova.

I can not agree with Ryder (1785), however, that "the ovary itself seems to have no exterior investment, so that the follicles lie directly within the abdominal cavity, the young fishes upon the completion of their development rupture them and escape into the latter, and from thence through the abdominal pore into the outer world." [a] The ovary, as stated above, is a tubular organ which opens directly into the urogenital sinus. When distended with advanced embryos, the exterior walls of the ovary are very tenuous. The young fishes do not, however, break out into the abdominal cavity, but pass out of the ovary directly through its opening into the urogenital sinus, thence to the exterior. There is no aperture leading directly from the abdominal cavity to the exterior. Furthermore, examination of the ovary of a female immediately after she has given birth to a brood of young shows the walls of the ovary intact. No ruptured ovarian follicles communicate with the cœlom. When the exterior walls of the ovary are dissected off, the ruptured ovarian follicles are found in place in a somewhat shrunken condition.

It may not be amiss at this point to call attention to an error which appears in the recent paper by Seal (1911) referred to above. "The ova of a full-sized *Gambusia* are," he says, "when fully developed, about an eighth of an inch in diameter, transparent and nonadhesive. Each one is held, apparently, by a thread of membrane to a central nucleus, the character of which could only be determined by microscopic observation. The young fish can be seen fully formed, their eyes moving as they turn around in the egg." [b]

That the author quoted above has mistaken the ovarian follicle for the egg is obvious. The embryo is developed at the surface of the egg, which has no investment of its own save the vitelline membrane. When the yolk has been absorbed by the embryo there remains no trace of the egg. The young fish is then inclosed in the ovarian follicle which is suspended to the central rachis by the structure referred to in the above quotation as "a thread of membrane holding the egg to a central nucleus."

In the paper quoted above (p. 93), Seal describes the extrusion of the young as follows: "They are expelled one at a time and the ejection of each fish is so rapid that they appear as though shot out with some force. This, however, might be due to the bursting of the follicle and the uncoiling of the fish as it is released from restraint. * * * The follicles are undoubtedly ruptured at the moment of extrusion, whether inside or out I have never succeeded in observing, but it appears the more probable that it is inside."

In view of the fact that the ruptured ovarian follicles are found in place in the ovary after the young fishes are extruded, it is obvious that the rupturing of the follicle occurs not only within the body of the parent but within the ovary. The young fish is, doubtless, uncoiled as soon as it leaves the follicle. This uncoiling could, therefore, add little to the force with which the young fish is extruded. The rapid escape of the

[a] Ryder, John A.: On the development of viviparous osseous fishes. Proceedings of the U. S. National Museum, vol. VIII, p. 148.

[b] Seal, William P.: Breeding habits of the viviparous fishes *Gambusia holbrookii* and *Heterandria formosa*. Proceedings of the Biological Society of Washington, vol. XXIV, p. 93, 1911.

young fish, if, as is usually the case, it comes out head foremost, may be readily explained by the tapering form of its body and by its own swimming movements. That some force is necessary, however, for the extrusion of the young is evidenced by the perceptible contractions of the muscles of the abdominal walls of the parent just before the young is extruded.

MALE.

Modified anal fin.—The male members of the species may be readily recognized by the modified anal fin, which functions as an intromittent organ. The third, fourth, and fifth rays of this fin are enlarged, greatly elongated, and variously curved. All of the rays are composed of segments. The diameter of each segment is slightly greater at the ends than in the middle. Thus each ray shows a series of slight circular ridges. These ridges are most prominent on the third ray, which is the largest of the elongated rays and has a slight backward curve near its proximal end. The distal portion of this ray bears a row of short, pointed spines on its anterior aspect, while posteriorly it is fringed, a short distance from the tip, by a dentate ridge apparently in the fin membrane.

The proximal portion of the fourth ray has a gentle forward slope until it comes into close proximity with the third. From this point the former ray extends distally parallel with the latter. The fourth ray is slightly longer than the third. Its distal portion is divided, the two divisions diverging for a short distance and again coming in contact with each other at the tip. The anterior division bears a few very small spines anteriorly. The posterior division bears a considerable number of short, slender spines posteriorly a short distance from the tip. The proximal ones of these spines are arranged in two groups of three spines each. The fifth ray makes a short, sigmoid flexure at its proximal end and then extends distally parallel with the fourth. Near its distal end it makes another slight sigmoid flexure and terminates in a small hammer-shaped enlargement which interlocks with a slightly recurved hook on the posterior division of the distal portion of the fourth ray. The third, fourth, and fifth rays of the anal fin are bound together by the fin membrane. The fifth ray may be brought forward at one side of the fourth until it comes into close or immediate proximity with the third. In this manner a groove or tube is formed through which the milt is transmitted into the genital aperture of the female. The first two and the last five rays of the anal fin are somewhat modified but not elongated.

The rays of the modified anal fin are illustrated in figure 2, plate XVI. Figure 3, plate XVI, shows the distal portion of the three elongated rays drawn in detail under higher magnification.

Mechanism controlling anal fin.—The modified anal fin is controlled by a powerful muscle which is inserted on the proximal ends of the rays of the anal fin and has its origin on the modified hæmal spines of the first three caudal vertebræ and a similar process projecting ventrally from the fourth to the last abdominal vertebra. This muscle stands in an almost vertical position and is so large that it causes a perceptible bulging of the body walls just above the vent. (Pl. XVI, fig. 1.)

The process projecting ventrally from the fourth to the last abdominal vertebra has a slight forward slope. The modified hæmal spines of the first three caudal vertebræ project forward into the abdominal cavity in an almost horizontal position. The first hæmal spine is nearly straight, having a slight downward curve near its distal end.

The second makes a slight forward curve near its origin. From this point a somewhat flattened forked process extends posteriorly, one prong of the fork passing on either side of the third hæmal spine. These two prongs terminate in footlike enlargements in the muscles of the anterior caudal segments. The third hæmal spine makes a somewhat stronger forward curve near its origin than the second. From its proximal portion a short, flattened, keel-shaped process extends posteriorly. The distal ends of these three hæmal spines are connected by a narrow band of cartilage. (Pl. XVI, fig. 2, HS.)

The interhæmals are correspondingly larger in the male than in the female and are embedded in the large muscle controlling the modified anal fin. The one articulating with the third ray of the modified anal fin is greatly enlarged and articulates loosely with the two anterior processes on which the muscle has its origin. (Pl. XVI, fig. 2, IH.)

The mechanism controlling the modified anal fin projects anteriorly into the abdominal cavity to such an extent that the space allotted to the air bladder becomes somewhat restricted. Consequently, the latter organ is relatively shorter and occupies a more oblique position in the male than in the female.

Ryder [a] (1885) has given us a brief description of the modified anal fin of *Gambusia* and the mechanism by which it is controlled, which is in many respects erroneous. A comparison of Ryder's description with the description given above will not be attempted in this paper. The former description, published more than a quarter of a century ago, was obviously not the result of an exhaustive study.

Testis.—The testis, like the ovary, is a paired tubular organ and is not distinctly divided. (Pl. XVI, fig. 4.) It is located in the abdominal cavity dorsal to the posterior portion of the intestine and just anterior to the large muscle controlling the anal fin. The testis does not extend as far anteriorly as does the ovary, but, like the latter organ, the left side of the testis is shorter than the right.

The spermatozoa are contained in spermatophores, which are rounded or spherical bodies, 0.1 to 0.2 millimeters in diameter. (Pl. XVI, fig. 5.) The walls of the spermatophores are exceedingly delicate. If the spermatophores are ruptured under the microscope, the spermatozoa may be seen to escape freely even though they are still immature and inactive. The spermatozoa are comparatively large. Each one is composed of a comparatively large, elongated, slightly curved and bluntly pointed head, a middle piece which is nearly as long but more slender than the head, and a long flagellate tail. (Pl. XVI, fig. 6.)

In most of the spermatophores observed, the spermatozoa were inactive and apparently curved around a small, bubble-like body, thus forming a more or less complete ring. When the spermatophores were broken many of the spermatozoa were released from this curved position and freed from the small, bubblelike body. The tails, however, still retained a slight curve. The heads of the spermatozoa may be readily observed in the spermatophores under moderately high magnification. They are closely aggregated but show no regular arrangement. While no spermatophores were observed in the genital organs of the female, it is highly probable that the spermatozoa are transmitted from the male to the female in these bodies.

[a] Ryder, John A.: On the development of viviparous osseous fishes. Proceedings of the U. S. National Museum, vol. VIII, p. 143, 144.

EMBRYOLOGY.

OVUM.

The mature ovum is a spherical body having a diameter of about 1.8 millimeters. It has a gold-yellow color and, being heavily laden with yolk, is quite opaque. It is invested by no distinct egg membrane such as invests the eggs of most of the oviparous fishes, but is covered only by a thin, vitelline membrane. Beneath the vitelline membrane the entire surface is more or less completely covered by oil globules of unequal size and distribution. (Pl. XVII, fig. 1.)

BLASTODERM.

The ova are fertilized within the ovarian follicles. Unless the time of fertilization can be controlled, it becomes difficult to secure the earliest stages of development. The earliest stages which were secured after fertilization showed a small blastoderm in the many-cell stage. This blastoderm appears as a small, almost circular cap of cells which is slightly elevated above the surface of the yolk. (Pl. XVII, fig. 2, B.) The distribution of the oil globules is not disturbed during the process of cleavage and numerous globules may be observed through the blastoderm.

As the blastoderm increases in size, the cleavage cavity becomes plainly visible. The germ ring is never well defined, but appears as a slight thickening of the periphery of the blastoderm. The cleavage cavity, as observed through the overlying blastoderm, soon assumes a somewhat triangular outline. The blastoderm becomes slightly elongated along the axis, which becomes the future axis of the embryo. At the side of the cleavage cavity on which the thickened area at the periphery of the blastoderm is broadest, the blastoderm becomes thicker and more opaque. This area is symmetrically divided by the long axis of the blastoderm and, inasmuch as it gives rise to the embryonic shield, may be recognized as the posterior pole of the blastoderm. This area increases in size and distinctness until the embryonic shield is well outlined. (Pl. XVII, fig. 4.)

DIFFERENTIATION OF THE EMBRYO.

From the posterior pole of the embryonic shield a narrow thickened area grows anteriorly. This thickened area alone represents the embryonic area, while the thinner lateral areas represent the extra-embryonic area of the embryonic shield. (Pl. XVII, fig. 4.) The embryonic area continues to grow anteriorly over the cleavage cavity and becomes gradually enlarged at the anterior end. In this manner the head of the future embryo becomes outlined.

While the embryonic area is becoming differentiated the blastoderm spreads rapidly over the yolk until the latter is completely covered. The progress of the growth of the blastoderm over the yolk could not be observed satisfactorily, partly because the germ ring is not well defined and not easily observed on this very opaque egg and partly because not all the desired stages of development could be secured. A careful study of the stages available, however, seems to indicate that the differentiation of the embryo of *Gambusia* takes place in a manner which is quite typical for teleosts.

LATER DEVELOPMENT.

After the formation of the embryonic area the embryo soon becomes well outlined. Plate XVIII, figure 5, illustrates a stage at which the tail bud has already grown out and the anlage of the neural axis is apparent throughout the entire length of the embryo. The optic vesicles are well formed and from 3 to 4 somites are already apparent.

Plate XVIII, figure 6, represents an embryo in which the divisions of the brain are becoming distinctly outlined. The auditory vesicles and from 12 to 14 somites are already present. At this stage the heart is becoming differentiated as a simple curved tube. The heart soon begins to pulsate, and a circulation is set up over the surface of the yolk. This circulation is at first slow and irregular but soon becomes very vigorous.

The growing embryo lies in a groove in the surface of the yolk and is inclosed only by the ovarian follicle. As development advances the ovarian follicle increases in size and becomes increasingly vascular. The space between the egg and the follicle becomes filled with a transparent fluid. Thus the embryo lives in a fluid medium. Although the ovarian follicle becomes highly vascular, a placental or pseudoplacental relationship such as exists in the selachians or even in some of the viviparous teleosts is not suggested. The embryo develops no structures which would seem to be adapted to absorb nourishment from a fluid medium. Furthermore, no fæces of any kind are ever observed in the follicle. The abundant yolk supply in the egg is, doubtless, sufficient to supply all the food material required by the embryo.

It is probable, as was suggested by Ryder (1885), that "the very intricate meshwork of fine vessels which covers the follicle supplies the developing fœtus with fresh oxygen, and also serves to carry off the carbon dioxide in much the same way as the placenta or afterbirth performs a similar duty for the young mammal developing in the uterus of its parent."[a] The analogy between the intra-follicular respiration of the developing embryo of *Gambusia* and the intra-uterine respiration of the young mammal must, however, not be carried too far. The embryo of *Gambusia* develops gills which apparently become functional very early. An examination of the gills of an advanced embryo removed from the ovarian follicle, as Ryder has already observed in the paper quoted above, shows that the gill filaments are already pinnate and that the pinnæ contains loops of blood vessels. This condition of the gill filaments, as is well known, is not attained by the larvæ of many oviparous fishes for a considerable interval after hatching. Furthermore, rythmical breathing movements may be observed as the embryo lies coiled in the ovarian follicle. It is probable, therefore, that the intra-follicular respiration of the embryo of *Gambusia*, at least during the later stages of intra-ovarian life, is more nearly analogous with the respiration of adult fishes than with the intra-uterine respiration of the young mammal, the fluid in the follicle, by which the embryo is constantly bathed, being aerated by the follicular circulation.

As the embryo grows, the tail extends posteriorly partly encircling the egg. Soon, however, it bends indifferently to the right or to the left. (Pl. XVIII, fig. 8.) This bending brings the tip of the tail into proximity with the head. Consequently, as the caudal fin is developed it overlaps the face of the embryo, sometimes partly or completely covering one or both of the eyes. (Pl. XIX, fig. 9.)

[a] Ryder, John A.: On the development of viviparous osseous fishes. Proceedings U. S. National Museum, vol. VIII, p. 147.

Pigmentation begins comparatively early. Scattered pigment spots first appear on the dorsal surface, being more closely aggregated on the posterior region of the head and along the dorsal mid-line of the trunk. These pigment spots become more numerous and more closely aggregated until at birth pigmentation is almost complete.

Embryos which still retain a yolk sac of considerable size when removed from the ovary show nearly all the characteristic markings of the adult. At birth the yolk sac is completely absorbed. The newborn fish answers fairly well, except with respect to dimensions, to the diagnostic description of the species. Its color is light olive, darker dorsally than ventrally. The number of scales in the lateral and transverse series, respectively, correspond to the number of scales in these series, respectively, in the adult. The number of rays in the dorsal, anal, and caudal fins also correspond to the number of rays in these fins, respectively, in the adult. The fine dark line along the side is already present. The two or three transverse rows of dark spots on the dorsal, the dark margin on the anal, and the three or four irregular rows of dark spots on the caudal fin, characteristic of adult females, are already becoming differentiated. The dark purplish blotch on the side above the vent (absent in males) is not yet apparent. The modified anal fin of the male was not observed in newborn fishes.

The newborn fishes are 9 to 10 millimeters in length and are very vigorous. Having been protected from many of the dangers which beset the larvæ of oviparous fishes, they are now well prepared to enter upon an independent existence.

Embryos still carrying a yolk sac of considerable size, being removed from the parent, were able to swim freely in water, where they continued to live, the yolk sac being gradually absorbed. Such embryos were kept in small aquaria with occasional changes of fresh water for a period of 10 days. At the end of this time the yolk sac was completely absorbed and the young fishes were apparently in a healthy condition.

SUMMARY.

1. *Gambusia affinis* is known primarily as a fresh-water species, but occurs also in brackish water. Under experimental conditions, fishes transferred from brackish water to sea water were kept alive and apparently in a normal condition for a period of 10 days.

2. The ovary of *Gambusia* is a paired tubular organ without a distinct median wall, which opens directly into the urogenital sinus. Each ovum is contained in a separate cellular follicle in which fertilization takes place and the embryo is developed. At the completion of development the ovarian follicles which are attached to the central rachis by a slender stalk are ruptured and the young fishes are extruded directly through the urogenital aperture.

3. The modified anal fin of the male which functions as an intromittent organ is controlled by a powerful muscle which is inserted on the proximal end of the anal fin rays and has its origin on a bony process projecting ventrally from the fourth to the last abdominal vertebra and the modified hæmal spines of the first three caudal vertebræ. The third, fourth, and fifth rays of the anal fin are enlarged, greatly elongated, and variously curved, bearing short spines on the distal portions. The interhæmal which articulates with the third ray is enlarged and articulates with the two anterior processes on which the muscle controlling the anal fin has its origin.

4. The testis, like the ovary, is a paired tubular organ. The spermatozoa are contained in the spermatophores and are probably transmitted from the male to the female in these bodies.

5. The formation of the blastoderm and the differentiation of the embryo takes place in a manner which is quite typical for teleosts.

6. As development advances, the ovarian follicle becomes highly vascular, increases in size, and is filled with a transparent fluid in which the embryo is constantly bathed. This fluid is doubtless aerated by the follicular circulation. The gills of the developing embryo apparently become functional comparatively early. During the later stages of intra-ovarian life, rhythmical breathing movements of the embryo may be observed.

7. The young are born in an advanced stage of development and show nearly all of the diagnostic characters of the species. They undergo no marked metamorphic changes after birth.

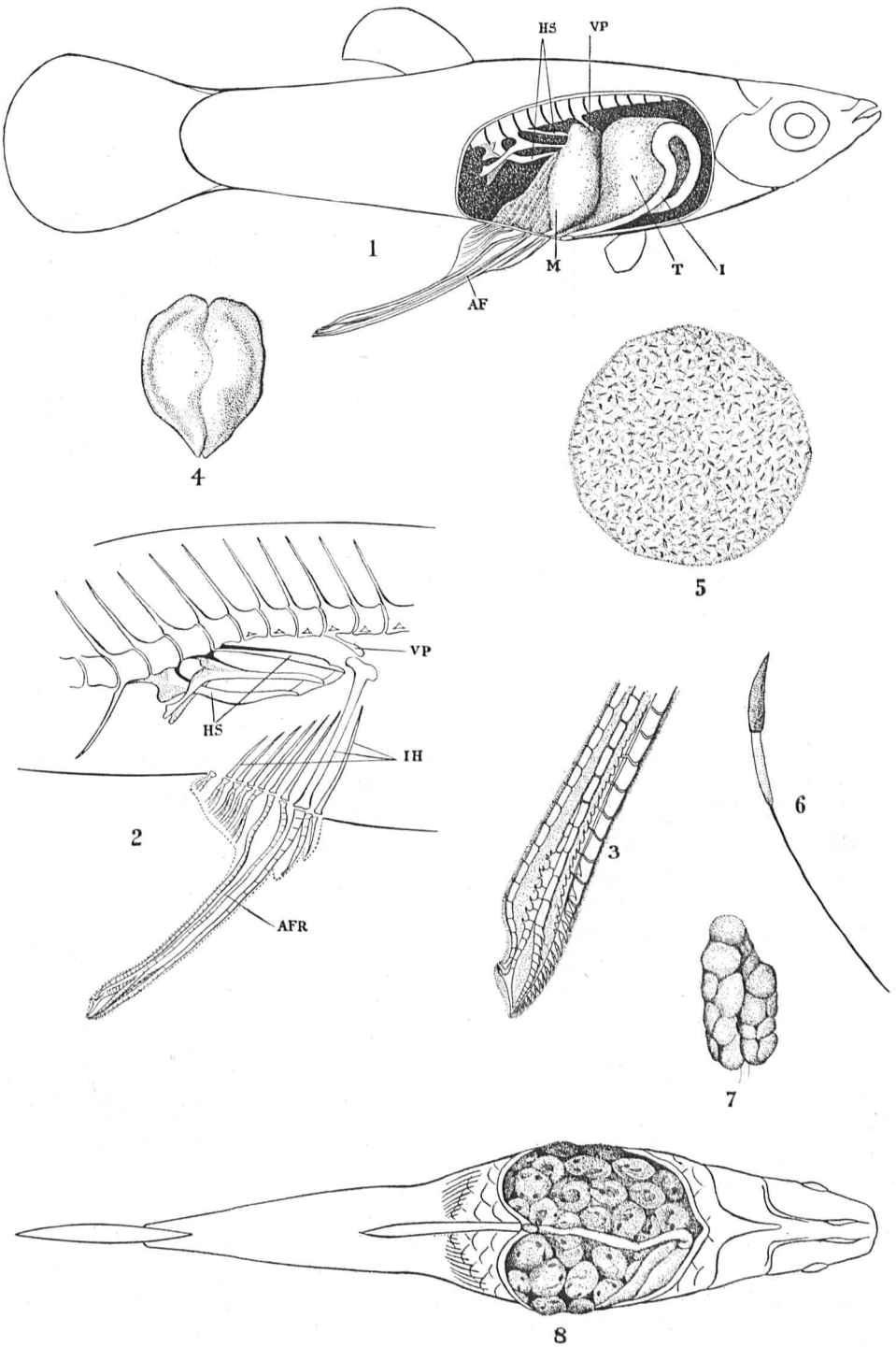


FIG. 1.—Dissection of male *Gambusia*, showing testis and mechanism controlling anal fin, x 7.2; *AF* modified anal fin; *HS*, modfied hæmal spines; *I*, intestine; *M*, muscle controlling anal fin; *T*, testis; *VP*, ventral process of abdominal vertebra.

FIG. 2.—Skeletal parts of mechanism controlling modified anal fin; *AFR*, anal fin rays; *HS*, hæmal spines; *IH*, interhæmals; *VP*, ventral process of abdominal vertebra.

FIG. 3.—Distal portion of modified anal fin greatly enlarged.

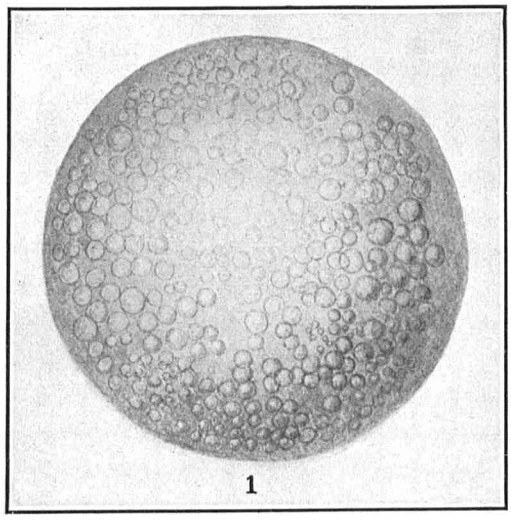
FIG. 4.—Testis, x 9.

FIG. 5.—Spermataphore, x 385.

FIG. 6.—Spermatazoon, x 3,000.

FIG. 7.—Ovary, x 4.

FIG. 8.—Dissection of female *Gambusia*, showing ovary distended with advanced embryos, x 3.6.



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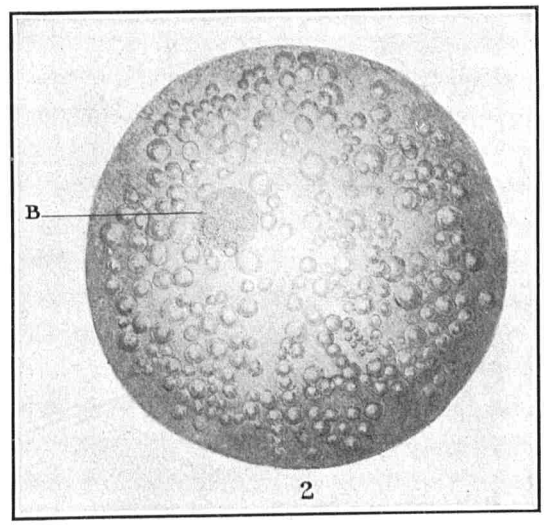

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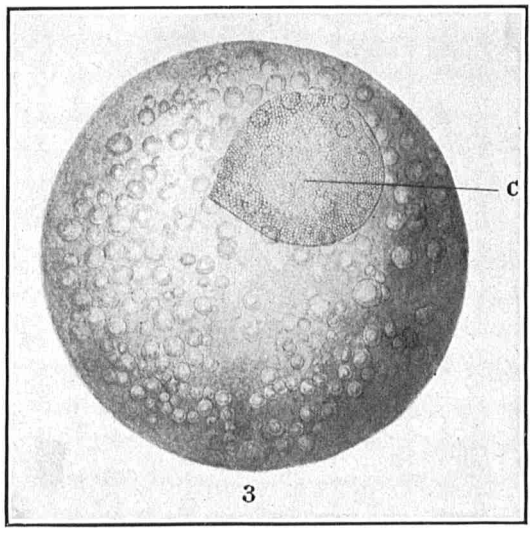

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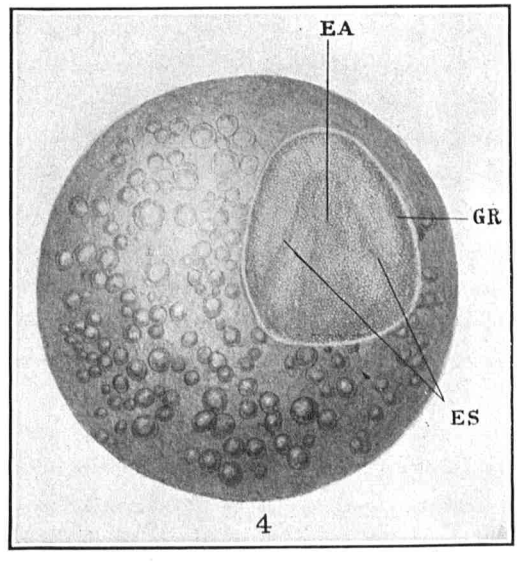

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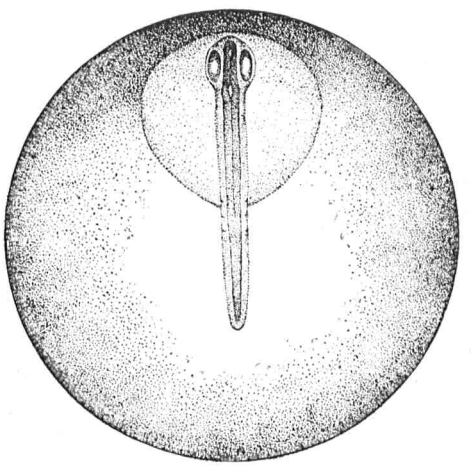
FIG. 1.—Mature ovum, x 45.
FIG. 2.—Ovum with early blastoderm, x 45; *B*, blastoderm.
FIG. 3.—Ovum with later blastoderm, x 45; *C*, cleavage cavity.
FIG. 4.—Ovum with blastoderm, showing embryonic shield, x 45; *ES*, embryonic shield; *EA*, embryonic area; *GR*, germ. ring.



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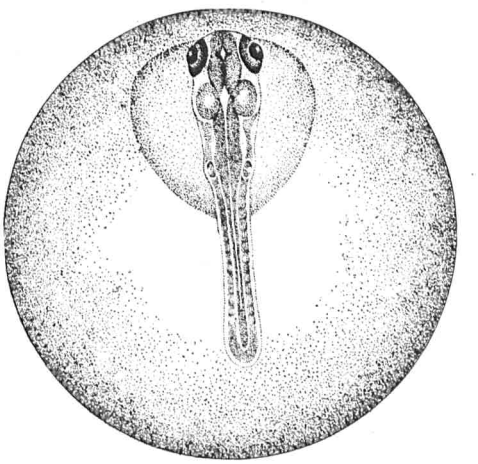

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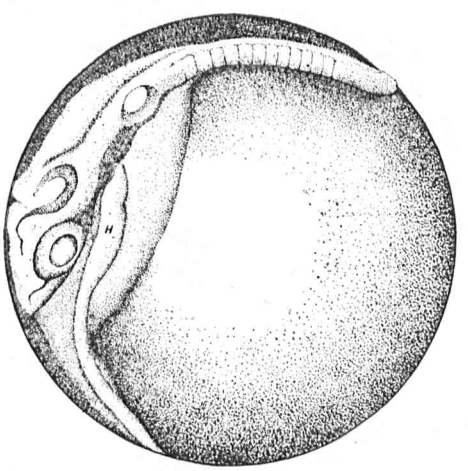

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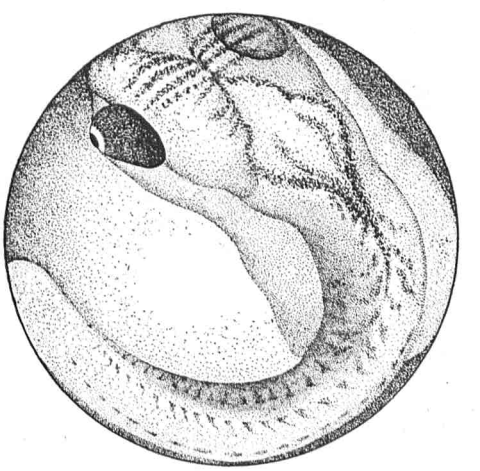

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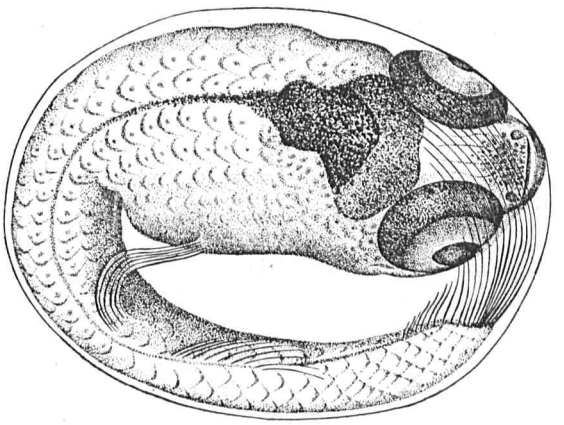
FIG. 5.—Embryo with 3–4 somites, x 50.
FIG. 6.—Embryo with 12–14 somites, x 50.
FIG. 7.—Embryo with about 12 somites, side view, x 50; *H*, heart.
FIG. 8.—Embryo with pigmentation started, inclosed in ovarian follicle, x 45.



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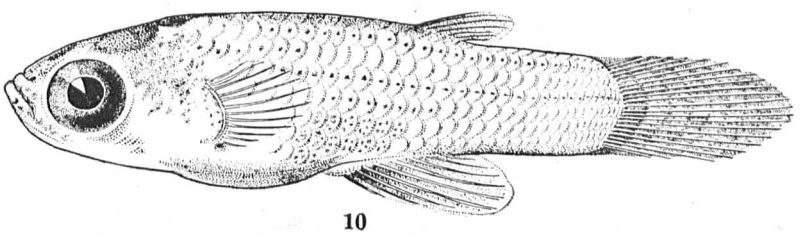

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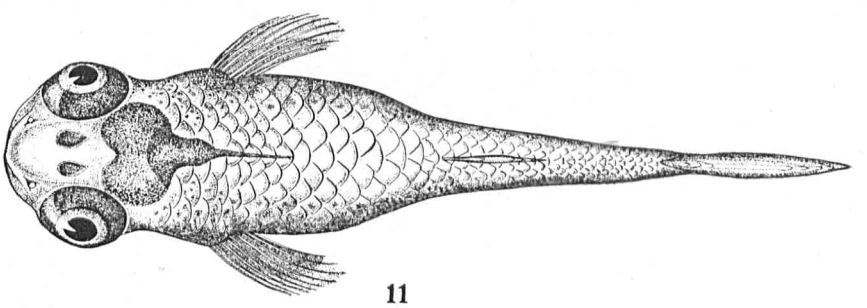

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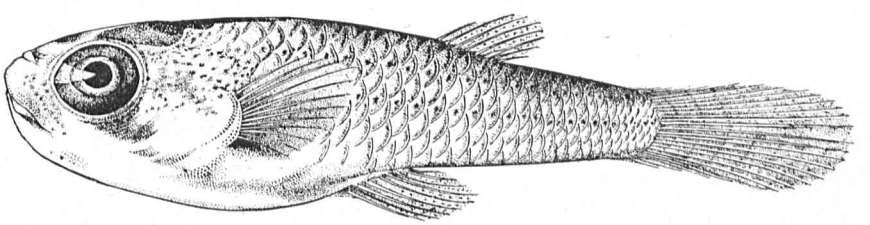

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FIG. 9.—Advanced embryo inclosed in ovarian follicle, x 30.
FIG. 10.—Embryo with yolk sac nearly absorbed, removed from ovarian follicle, x 18.
FIG. 11.—New-born fish, dorsal view, x 15.
FIG. 12.—New-born fish, side view, x 15.

SPOROZOÖN PARASITES OF CERTAIN FISHES IN THE VICINITY OF WOODS HOLE, MASSACHUSETTS

By C. W. Hahn

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SPOROZOÖN PARASITES OF CERTAIN FISHES IN THE VICINITY OF WOODS HOLE, MASSACHUSETTS.

By C. W. HAHN.

While studying the Sporozoa in different species of fish at Woods Hole, Mass., in 1909, the myxospore of one was observed in diseased killifish, *Fundulus heteroclitus* and *Fundulus majalis*. Additional material was obtained and some special experiments were carried out during the seasons of 1910, 1911, 1912, and 1913, the United States Bureau of Fisheries providing the facilities for this and other similar studies at its Woods Hole biological laboratory. [a]

OCCURRENCE OF DISEASE.

When a number of *Fundulus* of either of the common species (*heteroclitus* or *majalis*) are confined in aquaria for a few days during the warm season, one or more thickened white or pink areas appear upon the integument of some of the fishes. The scales of these patches are more or less loosened. They increase in size and number, and the number of afflicted fishes also increases. The fins when involved become bloody and the fin-rays are exposed. Elsewhere the integument disintegrates and the flesh is laid bare. Considerable excavations into the body muscle are not uncommon. The largest cavity of this kind observed was in the head region, measuring about 10 to 12 mm. in diameter and 2 to 3 mm. in depth. Such excavations expose large areas of the skull. When other parts are attacked, loss of blood or penetration of vital parts causes death before the lesion becomes conspicuous externally. The integument is thickened around the sores where the scales are loose. Its color is pink or white. The scales fall out at the edge of the sores. The caudal fin may be completely removed, also the flesh and integument of the tail, thus exposing the vertebræ, before the fish succumbs to the disease. Fish frequently give evidence of weakness and depression even before the flesh has been exposed. There is nothing peculiar about the locomotion except a diminished activity. In certain cases where there is conspicuous inflammation of the integument, especially under the head, the fish may be observed to dart downward, and, with a slight rotation or twist of the body, to scrape the ventral or lateral portion of the head upon the bottom of the aquarium. The fish slowly lose strength, the smaller ones first, and the larger ones not until they are greatly mutilated. Apparently all afflicted fish die unless special care is given to cleanliness, water, and food.

The proportion of fish that are diseased when caught has not been ascertained. The ratio of those that develop integumentary sores in the first day or two to those that are healthy depends to a great measure upon the injuries received in handling the

[a] Valuable assistance from Dr. Edward Linton and Mr. Vinal E. Edwards is gratefully acknowledged.

catch. Sometimes 50 per cent of the *Fundulus* that have been roughly handled, as when stripped for eggs, will become diseased in 24 hours. Of these, half may be dead within 12 hours. If a few crabs happen to be confined in the same aquarium with a large number of *Fundulus*, they inflict injuries upon practically all the fish and all are soon diseased. Uninjured *Fundulus* develop the disease infrequently. (See p. 196.) Roughly speaking, 3 to 4 per cent of the *Fundulus* that are brought into the laboratory at this season (July and August) and confined in small aquaria having but a liter or two of water for each fish, will be found diseased within two days. Within another day or two some of these fish die and a large number die in the course of a week. Diseased *Fundulus* are therefore almost constantly available.

METHODS OF STUDY.

Both fresh and preserved tissues were examined microscopically, the method of handling the tissues being as follows: The scales having been removed with forceps, the edge of a slide is drawn over a diseased area with a little pressure, and the mucus and cellular material thus obtained is spread evenly over the surface of another slide; or, a portion of integument or muscle which has been removed with a scalpel is ground between two thick slides by giving to the upper slide a circular motion. It is necessary to use considerable pressure, and at times cut tough fragments with the sharp edge of the upper slide. Under these conditions, both slides may be preserved for observation and still others made from the ground-up material. Some of the smear preparations made in this manner were examined while fresh and others were fixed and stained. Altogether about 85 fish were examined microscopically. Fresh smears which were sometimes supplied with bile and serum were sealed with vaseline, and could then be examined from time to time, during a period of 24 hours.

For sectioning, tissues were fixed in a saturated solution of corrosive sublimate in 35 per cent alcohol with 0.2 per cent acetic acid and 6 per cent formaldehyde; also in the ether-formalin-alcohol mixture given below. Some of the smears were fixed in the same sublimate mixture; others in a solution of corrosive sublimate in 2 parts absolute alcohol and 1 of ether; still others in a mixture of absolute alcohol (60 per cent), ether (35 per cent), and strong formaldehyde (5 per cent). The mercury preparations are stained with a modification of Mayer's hæmatein. (See Hahn, Archiv für Protistenkunde, bd. XVII, no. 3, p. 316, footnote.) Usually the alcohol and formalin preparations are stained in methylene blue or Giemsa's stain. The methylene blue was extracted in a saturate alcoholic (70 per cent) solution of both eosin and orange G. The Giemsa was washed in water, allowed to dry, and decolorized in carbol-xylol, without the use of alcohol. Some of the more recent preparations fixed by either of the above fluids have been more successfully stained by first treating with hæmatein for several hours, then decolorizing in 70 per cent alcohol with 1 per cent HCl, returning through the alcohols to water, and staining in methylene blue or toluidin blue. After dehydration they were left in a contrast stain (eosin and orange G) for a few minutes and rapidly run into 95 per cent alcohol, carbol-xylol, and two changes or xylol. Both smear preparations and sections are mounted in Canada balsam without cover glasses.

Searching is most satisfactorily carried on with an ocular of 1 inch and an objective of one-fifth inch focal distance. A one-twelfth inch oil immersion objective combined with the same ocular for ordinary observation is supplemented, when occasion requires, with a no. 2 compensating ocular. The one-fifth inch objective is not too high to be

used without a cover glass and reveals most of the details necessary to recognize the presence of Protozoa or other unusual histological conditions. A mechanical stage is indispensable.

Three organisms are involved in most of the *Fundulus* ulcers, rarely a fourth. A thick, short bacillus is the most abundant. A long, slender bacillus is less common. The Sporozoa are represented by a species of *Myxobolus*, and in one case a species of *Chloromyxum*. From the evidence in the following account it will be learned that the primary attack upon healthy tissues, in a certain proportion of the diseased fish, is probably made by the long bacillus. At least a few and probably many of the diseased fish are primarily attacked by Myxosporidia. The short bacillus is more or less incapable of rapid growth in living cells of any kind. While it is not within our province to make an exhaustive study of the fungus diseases, it has been necessary to ascertain to what extent they participate in bringing about these pathological conditions.

EXPERIMENTS TO DETERMINE CHARACTER OF INFECTION.

The following experiments were carried out in order to gain some accurate information as to the conditions whereby healthy fish are infected and the possibilities of their recovery. At the time it was not possible to discriminate between fish that were infected by a fungus and those that were infected by a sporozoön. It will be apparent that the experiments are not vitally affected by the kind of parasite present.

Forty fish were divided equally and placed in two 5-gallon aquaria. These fish had been seined in the usual manner and brought to the laboratory on board the steamer *Phalarope* in large milk cans. The trip from the collecting grounds (Menemsha Bight) usually requires about one and one-half hours. The cans accommodate from 200 to 300 fish each. A hose supplies them with fresh water. The 40 fish used in this case were examined carefully and found to be free from all visible integumentary disturbance.

First stage.—Aquarium no. 1 was carefully cleaned and sterilized. Aquarium no. 2 had contained diseased fish, and 2 diseased fish were allowed to remain with the 20 fish used in the experiment. Contaminated fish from other sources were always kept in this jar. Both groups were fed about every 48 hours. After a period of 11 days none of the fish in the clean jar showed any signs of disease. From this fact we concluded that they were free from the disease and suitable for experimentation of a different kind. After the same period (11 days) the contaminated jar had one fish with a conspicuous sore. It died a day later.

Second stage.—On the eleventh day one of the fish in each of the two jars was operated upon. A scale or two was removed and the integument pierced with a scalpel just back of and dorsal to the opercle. More diseased fish were introduced into aquarium no. 2. Five days later the fish in aquarium no. 1 which had been operated upon died. The integument, at the point where the incision had been made, had developed a typical sore. At this time the fish with the pierced integument in no. 2, being a large fish, had not developed a sore of noticeable extent.

Third stage.—On the sixteenth day of the experiment, all fish having recovered in both no. 1 and no. 2, scales were removed and the integument of all the fish was pierced in the same manner as was done with the two above mentioned. Two days later almost all of those in jar no. 2 had developed marked diseased patches at the very spot where the integument had been pierced. No noticeable change had taken place in the fish of the clean jar. Four days later one fish in jar no. 1 died from the effects of the rapidly

advancing disease. Subsequent examinations of the tissues showed that the probable cause of this disease is a myxosporidian belonging to the genus *Chloromyxum*, being unique in this respect. (See p. 205.) Four dead fish taken from jar no. 2 at this time included two that had been introduced for the purpose of spreading the disease. After seven days the fish in jar no. 1 were all recovering. The incised integument had closed and appeared a little white. Of those in jar no. 2, two were dead, three were seriously diseased and died within 24 hours, and the others had conspicuous sores. The remaining 14 fish from this time began to show signs of recovery, probably because they were not subjected to contamination and they were fed more regularly. Twelve fish remained in jar no. 1 and had completely recovered before the experiment was discontinued.

In the above experiment the treatment given to the two jars was as far as possible the same. Some fish escaped from both jars by jumping out.

The first stage of this experiment, which corresponds with the first 11 days, was not conclusive. One fish, having contracted a fatal disease from a contaminated environment, demonstrates the possibility that fish with apparently healthy integument may acquire the ulcers. The second stage of the test, covering six days, was still less conclusive. But the third stage, covering seven days, showed beyond doubt that the infection enters a lesion of the integument, that contamination favors its entrance, that some of these diseases may be contracted in tolerably pure water, and that lesions which are not contaminated heal completely.

Another experiment of this character was then started, making use of some of all the lots of fish that had been under observation. All were in good condition. Eight fish of fair size were carefully removed from this stock and, by means of a small sterilized scalpel, an incision was made back of the head and a pocket then made under the integument so as to disturb the tissues as little as possible. Into this pocket was inserted a bit of the diseased flesh from sores of four fish taken from different aquaria. As a control, eight more fish of the same size were similarly cut, but nothing was introduced into the pockets. Of the contaminated fish, four died from the disease in two days, the balance in four days. In this case the disease spread over the whole upper part of the body and assumed the characteristic appearance usually encountered. Only one of the controls died. The others healed and recovered completely. From time to time the diseased fish which were introduced into the contaminated jar and those used for the inoculation experiments were examined microscopically. All were infected with bacteria.

This last experiment, covering a period of four days, confirms the results of the previous experiments as to the infectious nature of the disease as well as the inability of the fish to throw off strong cultures of the causal agents. We also learn that when the fish is well nourished and in a wholesome environment, it has considerable natural immunity and recovers readily from the affliction.

In order to prevent the customary mortality from this kind of affliction, care should be taken not to injure the fish while collecting; no crabs or other carnivorous enemies should be confined in the same tanks with the *Fundulus*, and after establishing them in an aquarium without crowding, they should be fed on alternate days. The aquarium should be kept free from dead and diseased fish. With proper circulation of water, this treatment will no doubt reduce the mortality to a negligible quantity and preserve the fish for several months.

PATHOLOGICAL CONDITION OF THE TISSUES.

Those typical sores in which Sporozoa can not be positively demonstrated, and of which a part may be due to bacteria, present the following histological conditions. They are probably primarily exogenous ulcers in which there is at times abundant granular degeneration derived both from lymphocytes and hæmocytes. Sometimes at the nidus of the necrotic area there are small cysts or abcesses containing small lymphocytes. Usually the vascular tissues abound and erythrocytes preponderate. There is a decided tendency at times for the epidermis to form a cicatrix. Again it gives evidence of sloughing off. But so far as the muscle tissue is concerned universal necrosis is common.

The involved epidermis contains numerous nonstaining globules or masses of variable size (fig. 36, pl. XXI), as to the exact nature of which we are yet in doubt. They are also to be found in the connective tissue of the dermis and in certain partly atrophied muscle fibers when adjacent to degenerate tissue. They seem to be more numerous in the epidermal cells wherein there are obvious signs of disintegration (pp. 198, 201, 203). Inasmuch as there is a nonstaining zoöglœa or secretion about some of the bacilli that are commonly found in these parts, which frequently prevents them from staining (see p. 200), it is possible that these bodies are of the same nature and contain one or more of the bacilli. No doubt many are fat globules, but some are certainly not. Some of these bodies in sections of muscle containing myxoplasms possess a well-defined nucleus. (Fig. 12, pl. XX.)

In smears of integument, it is occasionally possible to find fragments of considerable size having the epidermal cells more or less filled with the short bacillus referred to above. It is not difficult to prove, by the observation of fresh material or by comparison of tissues of different stages of degeneration, that the short bacillus is seldom found in normal living cells. It is therefore not probable that the primary attack upon the epidermis is caused by this particular organism. The long slender bacillus is less commonly encountered in the dermis and epidermis. There is but little evidence in support of the view that it is the initial cause of epidermal decadence.

The muscle fibers beneath these infected areas present an interesting condition. To the naked eye there seem to be numerous white threads running parallel with the muscle cells. This is especially true of well-advanced ulcers. When seen under the microscope, such flesh has but few normal fibers with fibrillæ and cross striæ. Most of them have the sarcolemma and interfibrillar connective tissue still sufficiently intact to retain the general external structure of the separate fibers, but the myoplasm is in various stages of degeneration. We conclude, therefore, that the parasite is intracellular and does not pass readily from one fiber to another. The muscle fibers sometimes undergo degeneration more or less uniformly throughout their length. In some cases it is more rapid in the immediate vicinity of the parasites. This we know from sections where the fibrillæ show in places adjacent to degenerate myoplasm in which Sporozoa are numerous. One side or the middle may be far more degenerate than the rest of the fiber. The parasites have probably passed through these regions. The first indication of change is the loss of fibrillation. It is rather difficult to find a parasitized fiber showing normal fibrillation (fig. 13, pl. XX). The pale bands of muscle fibers next become granular (figs. 1 and 2, pl. XX) and at length the sarcous elements break up into large pieces. Eventually there is total granular atrophy of the fiber within the sarcolemma. In certain cases, usually

when the atrophy is hyalin, there are considerable clefts in the sarcoplasm. (Fig. 4, pl. xx). These spaces may come to be more or less closely packed with erythrocytes or leucocytes, or both, so that when the cytoplasm of the blood cells has degenerated a third and common condition is encountered. The nuclei in various stages of degeneration become densely packed and enlarged. They assume amœboid shapes, large alveoli appear in them, and eventually they fall a prey to the short bacillus (fig. 5 and 6, pl. xx) elsewhere encountered.

The conditions thus presented are such as to suggest an amœboid parasite which has demolished a muscle fiber and simultaneously broken up into innumerable bacillus-shaped spores by schizogony. (See fig. 10, pl. xx.) The connective tissue nuclei of the flesh and integument and the nuclei of the gill epithelium give rise to the same degeneration phenomena. Such nuclei may be about equally hypertrophied and massed in such a manner as to completely disguise their true nature. Both muscle and vascular nuclei may occur in abnormal numbers under the sarcolemma of fibers which are in almost any state of atrophy but without clefts. (Fig. 5, pl. xx.)

In both fresh and stained muscle the evolution of a curious artifact was observed. It appears as a dense hyalin body in the sarcolymph, between fibrillæ. (Fig. 3, pl. xx.) Assuming an amœboid form it resembles a rapidly growing organism. (Fig. 2 and 7, pl. xx.) But the regular distribution (fig. 2 and 3) and numerous variations toward a crystalline rosette structure are conclusive evidence of their lifeless nature.

Whatever the active cause of the degeneration of muscle fiber, be it bacteria or Protozoa, the atrophy advances far into one or more muscle fibers without causing any damage to the adjacent fibers. In cross sections of such tissues there may be a small group of normal fibers cut in section amongst numerous others that are wholly degenerate. Capillaries, arteries, veins, and sheets of connective tissue, entirely normal in appearance, may also penetrate these necrotic masses. This is no doubt due to the restraining influence of the sarcolemma upon either the parasite or toxin. As we have already noted, the sarcolemma retains its normal relations in completely atrophied fibers.

Restricting our statements to tissues known to be infected by Sporozoa, there are but two kinds where their action has been observed, namely, muscle, and the connective tissue of the gill. The pathological condition of the muscle tissue, in such cases, is not distinguishable, as far as we know, from that which results from the action of bacteria; but if the pathological changes are to be considered as characteristic of a parasite when it is known to be the cause of the atrophy, a careful study of those cases where bacteria are a negligible factor is important. The myxospores, which are the most easily recognized stages of the Myxosporidia, are common only in smear preparations and only those which include more or less diseased muscle fibers. These same smear preparations also contain cells identical in appearance to myxoplasms, pansporoblasts, and sporoblasts, which happen to be the only representatives of the Sporozoa that we have encountered in sectioned material, thus suggesting their myxosporidian character.

Several fragments of tissue, the integument of which was slightly diseased, were sectioned. They give no evidence of myxospores, but the muscle fibers present practically the same degenerative changes to be seen elsewhere. The dermis contains numerous minute unstained lens-shaped structures similar to those described on page 197. These extend into the ends of the adjacent muscle fibers, becoming less numerous in the deeper parts. Such fibers show obvious signs of atrophy. Elsewhere

there are numerous deep fibers containing many large cells, which vary in size and have conspicuous nuclei. (Fig. 18, pl. XXI.) These are confined by the sarcolemma to a very few fibers and extend for a long distance through them. A small cavity only is excavated about each cell. They are usually isolated, though two or more may occupy the same cavity. The sarcoplasm in such cases is much atrophied, being uniformly granular or homogeneous. A sharp line of demarcation exists between the infected and uninfected parts of the muscle fiber, the former being degenerate and the latter striated and normal. Situated amongst the fibers containing the Protozoa are others lacking them but atrophied in a typical manner, the sarcoplasm being broken into irregular fragments. There are several other foreign and unnatural structures in the sections just referred to, about which the details are given on page 203. Muscle fibers packed with blood tissues and degenerate nuclei have not been found in any of the sectioned tissues which contain unmistakable cases of Myxosporidia; but no special significance has been attributed to this fact.

Smears of gill filaments stained with Giemsa stain present the following conditions: Both normal and degenerate tissues are encountered. In some places the cartilage supporting structures have been attacked and are partly disintegrated. The general external form of the supporting tissue, including the surrounding connective tissue and epithelium, are, as a rule, partly maintained; but elsewhere the degeneration is complete. Epithelium and connective tissue cells disappear completely, leaving the elastic fibers and blood elements. Here, as elsewhere, the nuclei of the latter are most persistent, especially those of the erythrocytes. A large portion of the expressed fluids is composed of an acidophile substance containing odd-shaped portions of the fused nuclei. The spaces between the chromatin threads of the latter having become much dilated, fuse and form large masses of network. These are mechanically separated on crushing the tissue. Such masses of nucleic acid or degenerate chromatin have unbroken connections with the normal blood in the arteries or veins of the less disturbed tissue. Where the blood emerges from partly degenerated blood vessels, they are filled with atrophied erythrocyte nuclei. It seems probable that very large masses of homogeneous eosinophil material, which are constantly associated with the degenerate gill tissue, are derived from hæmoglobin, lecithin, etc., of the stroma.

Myxospores abound in these degenerate gill tissues, especially in the purulent residues of degeneration where nothing else remains recognizable. They also occur deep in the connective tissue near the cartilage and amongst the capillaries. The spores, developing spores, sporoblasts, and pansporoblasts, in all stages, are clearly defined, apparently unaffected by the conditions where tissue cells have become wholly atrophied. This fact, together with the great abundance of myxospores and developing myxospores, both occurring in considerable clusters, prove beyond question that the primary cause of necrosis in this case is the *Myxobolus*. No bacteria or other possible agents have been encountered.

BACTERIA ASSOCIATED WITH ATROPHIED TISSUES.

The small bacillus above referred to (p. 195) varies greatly in size. The smallest (fig. 8, pl. XX) measure less than 0.7μ in thickness and 1.5μ in length. The large ones (fig. 9, pl. XX) average 1.5μ in thickness and 7μ in length. The former are homogeneous when stained. The latter frequently appear to have very conspicuous granules just

inside the cell wall. These are probably artifacts. The older bacilli (fig. 9, pl. xx) taper at one end. They were at first taken for protozoan spores. These bacilli occur by thousands in and near degenerate epidermis and muscle tissue. It is not unusual to find them grouped in the form of the cell which they have completely destroyed. They are then of nearly uniform size (fig. 10, pl. xx); but between individuals of separate groups, there is often a great difference in size. They stain, as a rule, with methylene blue, gentian violet, toluidin blue, and Giemsa stain. Inside the host cells (fig. 6, pl. xx) and when first set free from them they stain, if at all, with great difficulty. This may no doubt be due to a zoöglœic condition. In smears, the stretching of this secretion causes the bacilli to be drawn into long parallel rows. The secretion then resembles elastic connective tissue fibers and the bacteria replace the connective tissue nuclei. At times the zoöglœa is not noticeable. (Fig. 8 and 10.)

To what extent toxins emanating from the short bacillus are the cause of the death and disintegration of the host tissues we can judge from the following facts: As already stated, this bacillus is not to be found throughout large areas of atrophied muscle and integument. If the toxin emanating by diffusion from a localized organism brought about the decadence of a tissue, one would expect the evidences of such decadence to indicate a uniform advance of said toxin in the same direction through a given tissue; but, as we have seen, the atrophy of muscle fibers is limited to a certain few in a large number of normal cells, or there may even be a few normal fibers extending through and far into a necrotic region. The same relations prevail more or less in the epidermis. If the short bacillus is to be regarded as a saprophyte, then some more virulent primary organism must be present. In the diseased gills the abundance of *M. musculi* and the extent of injury in its immediate presence point to the sporozoön as the primary agent. There are a few places in the gill tissue where the short bacillus is abundant, but, as would be expected of a saprophyte, in very degenerate tissue only. Such seems to be its relation to all the tissues.

There are also tissues in which nothing but the long bacillus can be recognized as the agent of primary degeneration. While never abundant, it may be observed more frequently than the short bacillus in fresh smears of infected tissue. After about 24 hours the latter appear in clusters in the muscle fibers occupying excavations of regular ovoid contour. The long type occurs less frequently in tissues that are completely atrophied than in those which just begin to show signs of decadence. Fresh muscle, in the latter condition, may have the long bacilli more or less abundantly distributed under the sarcolemma, but never in compact groups, a condition which is characteristic of the short form. In sections, the long type has been encountered, one or two at a time, in muscle fibers at or near the region of advancing degeneration, and occupying irregular transverse clefts in the scarcoplasm (similar to those in fig. 4, pl. xx). But these cavities seem to be much too large to be considered the excavations of so few of these minute organisms. Their toxins may precede them and the transverse cleavage of the muscle fiber may be due to subsequent mechanical forces. On the other hand, the bacillus is quite as likely to creep into the crevices in the sarcoplasm as are the blood tissues (p. 198). Its presence is therefore not necessarily evidence that it is the cause of the crevices. In one stained smear, some of the muscle fibers of which are completely hypertrophied, the long bacillus is very abundant, especially in the connective tissue. There is no evidence of the admixture of fluid from purulent tissue such as is frequently

common when the short bacillus occurs abundantly; nor are there any of the short bacilli. The normal striated fibers possess few if any of the germs and they seem to be numerous in proportion as the sarcoplasm is degenerate. These are not the conditions we would expect of a virulent parasite unless its primary attack is through the agency of a toxin. There is a second factor to be considered, however, inasmuch as numerous myxoplasms and autospores of *M. musculi* occur in some of the less decomposed portions of the same tissue. With the evidence at hand bearing upon the virulence of the two bacilli, the most natural conclusion is that the short bacillus is a saprophyte, that the long bacillus is either a facultative parasite upon the post tissues, which has been reduced in vigor by the Sporozoa already established therein, or perhaps a true parasite, in which case there are frequent double infections, the long bacillus and Myxosporidia together preparing the way for the saprophytic short bacillus.

The long and short bacilli are easily distinguishable by their size, shape, and habits. The long bacillus is 0.7μ in diameter and usually at least 2.5μ long, but it may be 22μ long, without any noticeable increase in diameter. (Fig. 11, pl. XX.) They have tapered ends, especially those which have but recently divided. Sometimes the long type divides, forming short rods, but they are then in chains. They never occur in clusters as in figure 10, plate XX. The short type is never coiled, never so long, and always thicker than the long bacillus. They are both encountered in smears which include the fluids of completely broken-down tissues, but the short form is always abundant in such fluids, while the former is rare. One is frequently clustered and in regular pockets, the other isolated or scattered and, if in cavities at all, they are irregular crevices.

SPOROZOA ASSOCIATED WITH ATROPHIED TISSUES.

From the evidence in the foregoing pages and borne out by that which is to follow, it is certain that a sporozoön causes the primary degeneration of muscle, gill, and possibly integumentary tissues, resulting in pathological conditions which are quite as characteristic as when the bacillus is the primary parasite. In one tissue which was sectioned (fig. 18, pl. XXI) the degeneration of the muscle fibers is identical to that where bacteria alone have been observed (p. 200). The atrophied fibers, which contain numerous scattered Sporozoa (p. 198, 203), occur in groups of two or three here and there throughout the fragment of flesh. Frequently, in both sections and smears, degenerate muscle fibers occur in which there are cells similar to the above but with neither nucleus nor cytoplasm stained; also large amœboid masses of granular cytoplasm without any visible nucleus (fig. 13, pl. XX). Usually such foreign cells occur in tissues when either myxospores or multiplicative stages are more or less abundant.

In one or the other of the above stages the sporozoön has been positively identified with the disease in 18 of the 85 fish which have been examined. On the other hand, many degenerating fibers have been encountered both in smears and sections in which neither Protozoa nor bacteria could be found. In such cases there is about equal lack of evidence that either of the above are the causal agents of such disintegration. While it is probable that the majority of the sores are caused by the inoculation of a wound by a germ, there is less evidence of a primary attack upon the tissues by the bacteria, except through a widespread toxin, than by Sporozoa. In this connection there is probably a significant difference in the external appearance of diseased tissues which are primarily due to the sporozoön attack and those which are caused by bacteria.

Certain fish in which the diseased parts were conspicuously congested (ventral part of the head, around the anus, and about the eyes) were almost invariably found to contain a large number of myxospores. When we consider the unknown stages of the Sporozoa which, according to the cyclic habit of these organisms, advance from stage to stage in a given culture at nearly the same rate, there is reason to attribute to them more destruction than our observations warrant. Our present lack of knowledge is no doubt due in part to the inadequate stains that have been employed and in part to the confusion of tissue cells with certain stages of the myxosporidian cycle. (See also p. 205.)

STAGES OF MYXOBOLUS MUSCULI.

Mention is made in the literature of but one other case of mxyosporidian disease of the integument and flesh which is closely allied to that of the *Fundulus*, namely *M. lintoni* of *Cyprinodon variegatus* (Linton, 1889–1891). With this one exception, similar diseases in other American and European salt-water minnows, as far as we can learn, have not been described. The *M. lintoni* of the *Cyprinodon* was at first supposed to be identical to the *M. musculi* of *Fundulus*. But very recently a tumor of the variegated minnow was encountered. (See p. 206.) Both the spore and the tumor are markedly different from the common condition of *Fundulus*.

The myxoplasm of *M. musculi* produces a great many pansporoblasts, each with a single spore. There is a large vacuole in most of the spores which is the characteristic iodinophilous vacuole of the genus *Myxobolus*, to which the parasite undoubtedly belongs.

Of the life history we have the spore, pansporoblast, possibly the myxoplasm, schizont, and multiplicative or autospore. In but 3 of the 18 fish which harbor Sporozoa have we stages (figs. 20, 21, 26, 27, pl. XXI) that can be unmistakably connected with the spore. By association in the same tissue or by the appearance and staining reaction we have probably identified the myxoplasms and autospores.

According to Auerbach's (1910) description of *M. bergense*, the spore terminates the life cycle in a given host and starts a new cycle in a new host. We can but assume that the trophoplasm of *M. musculi* likewise arises in some way from a primary myxospore. The trophoplasm (fig. 12, pl. XX) is difficult to stain, and therefore its sporozoön properties are not always certain. (See also *Chloromyxum* properties, p. 205.) Spherical or oval spaces in the diseased myoplasm and in the epidermal cells (possibly identical, fig. 36, pl. XXI, and p. 197) are very abundant. These are probably multiplicative trophoplasms, unless we have confused them with fat or other nonstaining substances. Sometimes these bodies have nuclei (fig. 12) which, though usually faint, may stain deeply. It is not impossible that some of these small trophoplasms may be those of the *Chloromyxum*. When large, the trophoplasms have a granular structure (fig. 13, pl. XX) and are doubtless preparing to undergo schizogony. We have encountered but five or six such schizonts. In one series of sections they are associated in diseased muscle fibers with cysts containing many spores. (Fig. 14, pl. XX.) The amœboid form of the mature schizont is characteristic and distinguishes it from the smaller forms. The schizont in figure 13 is 33μ wide by 74μ in length. Some of the cysts are about this size, but figure 14, which is 19μ wide and 24μ long, is a section through the small end of a cyst of only moderate size. The cysts are found both within and between the muscle fibers. They contain several hundred spores, the nucleus of which, like that of the trophoplasm, has at times little affinity for the stains we have employed. The spores sometimes

appear to be spherical in form (fig. 14, pl. XX) and vary somewhat in size. They have a small faintly-staining nucleus and hyalin nonstaining cytoplasm. Isolated spores and masses of spores recently discharged from the cysts also occur in the smear preparations associated with the intrafibrillar masses of material that appear to be equivalent to schizonts. These spores also occur in small numbers in the diseased gill where myxospores and sporoblasts are to be found in very great numbers.

The occurrence of a multiplicative process of reproduction amongst the Myxosporidia in the manner here described is not uncommon. We have authentic cases in gall parasites of the flounder, and they have been described in *M. pfeifferi* (Keysselitz, 1908) and in *Henneguya gigantea* (Nemeczek, 1911). While there is no question but that there are multiplicative spores, our evidence that the spore here described is such is, as with the trophoplasm, far from conclusive. Judging from the meager evidence at our disposal, there is about equal reason for considering it a young sporoblast or a young trophoplast. It is more harmonious to regard these amœboid spores as the progenitors of both multiplicative and propagative trophoplasts and the oval spores, which are described below (p. 204) as sporoblasts, more especially since they apparently arise by free cell formation and in smaller numbers.

The propagative and sporoblast stages have been encountered frequently in both sections and smear preparations. One series of sections of diseased integument and muscle (referred to on p. 198), which were cut approximately at right angles to the body surface, contains numerous large cells (fig. 18, pl. XXI) with small well-stained (with hæmatein) nuclei. Some of the muscle fibers are cut obliquely. They lie in the midst of healthy tissue and under integument which is apparently healthy. These myxoplasms are of oblong or spherical form with more or less even surface. The cytoplasm is tolerably homogeneous and does not retain the stains. The karyoplasm is also unstained, but the chromatin is somewhat conspicuous. These cells occur abundantly throughout 60 or more sections, in 6 to 8 adjacent fibers, also in other distant fibers. Upwards of a hundred perfectly normal fibers around them have not a single foreign cell. Such cells are always intracellular. The sarcoplasm is considerably modified. The fibrillar structure is lost and the appearance is almost homogeneous.

A cell similar in every respect to the myxoplasm just referred to, occurs abundantly in smears of muscle tissues. It stains less readily than do leucocytes and has smaller nuclei with less conspicuous chromatin. Myxospores and pansporoblasts have been found in their midst, in fact are to be found on slides where this type of cell occurs and not elsewhere. In this connection it is interesting, and perhaps additional evidence of relationship, that the same sore from which the sections containing these myxoplasms (fig. 18, pl. XX) were made also supplied a smear preparation containing numerous myxospores and pansporoblasts represented in figure 26, plate XXI. The sporoblast resembles the myxoplasm of smear preparations in shape, clear, nonstaining cytoplasm, size, and feebly staining (with methylene blue) nucleus. It is for the above reasons that this type is assumed to belong in the propagative cycle.

There is a wide range of conditions to be seen in the nuclei of these myxoplasms, as well as some variations in size. Some densely-staining, cigar-shaped bodies (fig. 17, pl. XXI) almost devoid of protoplasm are embedded in the sarcoplasma, and others are closely applied to the myxoplasms (fig. 18, pl. XXI, near right-hand upper corner). The

conditions suggest conjugation, but the stages are too few to indicate a succession of events. One myxoplasm contains two oblong spores. Elsewhere, replacing a degenerated muscle fiber, are numerous small cysts (12μ in diameter) with eccentric nuclei, which contain from four to ten or a dozen clearly defined oblong spores (fig. 16, pl. XX). These spores are found abundantly in other fish. (Fig. 19, pl. XXI.) They appear to arise by free cell formation. They are characterized by a transparency and a failure to stain that recall both the trophic stages and the sporoblasts. The nucleus, however, does stain faintly. It is quite large when the spores are set free. The latter measure 4μ by 2.5μ and sometimes assume a spherical or amœboid form. Between this condition and the mature sporoblast we lack recognizable connecting stages. They are not far removed, however, from the latter, which are spherical cells with very large nuclei. (Fig. 35, pl. XXI.) These occur in the gill above mentioned and have there been definitely connected with the myxospore. The pansporoblast has been encountered, along with spores and sporoblasts, in fresh smears of muscle. These are apparently identically homologous to those described for *M. pfeifferi* in the gills of the barbel (Keysselitz, 1908). If so, the sporogenesis there related would appropriately apply to *M. musculi*. Many stages in the genesis of the spore are represented in one of our smear preparations. These have propagative stages (Keysselitz, 1908) as follows: First the sporoblast with large nucleus (fig. 35, pl. XXI) and two-parted pansporoblast (sporocyst) (fig. 22, 23, pl. XXI), which, according to Keysselitz, arises after a process of autogamous conjugation. The sporocyst apparently sets free the sporocytes before sporogenesis has proceeded far (fig. 21, pl. XXI). Giemsa stain does not reveal all the nuclei concerned in sporogenesis. Valve cells are formed (fig. 24, pl. XXI) before the polar capsules appear as large spherical bodies (fig. 25, pl. XXI). Later the myxospore becomes elongated and tapered (fig. 20, 26, pl. XXI). Two preparations have multitudes of immature spores. They are all free from the sporocyst protoplasm and have thick valves. It is therefore rather perplexing to explain figures 20 and 26. Perhaps the spore is about to be discharged in figure 20. Considerable variation in this respect occurs amongst some of the gall Myxosporidia.

There are myxospores in 12 of the 85 fish examined. In but 3 of these do they occur in great numbers. With two exceptions (in diseased gills), the myxospores are not assembled in a manner that would suggest their origin from cysts or masses of pansporoblasts, as is common in other species of Myxosporidia. The two cases referred to may not be interpreted as evidence of this condition, but rather that the pansporoblasts, where very numerous, have been packed close together. There are at least a thousand well-stained spores in the preserved tissues. Not one occurs in the 10 tissues of which sections have been made. But those same tissues which contain spores have supplied all the propagative myxoplasms.

The myxospores are very small (fig. 28, 29, pl. XXI). They average 14.3μ in length and 6.7μ in width. In one fixed individual the plane at right angles to that passing through the polar capsules is presented. It measures 6.7μ in thickness, from which we conclude that they are approximately circular in section. But another fresh spore was flattened in a plane perpendicular to that of the polar capsules and sutures to about two-thirds its width (fig. 30, pl. XXI). The polar capsules of myxospores average 6.5μ in length and 2μ in thickness. When extruded the filament is three to four times the length of the spore (fig. 29, pl. XXI). Coiled within the polar capsule, the filament makes from

10 to 14 turns (fig. 26, 28, pl. XXI). In young spores the valves are quite thick and may be seen at the edges as a pale border to the spore, but in mature spores they are thin and almost invisible. Young spores are shorter (12μ) and wider (7.5μ) than the mature spores and the polar capsules are not so long (6μ). They lengthen out as they approach maturity. When young, the nuclei stain with great difficulty, if at all. The sporoplasm occupies all the space at the large end of the spore. A large vacuole is nearly always visible in the sporoplasm. There are also dense areas and from 1 to 10 nuclei. (Fig. 20, 28, pl. XXI.) The nuclei are unstained in figure 29, plate XXI. There are sometimes seven greenish-blue nuclei (fig. 28, pl. XXI) and three rather irregular dark-blue bodies between the polar capsules. It is not possible to be sure that this (10) is the maximum number as some of these are ill defined. A number of spores have their nuclei attached near the large end of each polar capsule, thus identifying them as the "polar capsule" nuclei. Probably the remaining four belong to the sporoplasm. It is not possible to recognize the "wall nuclei" at this stage, and the "resting nuclei" of the pansporoblast are doubtless lost.

CHLOROMYXUM FUNDULI.

The Chloromyxa which have been observed in the muscle of other fish (p. 208) are not identical to that found in *Fundulus*. *C. quadratum*, which resembles the latter, has myxospores measuring 6μ in diameter by 5μ along the polar axis, while the *Chloromyxum* of the *Fundulus* measures 7.5μ in diameter and 6μ along the polar axis and differs in shape. The spore of *C. quadratum*, when seen in line with the polar axis, has the sides deeply concave, and in the other plane it is more pointed. The polar capsules are also much shorter. They also differ in the relation of the spore to the pansporoblast and in the pathological effects. (For description of spore of *C. funduli* see p. 208.) No reference to a myxospore of this character has been found by the writer. The name *C. funduli* has therefore been applied to this species.

The myxospores of *C. funduli* have been encountered in but one fish. If the myxoplasm occurs in other preparations, it has not been possible to identify it, although many suspected myxoplasms exist. It is not very probable that they are at all uncommon. They do not take up a particle of such stains as we have employed. The single slide containing this species is a smear preparation made from the diseased flesh of a fish which died in jar no. 1 of the experiment reported on page 196. It is stained with Giemsa stain.

The muscle of this fish is in an advanced stage of decomposition. When the fresh slides were examined no myxospores were noticed, being difficult to see without a stain, but the sporoblasts were observed without recognizing their importance.

Bacteria are present on the slide but lacking in the muscle fibers. The decadence of the muscle must in this case be ascribed to the *Chloromyxum*, which is abundant in the hypertrophied muscle. The muscle is full of cavities containing unstained myxoplasms and sporoblasts which are identical in appearance to those of many other preparations of diseased *Fundulus*. While this case introduces the possibility that many of the *Fundulus* cancers may be caused by *Chloromyxum funduli*, it gives very substantial support to the agency of Sporozoa as the cause of these diseases. Since *Chloromyxum* and *Myxobolus* are not uncommon in muscle tissue, double infections are to be expected. But having failed to encounter myxospores of the *Chloromyxum* in over 100 stained

preparations that have been examined, we are inclined to consider the *Myxobolus* more abundant and therefore the more common causal agent.

The myxospore of *C. funduli* is about 7.5μ in diameter, with a polar axis somewhat shorter (6μ). At right angles to the polar axis, it is circular. There are four polar capsules, which taper to the apex of the spore, curving so as to conform to the constriction of the spore, which provides it with a blunt pointed apex (fig. 31, 34, pl. XXI). There are four conspicuous nuclei (black), one near the base of each polar capsule. The sporoplasm is stained a pale blue by the Giemsa. The polar capsules do not stain (fig. 31, 34, pl. XXI). There are occasional myxospores of considerable size to be seen inside the sporoblasts when the latter do not take up a particle of stain. Such clear hyalin amœboid pansporoblasts are numerous throughout the sarcoplasm. They vary in size from a diameter of about 2μ to four or five times the diameter of the spore.

PROTOZOA RELATED TO THOSE HERE DESCRIBED.

Numerous Myxosporidia parasitic upon either integument, gill epithelium, connective tissue, or muscle of fish have been described by other authors. About most of them we have very meager information. *M. lintoni* (Linton, 1889; Gurley, 1893) of *Cyprinodon variegatus* (short minnow), as already stated (p. 202), more closely resembles the parasites of *Fundulus* than any other species of which we know. The difference at first seemed to be slight and to be easily accounted for by a difference in the age of the spores. But when a case of the *Cyprinodon* tumor was finally obtained and examined, the indentity of the parasites in the two hosts, as well as the nature of the lesions, was found to be different. The "irregular fungoid elevations," described and figured by Linton and observed again by the writer, are of the nature of cysts containing spores, located in the integument, whereas the elevated scales in *Fundulus* are due to an infection of the epidermis by bacilli and a subdermal atrophy of the muscle. No tumor or spore-filled cyst has ever been encountered. The *Cyprinodon* tumor which we examined developed in a comparatively short time, probably less than a week, though the period can not be accurately stated. It caused the death of the fish the day following that on which it was first noticed. After the death of the host, the tumor was 8 mm. wide by 10 mm. long, and caused a conspicuous elevation from the back of the fish anterior to the dorsal fin, about 2 to 2½ mm. thick. It was of a yellowish-pink color when seen through the slightly pigmented integument. The scales were practically undisturbed and the integument was completely intact, in this respect differing remarkably from the *Fundulus* sores. Beneath the tumor, the flesh contained intrafibrillar myxoplasms and sporoblasts with occasional spores, while the tumor itself was almost wholly a mass of myxospores, the latter numbering millions. We have already described (p. 197) a totally different condition in the *Fundulus*, resulting from the *M. musculi*.

There is such a difference in the appearance (Linton, 1889, fig. 3) of the spores that they are readily distinguished. One can not be certain, however, that such differences are not due to the comparison of different stages in the development of spores of the same species. We have shown that the spores of *M. musculi* grow longer as they mature and the spore wall becomes thinner (p. 205). This fact would explain in part the discrepancy in the dimensions of the spores from the *Fundulus* and *Cyprinodon*. But, since the spore of *M. lintoni* measures 13.9μ in length, 11μ in width, and 8μ in thickness (at right

angles to the planes of the two polar capsules), and the mature spore of *M. musculi* measures 14.3μ in length by 6.7μ thick, and from 4 to 6.7μ in width (see p. 204), in individuals of apparently the same stage of development, it still seems that a sufficient discrepancy in size exists to supplement the marked differences in the pathological conditions. It may yet prove that the latter are due to the influence of different hosts, inasmuch as we have one case of a *Fundulus* with a typical *M. musculi* lesion, but having spores indistinguishable from those of *M. lintoni* in either size or appearance.

M. lintoni presents another contrast to the conditions in *Fundulus*. In the former, calcareous bodies were observed amongst the spores by Linton (1889) and the writer, whereas nothing of the kind has ever been encountered in the hundreds of *Fundulus* tissues which we have examined.

Although the name *M. lintoni* was for a time retained for the *Fundulus* parasite, the present state of our knowledge will not permit of this assumption. The species "*musculi*" has been adopted because of the interesting and characteristic attack which the trophoplasm makes upon muscle fibers.

The spore of *Myxobolus oviformis* (Thelohan) resembles *M. musculi* very much in appearance, but is less tapered and shorter (Thelohan, 1894).

The following, for one reason or another, are also of interest in their bearing upon *M. musculi*. A "Myxosporidian" of unknown genus and species was found by Linton (1899) in the connective tissue of the entire body of *Notropis megalops* Rafinesque (*albeolus* Jordan), the shiner. The epidermis is marked by dark purplish blotches. The scales are absent in most cases. A "Myxosporidian" of unknown genus and species was observed by Lieberkühn (1854) in the connective tissue of *Gastereosteus aculeatus* (stickleback). The skin is said to have contained cysts. The conditions seem to be unlike those in *Fundulus*. *Cyprinus leuciscus* (Müller, 1841) has been observed with tumors in the integument caused by a species of *Myxobolus*. *M. oblongatus* Gurley produces cysts under the scaleless skin of the head region in *Catostomus tuberculatus* Le Sueur (Gurley, 1891, 1893, p. 234). *M. transovalis* Gurley (1893) of *Phoxinus* (*Clinostomus*) *funduloides* Girard, occurs under the scales and external to the epidermis. "It forms a thin discoidal mass situated in the center of the concave undersurface of the scale." That it is not identical with *M. musculi* is certain from the dimensions of the myxospore (length 6μ, breadth 8μ), the diameter of which, at right angles to the polar axis, is greater than through the polar axis. We have very scanty information concerning the *M. strongylurus* (Gurley, 1893, p. 247), which is found encysted in the skin of the head of *Synodontis schal;* of *M. momurus* (Gurley, 1891, p. 416), known from cysts in the subcutaneous intermuscular tissue of *Aphredoderus sayanus* Gilliams; of *Henneguya nüsslini* Schuberg und Schröder (Leger, 1906), which is found in the connective tissue of the dorsal fin of the trout; of *M. gigas* Auerbach (1907), which thickens the integument at the ventral angle of the gill in *Abramis brama* Linnæus (bream); and of a *Myxobolus* of unknown species described by Borne in 1886 (Gurley, 1893, p. 244), which causes great tumors over the surface of *Leuciscus rutilis*.

In *Coregonus fera* there occurs a common disease of the integument caused by a species (*M. zschokkei*, Gurley, 1893; Zschokkei, 1884), the myxoplasm of which is not known. The cysts lie in the subcutaneous connective tissue and between the muscles. It causes irregular thick patches on the skin, from which the scales drop.

PREVALENCE OF MYXOSPORIDIAN INFECTION IN FISH.

The infection of muscle tissue by Myxosporidia is quite common in fish. A parasite belonging to the genus *Chloromyxum* occurs in the flesh of the young herring and young alewife (Linton, 1891). Both the pansporoblast and spores of a *Chloromyxum* have been found abundantly by the writer inside the fibers, and the spores also assembled elsewhere in large cysts. A fuller account of this species will be published later. The muscle cells of *Callionymus lyra* are also subject to an intracellular parasite (*Glugea destruens* Thelohan, 1891; Henneguy et Thelohan, 1892; Gurley, 1893), the myxoplasm of which has not been observed. It causes the muscle fibers to undergo degeneration. *Chloromyxum quadratum* (Thelohan, 1894) also occurs in the muscles of this fish. It is also reported in the flesh of *Coris julis*, *Syngnathus acus*, *Trachurus trachurus* (Minchin, 1903) and *Nerophis æquoris*. In *Cottus scorpio* the muscle tissue is attacked by *Pleistophora typicalis* (Thelohan, 1890, and 1891; Gurley, 1893). Both pansporoblast and spores have been found, but they are intercellular in position. The muscle fibers are displaced but do not degenerate. *Leptotheca perlata* (Gurley) occurs in the muscles of *Acerina cernua* Linnæus. Of these species there are none that closely resemble *M. musculi*. Numerous cases of Myxoboli are known to inhabit gill tissues. Auerbach (1911) lists 22 species of *Myxobolus* which have been described in the gills of fish. But we have encountered nothing that might be considered identical to *M. musculi*.

The disease of *Fundulus* is remarkably like that which has so frequently caused epidemics amongst the barbel (*Barbus barbus* Linnæus) of European rivers. The latter is caused by *M. pfeifferi* Thelohan (Raillet, 1890; Ludwig, 1888; Thelohan, 1894). It produces both tumors and ulcers and occurs encysted and free in muscle, liver, kidney, spleen, and connective tissue. The tumor when formed does not at all times break through, either into the body cavity or to the outside. It is not an integumentary parasite at the beginning as those of *Fundulus* seem to be. The tumor commonly occurs amongst the connective tissue and the muscles of the body wall. The parasite may be encysted in a thin restraining membrane produced by the host. Numerous individuals of about the same age tend to gather in groups and become isolated in tube-like cysts. The muscle fiber is invaded and undergoes a "vitreous alteration" (Thelohan, 1893) leaving "yellow granulations as degeneration products" (Keysselitz, 1908). Thelohan's figure 5, plate VII (Thelohan, 1894), representing a muscle fiber containing myxoplasms in transverse crevices recalls, very vividly the appearances we have encountered in the degenerate muscle of *Fundulus* (fig. 4, pl. XX). The tumors may soften and become a "stinking abscess containing spores" (Ludwig). *M. pfeifferi* passes through distinct cycles of development which is no doubt the case in *M. musculi*. In April it is in a vegetative stage in which the multiplicative reproduction prevails; later propagative reproduction is encountered and myxospores are developed. The rate of advance of the disease depends upon the temperature (Keysselitz).

Both Keysselitz and Thelohan describe bacteria in tissues of diseased barbel. Keysselitz says bacteria contribute liberally to the formation of the tumors. These bacilli are found only in the tissues infected by Myxosporidia. They prevent the growth of connective tissue and bring about degeneration (gangrene) of the tissue. These bacilli are "as long as the spore" (Pfeiffer, 1890) (6μ, Thelohan) and stain easily with methylene blue and gentian violet. (This is also true of the bacilli of *Fundulus*

diseases.) Pfeiffer mentions threads attached to these bacilli. A coccus is also occasionally found. The presence of bacteria is therefore not necessarily an indication that they are primary as causal agents of disease since *M. pfeifferi* is known to be the cause of the barbel disease.

GENERAL CONCLUSIONS.

I. The sores of *Fundulus* are usually caused primarily by lesions. These may occasionally be due to parasites such as leeches, distomes, and copepods, but usually to rough handling and carnivorous enemies.

II. At least four kinds of germs invade these lesions and bring about hypertrophy of the tissue elements and decomposition, namely, two species of bacteria and two species of Myxosporidia.

III. There is doubt as to the virulence of the bacteria. One species at least is saprophytic. There is no doubt as to the virulence of the Myxosporidia when present.

IV. Cleanliness, careful feeding, and aeration bring about recovery in practically all injured fish. It can not be claimed that fish which are known to have Myxosporidia are curable.

V. The trophoplasm of both species of Myxosporidia attacks the muscle fibers, that of the *M. musculi* also attacks the gill connective tissue.

VI. Blood elements, especially nuclei, give rise to abundant artifacts which are closely associated with the parasite involved.

VII. Sporogenesis of the *Myxobolus* occur infrequently in the muscle and gill tissues.

VIII. Multiplicative spores are probably formed in *M. musculi* in addition to primary sporocytes.

IX. The myxoplasm of both *C. funduli* and *M. musculi* are stained with difficulty and are therefore not easily found.

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EXPLANATION OF PLATES.

With the exception of figures 20, 27, 30, and 35, the drawings were made with the aid of a camera lucida. For figures 3, 5 to 11, 15 to 17, 19, 21 to 26, 28, 29, 31 to 34, and 36 a no. 12 Bausch & Lomb compensating ocular and one-twelfth inch oil immersion objective were used. For figures 1, 2, 12, 14, and 18 a Bausch & Lomb 1-inch occular was employed with the same objective. The 1-inch occular and a one-fifth inch objective were used in figures 4 and 13. All figures have been reduced to two-thirds the size of the camera images. The tube length was 160 mm. and the camera arm 90 mm.

The figures are numbered approximately in the order of development. Figures 2, 3, 5, 6, 7, 8, 9, and 10 are made from the same slide, and figures 13, 14, 16, 17, 18, 26, and 28 are from the same fish.

PLATE XX.

FIG. 1. A bit of infected muscle from a smear of a sore on the side of a small *Fundulus heterclitus* in the first stage of disintegration. Fixed in corrosive sublimate and acetic acid and stained with Mayer's hæmatein. The pale bands of the fiber are beginning to become granular at one end. Fibrin threads have been spread over it in making the smear preparation. (×860.)

FIG. 2. A bit of degenerating muscle fiber. Numerous artifacts and a degenerate erythrocyte nucleus occur in the sarcoplasm. The granular striæ are degenerated sarcolymph. Note the sarcoplasm is also becoming granular. (×860.)

FIG. 3. From a smear of a bit of degenerating muscle in a sore on the side of *Fundulus majalis*. The integument more or less disintegrated, scales entirely absent. Fixed in absolute alcohol, ether, and formaldehyde. Stained in methylene blue, orange G, and eosin. Sarcous elements have lost their sharp rectangular form and are becoming granular. A characteristic muscle artifact is distributed between the sarcostyles and some are just beginning to become amœboid in form. (×2000.)

FIG. 4. A characteristic appearance of a degenerating muscle fiber which may or may not be a later stage than those represented in figures 2 and 3. Neither bacteria nor Myxosporidia are necessarily present in these spaces. Both have been encountered there. (×400.)

FIG. 5. A fragment of degenerating muscle upon and into which erthrocytes and leucocytes have entered. The cytoplasm of the latter is disintegrated and the nuclei are in an advanced stage of degeneration. (×2000.)

FIG. 6. A typical mass of degenerate nuclei containing unstained bodies which are probably zoöglœa containing the short bacillus. There are cords of this material in which the bacilli are faintly visible. Such white areas are not merely transparent spaces but thick masses with stainable protoplasm above or below. (×2000.)

FIG. 7. Artifacts from decomposing muscle fibers. In fresh muscle these are common after 10 to 12 hours, appearing first between the sarcostyles. Older stages assume a more compact form. (See figures 3 and 2.) The stain is a homogeneous pale blue. Maximum length 8.9μ. (×2000.)

FIG. 8. The short bacillus. An isolated group near which are located cells containing white oval-shaped bodies like those in figure 6. Note the variation in size and shape. That one near the "×" sign measures 1.5μ by 7.4μ; that near the "+" sign measures 1.8μ by 1.1μ. (×2000.) (See also fig. 10.)

FIG. 9. Short bacillus older than figure 8. Nearly the maximum size. Note the taper toward one end and the stainable granules. The latter are probably artifacts. Left-hand upper one measures 5.2μ by 1.4μ. (×2000.)

FIG. 10. A cluster of long bacilli which have caused the complete breakdown of a tissue cell and rest in situ. (×2000.)

FIG. 11. Several of the long type of bacilli which are located just under the sarcolemma of a muscle fiber that shows the first signs of degeneration. The small individual in the middle below has dimensions as follows: Length, 4.8μ; thickness, 0.7μ. (×2000.)

FIG. 12. A section cut diagonally through a muscle fiber. This fiber is adjacent to the dermis. On the inner side the sarcoplasm is hypertrophied, on the outer side it retains the fibrillation. The oval bodies are interpreted as trophoplasms of the *M. musculi*. The large one has several spherical bodies which take a deep hæmatein stain, presumably nuclei. (×800.)

FIG. 13. A muscle fiber in which there are the first evidences of disintegration. It contains two or more large trophoplasts or schizonts. The appearance of the cytoplasm is like that of other stages, pale and unstained, there being no sign of the nucleus. There is evidence of a complex system of pseudopodial extensions of the cytoplasm which is characteristic of the Myxosporidia. Large individual 84.7μ by 192.5μ. (×400.)

FIG. 14. Multiplicative spores of *M. musculi*, presumably derived from a large trophoplasm such as figure 13. There is no cyst wall. In adjacent sections are fragments of the schizont nuclei mingled with the spores. The spores stain feebly with eosin and orange G. The nuclei are not stained deeply. 19.3μ in diameter. (×860.)

FIG. 15. A myxoplasm of *M. musculi* in muscle from a smear preparation fixed with absolute alcohol and ether and stained with methylene blue. One side overlies a nucleus of the muscle fiber. The pale bands of the muscle fiber may be seen. The muscle stained deeply and the parasite pale. The protoplasm is finely granular and there is only a suggestion of a cytoplasmic network. The nucleus is vaguely stained. 13.4μ by 18.6μ. (×2000.)

FIG. 16. Formation of sporoblasts of *M. musculi*. This cyst is one of a mass numbering several hundred which occupy a position where a muscle fiber has been completely destroyed. The 10 spores stain very feebly. They lie in slight cavities of the protoplasm. Diameter of cyst 12μ; length of spore 4μ. (×2000.)

PLATE XXI.

FIG. 17. A possible microgamete of *M. musculi* from amongst the numerous myxoplasms of muscle fibers adjacent to that shown in figure 18. The motile shape of several such structures, the small amount of cytoplasm, and close approximation to some of the large myxoplasms are noteworthy. (See right-hand upper region of fig. 18.) 6.5μ by 2.2μ. (×2000.)

FIG. 18. A section of a muscle fiber of *Fundulus heteroclitus* cut crosswise at a slight angle. The scales in the region of this infection had dropped off, and the area was almost white, being slightly discolored by blood. The tissue was fixed in corrosive sublimate and acetic acid and stained first in Mayer's hæmatein, then in methylene blue, later in eosin and orange G. One of the structures in the sarcoplasm, that to the left in the middle, is the nucleus of a muscle fiber. The others are stages in the propagative cycle of *M. musculi*, primary and secondary sporoblasts. The large one in the middle, at the top, is 12.6μ in length and 5.9μ in width. (×860.)

FIG. 19. Three young sporoblasts of *M. musculi* from the smaller type of cysts represented in figure 16, plate XX. Note the increase in the size of the nuclei. They are typically free from cytoplasmic stain. (See *C. funduli*, fig. 31.) Lower individual 4μ by 2.5μ. (×2000.)

FIG. 20. A fresh sporoblast of *M. musculi* containing a spore which is almost mature. From a deep cavity in the flesh back of the head. Interesting in connection with figure 26. (Free-hand drawing, not to scale.)

FIG. 21. Sporocyte of *M. musculi* expelled from pansporoblast. It forms the first stage in the series represented by figures 23, 24, and 25. The nucleus is small and faintly stained, as is the rest of the cytoplasm. It has no external envelope. Diameter 11.9μ. (×2000.)

FIG. 22. A pansporoblast of *M. musculi* (sporocyst) with two daughter cells, the nuclei of which are undergoing autogamous conjugation. (×2000.)

FIG. 23. A pansporoblast of *M. musculi* after the autogamous conjugation and subsequent division of the nuclei.

FIG. 24. A sporocyst of *M. musculi* which has been set free from the pansporoblast. Apparently the sporoplasm remains attached to one myxospore (fig. 20), and the other is almost devoid of external protoplasm. The two wall cells are clearly visible, but without nuclei. The capsule nuclei are probably formed but do not stain. One of the 12 nuclei happens to be in a suitable condition to take the stain. 11.9μ by 13.4μ. (×2000.)

FIG. 25. A myxospore of *M. musculi* with a remnant of protoplasm. Two polar capsules are beginning to form. (×2000.)

FIG. 26. A sporocyst of *M. musculi* from a smear of diseased integument of the mouth and head in front of the eyes. Elsewhere the sporocysts have less cytoplasm. It is the only one encountered in this condition. The failure of the nuclei to take the stain is characteristic. The myxospore is immature, being less slender than older myxospores. The details of the polar capsules are very transparent and stain dark blue, while the spore wall is a very pale blue. The vacuole and sporoplasm are prominent, but the nuclei of the spore can not be clearly discerned. Sporocyst, 17.8μ by 23.8μ; spore, 14.8μ by 7.4μ; polar capsule, 7.4μ by 2.2μ. There are 13–14 spirals in the filament. Fixation: Absolute alcohol, ether, corrosive sublimate, acetic acid. Stain: Mayer's hæmatein, methylene blue, orange G., eosin. (×2000.)

FIG. 27. A sporoblast of *M. musculi* from a fresh smear of degenerated muscle taken from a deep cavity (the same as fig. 20). Easily distinguished from tissue cells by the three nuclei. Protoplasm contains much coarsely granular matter. (Drawn free-hand, not to scale.)

FIG. 28. Myxospore from the same slide as figure 26. The mature spore, when compared with that in the pansporoblast, is longer and more pointed at the polar end. The vacuole is probably an iodinophilous structure. The coiled filaments make 11 to 12 turns. The polar capsule wall is visible, but the spore wall can not be clearly seen. The valves and sutures are also indistinguishable. While there are as many as 12 blue and green bodies present, one can not be sure that all of them are nuclei. Seven or eight bodies are moderately conspicuous. Two lie in the wall of the polar capsules and are doubtless the capsule nuclei. 14.8μ by 6.2μ. (×2000.)

FIG. 29. A myxospore of *M. musculi* from large sores on each side of the tail of a *Fundulus heteroclitus*, caudal fin entirely gone. Fixed in absolute alcohol and ether, stained with methylene blue. Six unstained nuclei in the sporoplasm and one large vacuole. Filament discharged. Spore, 7.4μ by 16.4μ. Polar capsule, 2.2μ by 7.4μ. (×2000.)

FIG. 30. Diagram of the cross section of a fresh myxospore of *M. musculi* as if seen from the end. The specimen was lying so as to present the edge of the valves to view. It is obviously flattened. The polar capsules also appeared to be, but one can not be certain about this. The sutures are straight and symmetrical. Fixation: Alcohol, ether, formalin; Giemsa stain. (This drawing not made to scale.)

[Figures 31 to 34 are all from the same smear preparation of diseased muscle from a dead fish, being one of those taken from jar no. 1 (see pp. 195, 196).]

FIG. 31. Pansporoblast of *Chloromyxum funduli* embedded in a degenerated muscle fiber. The contained myxospore has taken up the stain, but the protoplasm of the pansporoblast is absolutely devoid of visible structure. Note the even contour of the characteristic lobose pseudopodia. 15.2μ by 12μ. (×2000.)

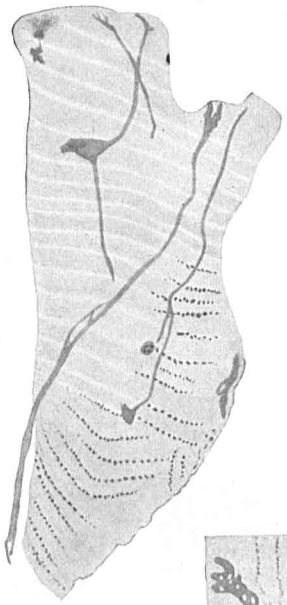
FIG. 32. One of a group consisting of free young myxospores of *C. funduli*. Like the mature myxospores, they stain readily, but their nuclei are not differentiated. They are, as a rule, not quite so irregular, but the pseudopodia are always small and angular. Note the contrast between these and the pansporoblasts. 3.7μ by 4.5μ. (×2000.)

FIG. 33. Myxospore of *C. funduli*. The outline is approximately circular. The sporoplasm is homogeneous but dense around the four polar capsules, doubtless because of the greater thickness at this point. The four nuclei are always associated with the polar capsules, hence are doubtless capsule nuclei. Diameter, 8.9μ. (×2000.)

FIG. 34. Myxospore of *C. funduli* seen from the side. Note the sporoplasm is not much denser about the polar capsules. The sporoplasm tapers to a blunt apex. In many it is more pointed. The polar capsules have long, curved, tapering necks with the large ends far apart. The capsule nuclei alone stain. 8.2μ by 6.7μ. (×2000.)

FIG. 35. A fresh sporoblast of *M. musculi* from the same slide as figures 20 and 27. The cytoplasm is rich in granules. The nucleus is very large and has a conspicuous karyosome. (Not drawn to scale.)

FIG. 36. An isolated epidermal cell derived from a mass near the margin of an advanced ulcer, most of which have numerous unstained bodies like those in the muscle fibers (fig. 12, pl. XX). From sections. The epidermal cell is not typical in appearance, but the unstained bodies are, and are identical to those in the adjacent slightly atrophied epidermis. (×2000.)



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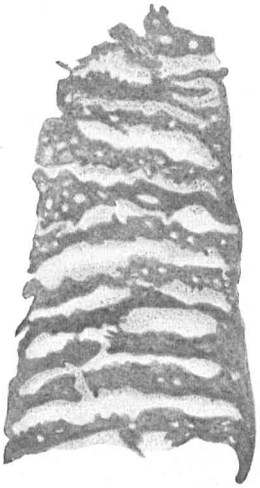

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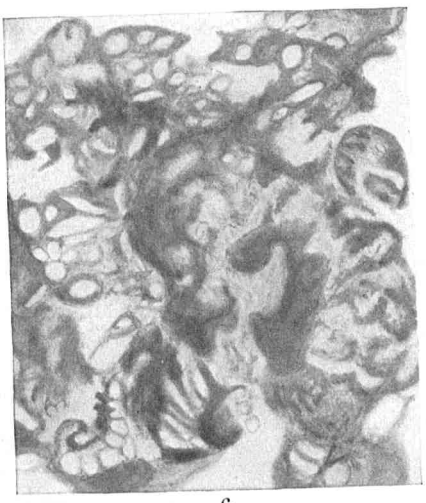

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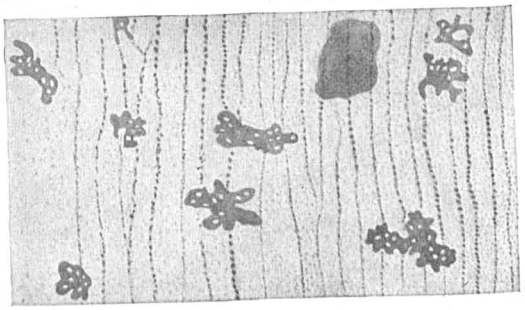

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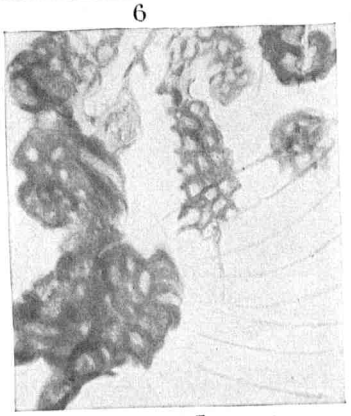

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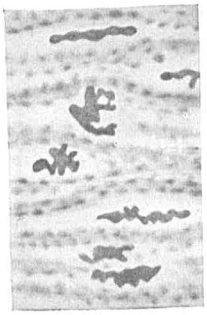

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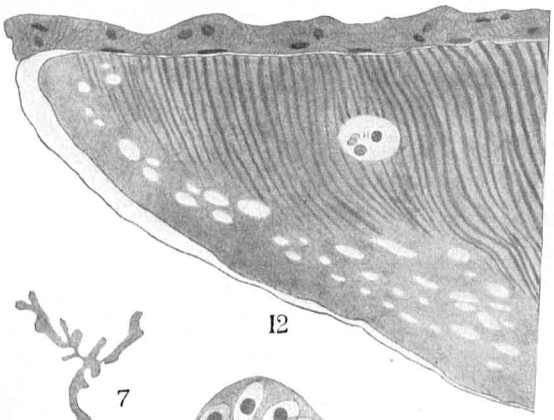

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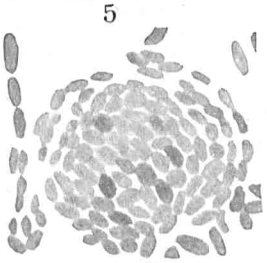

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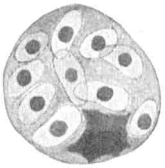

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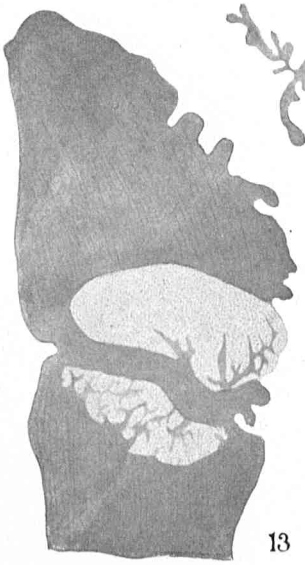

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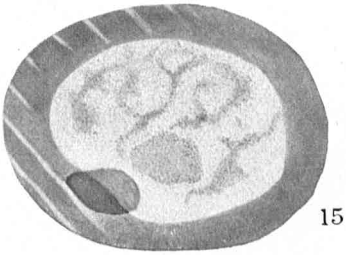

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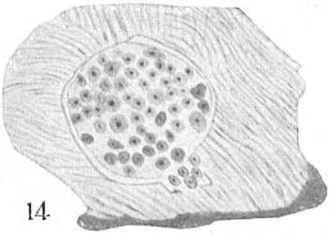

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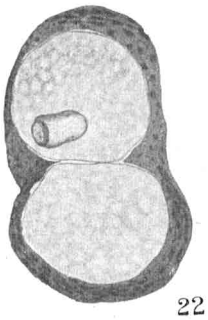
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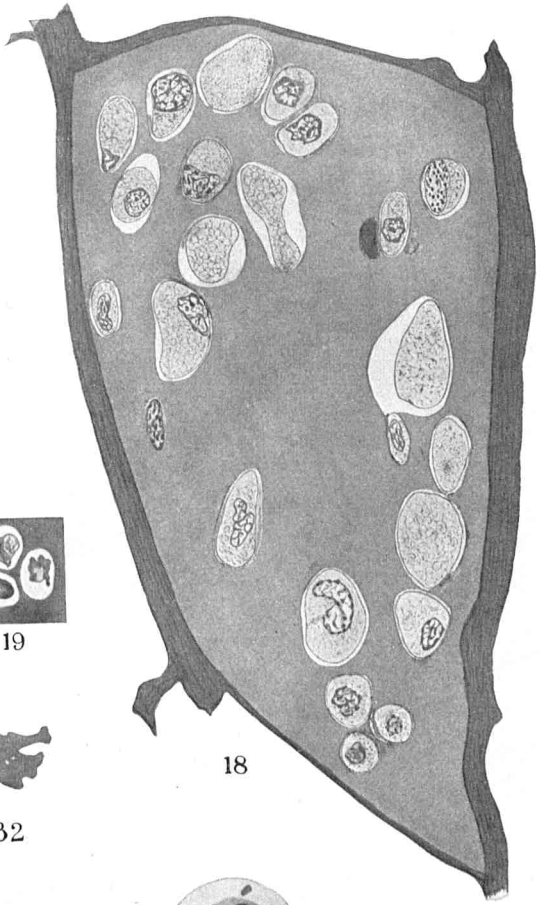
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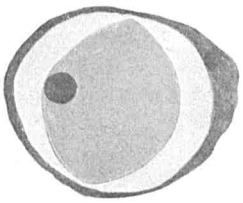
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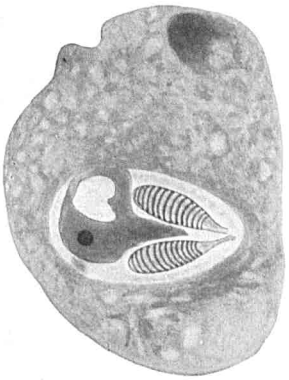
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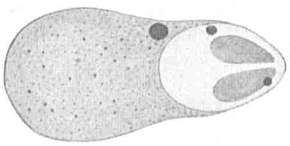
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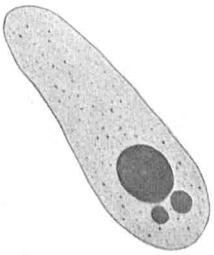
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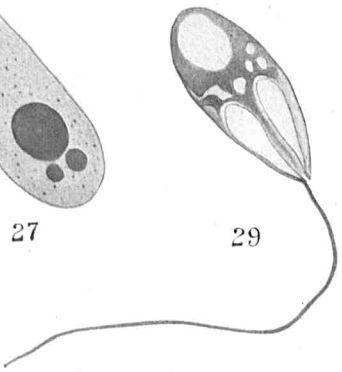
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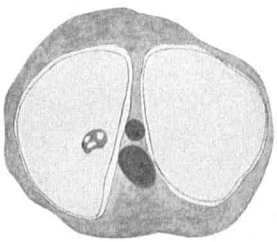
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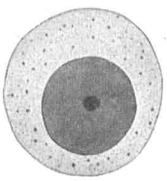
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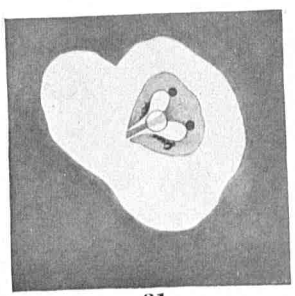
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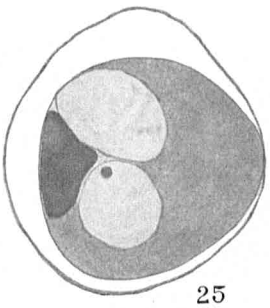
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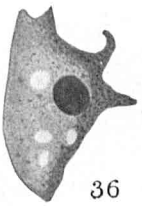
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AN ECOLOGICAL RECONNOISSANCE OF THE FISHES OF DOUGLAS LAKE, CHEBOYGAN COUNTY, MICHIGAN, IN MIDSUMMER

By Jacob Reighard

Professor of Zoology, University of Michigan

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AN ECOLOGICAL RECONNOISSANCE OF THE FISHES OF DOUGLAS LAKE, CHEBOYGAN COUNTY, MICHIGAN, IN MIDSUMMER.[a]

By JACOB REIGHARD
Professor of Zoology, University of Michigan.

INTRODUCTION.

During the summers of 1909, 1911, and 1912 I was in charge of the biological station of the University of Michigan, situated on Douglas Lake. The little time that remained to me after my routine duties was given to collection of data bearing on the ecology of the fishes of the lake. It was my hope after a considerable number of years to reach general principles by the analysis of data thus collected. It is unlikely that I shall continue the work. It seems, then, worth while to put on record such facts as I have. They are few, and the inferences that may be drawn from them are tentative; yet they may furnish a starting point for some one else or suggest a method. The records of 1912 were made under my direction by an assistant, Mr. M. E. Houck.

Douglas Lake—Turtle Lake on many older maps—(fig. 1), lies at about latitude 46° 30′ N., in the Southern Peninsula of Michigan, at an altitude of 712 feet above sea level. Its northern shore is some 15 miles in a direct line from the Straits of Mackinac. Its greatest length from east to west is 3½ miles, its greatest width 2⅓ miles. The lake has somewhat the form of a fish, the flukes of whose tail form North and South Fishtail Bays at its eastern end. The total area of the lake, exclusive of Fairy Island, is about 5.1 square miles; its shore line, including that of Fairy Island, measures 14 miles. The shores are nearly everywhere a mixed sand of granitic origin. The water deepens gradually over a terrace or shoal until it is 3 to 6 feet deep. The terrace varies in width from a few yards to a hundred or more. The bottom then drops rapidly, in most places into deeper water, forming the "slope" or margin, which is as steep as loose sand can lie. The slope is that part of the bottom on which vegetation ordinarily grows. It extends to the lakeward limit of vegetation, usually at a depth of not more than 25 feet. The depth of water at the lakeward limit of vegetation in Douglas Lake is unknown. The deeper water beyond the slope has a depth of 82 feet over a limited area near the southern end of South Fishtail Bay, and a depth of 89 feet at another point. The deeper water does not reach 80 feet over most of the lake and is not continuous but is interrupted by bars and shoals. Pending the completion of a hydrographic map, details are not available.

A white disk lowered into the water on August 12, 1913, disappeared at a depth of 12.5 feet. This indicates that the lake is not rich in plankton, but no plankton

[a] Contributions from the Zoological Laboratory of the University of Michigan, no. 143.

measurements have been made. The bottom temperature at a depth of 70 feet on July 10, 1912, was 47° F. (8.3° C.). At a depth of 82 feet in South Fishtail Bay a temperature of 6° C. has been recorded in July. There is a well-defined thermocline at a depth of 40 to 45 feet. Its unusual distance from the surface is due to the heavy winds which cause the surface waters to be intermingled. Above the thermocline the temperature rises until in August it reaches 20° C. at the surface.

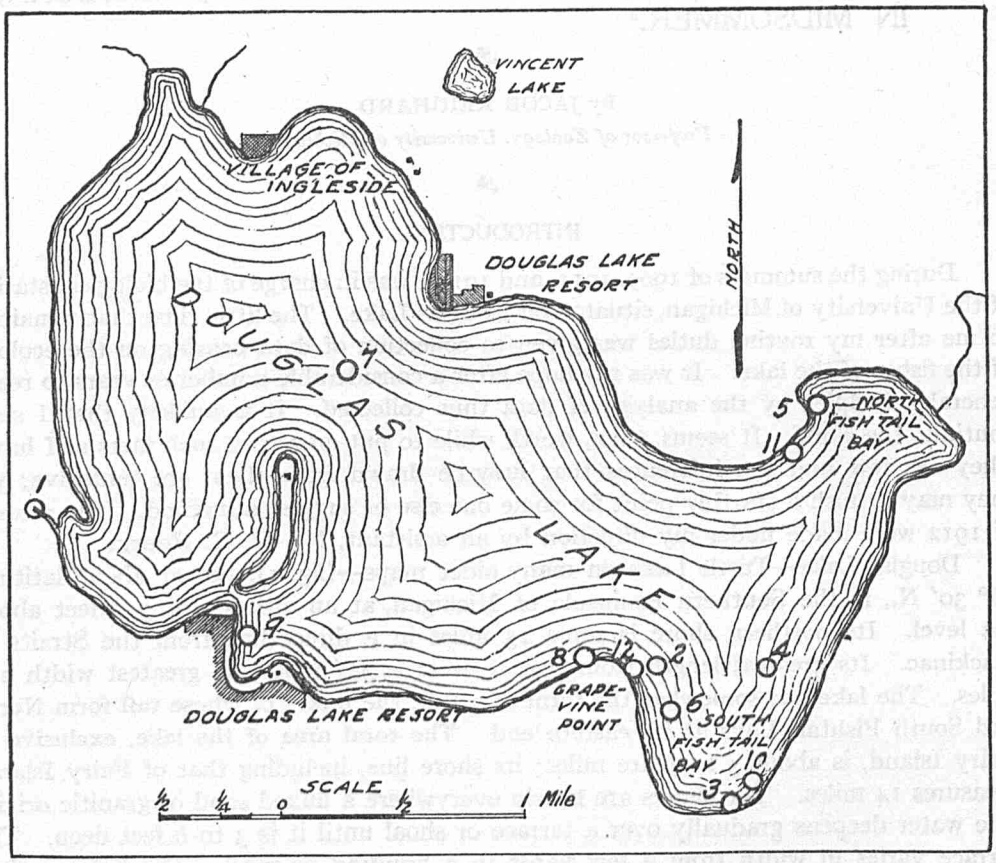

FIG. 1.—Map of Douglas Lake, Cheboygan County, Mich. The numbered circles show where collections were made. 1, Maple River; 2, two hundred yards east of Grapevine Point; 3, at the biological station; 4, sand bar; 5, protected cove on North Fishtail Bay; 6, deep channel east of Grapevine Point; 7, northeast of Fairy Island; 8, stony shoal on Grapevine Point; 9, protected bay at Bryant's landing; 10, east shore of South Fishtail Bay; 11, west side of entrance to North Fishtail Bay; 12, west side of South Fishtail Bay off Grapevine Point.

Above the thermocline in August there is abundance of dissolved oxygen, about 5.5 cc. per liter at the surface and 4.5 cc. at a depth of 33 feet. Below the thermocline the amount of dissolved oxygen is diminished. It varies in August from about 0.6 cc. per liter at a depth of 50 feet to nothing at a depth of 63 feet or more (Tucker, 1913). Below a depth of 45 feet the lake does not afford, in summer, enough oxygen to make it a suitable habitat for fish.

Little is known of the distribution of the vegetation in the lake. It is briefly discussed under fish habitats, but should be made the subject of special study.

Douglas Lake was at one time continuous with the Great Lakes and with Burts, Mullet, and Crooked Lakes to the south of it. The latter lakes continue to be broadly connected with Lake Huron by means of Crooked and Cheboygan Rivers. Douglas Lake, on the other hand, was long since separated from the other lakes. It has no direct connection with the Great Lakes, but is connected with Burts Lake by the Maple River. Thus its separation from the Great Lakes antedates that of Crooked, Mullet, and Burts Lakes and is more complete.

FISH HABITATS OF THE LAKE.

The following four fish habitats of the lake are provisionally recognized. A possible fifth habitat is suggested on page 246.

BARREN SAND-SHOAL HABITAT.

Wherever the terrace is without stones (pebbles may occur in the sand) it may be referred to as a barren sand shoal. The sand is loose and shifting in the more exposed of these shoals and practically always shows ripple marks. In less exposed places the sand particles are loosely united by a deposit of marl, probably of algal origin. This gives a certain firmness to the sand, as though it were mixed with clay and makes it resistant to wave motion. Such protected sand shoals are often free from ripple marks. The shore bordering all sand shoals is low, without an ice rampart of stones, and the first land terrace or bluff is at some distance from it. The shoal may be narrow (only a few yards or more) or wide (a hundred yards or more). The slope on its seaward edge is steep, as steep as loose sand can lie, and the water over the seaward edge is commonly about 4 feet deep. Near shore there may be a sparse growth of bulrushes but there is no other vegetation.

BARREN STONY-SHOAL HABITAT.

Wherever the shore is bordered by a bluff or terrace which is being eroded the shoreward margin of the shoal contains stones or small bowlders. Along such a shore there is commonly formed by the action of the ice a rampart of stones, which borders the shore like a low stone wall. The stony shoal is apt to be wide and the slope beyond it less steep than that of the sand shoal. The water over its outer edge is often 6 or 7 feet deep. Its bottom may be of shifting sand or of sand agglutinated with marl. Its shoreward border may support a growth of bulrushes or may be without them. Regarded as a fish habitat, its salient feature is the stones. Where the shore of the lake shows a series of headlands with intervening valleys, it is being eroded along the headlands and built up between them. The headlands are bordered by stony shoals and the intervening low shore by sand shoals. The two pass into one another without sharp demarcation.

THE VEGETATION HABITAT.

If we neglect the scant growth of bulrushes which may occur on the shoals, the vegetation of the lake is largely limited to the slope. In places the slope is continued into considerable areas of nearly level bottom covered by water less than 25 feet deep and overgrown with vegetation. Such an area, known as the "middle ground," extends

eastward and a little north from the northern end of Fairy Island to the mainland. There are similar areas south and west of Fairy Island. The slope, the middle ground, and similar areas lie in the open lake and are subjected to severe wave action. The water is usually in motion and the conditions are not in this respect unlike those found in a stream. Among the fish is at least one characteristic stream form, *Notropis cornutus*, the common shiner. This habitat may be called the unprotected vegetation habitat.

The vegetation is not emergent and is characterized by absence of water lilies. On the slope the plants are found on the less steep portions and there form a discontinuous fringe or zone. On a still, bright day one may see that the plant growth of the slope consists of little groups of *Potamogeton natans*, millfoil, and perhaps other plants which are 1 or 2 feet apart and in most places do not make dense masses. There are considerable stretches of the slope that are without vegetation. One of these lies opposite the laboratory on South Fishtail Bay. There are a few places in which the vegetation is more dense. On the whole, it occurs in patches or islands and within these it is sparse.

Where the shoals are protected from the wave action vegetation gets a foothold, muck accumulates, and the conditions approach those of a pond with relatively quiet waters. This is the case on the east and west sides of North Fishtail Bay, in the bay directly south of Fairy Island, and at the mouth of Bessie Creek. Water lilies occur in such situations and the large-mouthed black bass is the characteristic but not abundant fish. The common sunfish is more abundant here than elsewhere. This habitat of bays and estuaries may be referred to as the protected vegetation habitat. It contains most of the species of fish to be found in the lake. It merges into the unprotected vegetation habitat. For the present it seems best to treat the vegetation habitat as a unit, although in the future it may be advisable to subdivide it.

THE DEEP-WATER HABITAT.

Beyond the slope near the bottom is the abysmal or deep-water region, where the bottom is of a soft, black ooze and where there are no large water plants. It extends from a depth of 25 feet (probably somewhat less) to 89 feet, the extreme depth of the lake, and comprises the bottom and the layer of water 1 or 2 meters thick above it. Above the thermocline this layer of water is agitated by the wind, is relatively warm and well lighted, and contains in summer an abundance of oxygen. Passing downward along the bottom through the thermocline we encounter within a vertical distance of 7 feet a drop in temperature of some 10° F. As we descend the temperature near the bottom continues to drop from about 54° F. at the thermocline (July 10) to between 43° and 50°, depending on the depth reached. The water below the thermocline is not only cold but relatively quiet, unaffected by wave action, and relatively dark. In midsummer it contains little oxygen at any level and none at all at a depth of 63 feet or more. We have taken no fish below the thermocline in midsummer. They are then to be found only in those parts of the abysmal region that lie above the thermocline between the lakeward border of the vegetation zone and a depth of about 45 feet.

THE FISHES.

Our data concerning the fishes are given below under each species. The locality numbers in the tables refer to the map on page 220. The numbers in the column headed "Water depth" give the distances below the surface at which the fish were taken. They

are usually the depths at which gill and fyke nets were set on the bottom. Lengths of fishes do not include the caudal fin.

LEUCICHTHYS ARTEDI (Le Sueur), *lake herring*, or *cisco*.—This species has not been taken in nets, but adult specimens are frequently cast up on the beach of South Fishtail Bay. Three of them measured 5, 6½, and 7 inches, respectively, the latter a male with slender white testes ⅛ inch broad. A male 6⅞ inches long and with large testes was picked up, still living, over deep water in South Fishtail Bay, September 18, 1911. Our small-meshed gill net has taken suckers of 7 or 8 inches when set on the bottom in water of 26 and of 42 feet depth. It should have taken lake herring if they had been present there. In midsummer the same net has taken no fish when set on the bottom in water deeper than 45 feet, although in September a single sucker was taken at 72 feet. The absence of oxygen in the bottom water below 45 feet in midsummer makes it impossible for fish to live there. The lake herring must therefore live in deep water at some distance above the bottom. Perhaps its habitat will be found in the neighborhood of the thermocline. This species is characteristic of the Great Lakes, where its average length is 12 inches. Our largest specimens are only 7 inches long.

CATOSTOMUS COMMERSONII (Lacépède), *common sucker*.—The records in table 1 show the suckers taken in 1912.

TABLE I.—RECORDS OF CATOSTOMUS COMMERSONII TAKEN IN DOUGLAS LAKE IN 1912.

No.	Weight.	Length.	Sex.	Water depth.	Apparatus.	Stomach contents.	Locality in Fig. 1.	Date.
	Ounces.	*Inches.*		*Feet.*				
1	12	11.2	Male	4.5	Fyke	Empty	2	July 29
2	14	11.6	?	4.5	Gill	Not examined	2	July 31
3	10	10.8	?	4.5	Fyke	do	2	Aug. 3
4	9	11	Female	4.5	do	Empty	4	Aug. 5
5	5	8	do	5.5	do	do	4	Aug. 6
6	4.5	7.6	do	5.5	do	do	4	Aug. 7
7	4.5	8	Male	5.5	do	do	4	Aug. 10
8	6	8.8	do	5.5	do	do	4	Do.
9	14.5	12.4	do	5.5	do	do	4	Do.
10	10	11.8	do	5.5	do	do	4	Aug. 15
11	6	8.8	Female	26	Gill	do	6	Aug. 16
12	4	8	Male	26	do	do	6	Do.
13	3	7.2	Female	43	do	do	6	Aug. 19
14	5	8.4	do	6	do	do	3	Aug. 2

Common suckers have been seined in Maple River and have been seen at the mouth of Bessie Creek. There can be no doubt, then, that they occur over the whole lake and are amongst its commoner fishes. They are found in all the habitats. On September 23, 1911, one was taken in a gill net drawn from a depth of 72 feet. In July and August suckers have not been taken below the depth of 43 feet. They are sometimes seen feeding on the sand shoals in water a foot or two deep. They may therefore occur at any depth in the lake, but are not known below the thermocline in midsummer.

Food.—The young of this species are seen on the sand shoals in July and August in company with the young of the yellow perch and the spot-tailed minnow. In a school of 475 of these young fish taken on September 1, 1911, five were suckers between 1¾ and 2 inches long. The alimentary canal, from œsophagus to anus, of an individual 2 inches long was found to measure 3 inches. Its contents formed a brown mass inclosed in a mucus pellicle. The whole of it could be easily stripped from the canal. The contents

when forced out of the pellicle, proved to be wholly shells of a species of cladoceran, apparently *Chydorus*. A three-sixteenth-inch piece was cut from the middle of the alimentary canal where it is of average size, and the Crustacea in an estimated fifth of this counted. From this the entire number in the alimentary canal was estimated at about 2,400. Only 2 or 3 copepods were found in the sample, or 48 for the whole alimentary canal; the rest were Cladocera and all of one species. There was no sand, so that it may be safely said that the young sucker is not a bottom feeder, but lives wholly on the plankton.

The stomachs of 12 of the suckers included in the table were examined and found to be empty. In August, 1912, Prof. Frank Smith saw the adults feeding on the materials encrusting the vegetation at the mouth of Bessie Creek. They were sucking off whatever adhered to the floating stems and leaves of the plants. They went from plant to plant and mouthed over each branch from base to tip until the whole plant had been gone over.

The adult suckers may sometimes be seen at dusk or daybreak feeding on the bottom over the sand shoals. When approached they ordinarily make off at once for deep water. On July 3, 1912, I found several feeding on the sand shoals at midday, and each was surrounded by a group of a dozen or more log perch. The log perch were at that time laying their eggs in the sand and the suckers were feeding on the eggs. Each sucker was surrounded by a group of log perch which were trying to get such scraps as might be left from its feeding. It would be interesting to know whether this commensal relation between sucker and log perch obtains at other seasons and in deeper water.

While the suckers are thus engaged it is not difficult to approach them until they are at one's feet and to watch closely their method of feeding. The sucker moves slowly over the bottom. At intervals it stops, raises its tail until, if in very shallow water, the caudal fin breaks the surface. It buries its snout in the sand, often to the nostril, but sometimes only half so far. The fish then withdraws its snout from the sand and without moving from the place, works its jaws for several seconds as though chewing. At the same time a thin stream of sand is seen to come from its mouth. At intervals there is a sudden spurt of water and sand from its mouth so violent that it disturbs the bottom. When the fish has ejected all the sand it moves a short distance with its pectorals in close contact with the bottom and repeats its feeding movements. Wherever it has thrust its snout into the bottom there is left a deep pit which is usually a sharp mold of its snout and the lower part of its head. The pits are connected by broad sinuous trails made by the pectoral fins of the fish. These suggest the tracks of a huge snail and show oblique parallel lines where the edges of the pectorals have pressed against the sand at each stroke. These pits and trails are very characteristic impressions, and are abundant in shallow water throughout the summer. They are more numerous in protected places where the bottom is made somewhat coherent by the formation of marl, and where it possibly contains a larger percentage of nutritive matter. These "tracks" of the sucker enable one to tell each morning where they have been feeding during the night and in what abundance.

Great numbers of dead suckers are thrown up on the beach in South Fishtail Bay in July and August. Many of these have the characteristic form of starved fish. The

back is thin and sharp instead of round, and the head is disproportionately large compared to the body. This is because the head is made up largely of bone, and emaciation can not so greatly reduce its bulk as it does that of the more fleshy body and tail. The emaciated fish do not appear to be diseased and are not usually parasitized heavily enough to account for their emaciation. Death seems to be due to starvation.

Hankinson (1908) collected 41 suckers in Walnut Lake and gives their average weight as 2.5 pounds. Their average length, including the caudal fin, is, from Hankinson's tables, 16.2 inches. From Forbes's and Richardson's (1908) figure of the common sucker, the length from tip of the snout to the base of the caudal is found to be 0.88 of the total length from tip of snout to tip of caudal fin. Applying this correction to Hankinson's average length, we get an average length of his suckers of 14.3 inches, measured in the usual way from tip of snout to base of caudal. In contrast to this the 14 fish taken in Douglas Lake have an average length of 9.5 inches and an average weight of 0.48 pound.

From the fact that all stomachs of the common sucker were found empty, from their habit of feeding on the comparatively innutritious materials of the lake bottom and on those covering the stems and leaves of plants, from the large number of deaths among them in midsummer, and from their relatively small average size, it may be inferred that the fish get insufficient food.

In Walnut Lake Hankinson (1908) found, as the result of the examination of the alimentary canals of 13 common suckers, caddis-worms and cases, small bivalve mollusks, amphipods, insects, marl, midge larvæ, and *Daphnia*. Of these, the caddis-worms, amphipods, and midge larvæ are commonly associated with vegetation. It is not unlikely that the relatively slight development of vegetation in Douglas Lake makes it an unfavorable habitat for suckers.

The breeding grounds of the Douglas Lake suckers are unknown. According to the writer's unpublished observations, suckers breed in streams where there is swift water and gravel bottom. These conditions are found in Maple River and in Bessie Creek. Young suckers less than 2 inches long are found in June on the shoals of South Fishtail Bay, about 6 miles by the shore from either of these streams. They are doubtless fish of the season and, if the breeding habits of the suckers of Douglas Lake are the same as elsewhere, the young must have wandered to the shoals from the breeding grounds in Maple River and Bessie Creek. It is possible, however, that the essential requirement for breeding is suitable bottom, not running water. Bottom suitable for suckers is plentiful in Douglas Lake on the shoals, and the young suckers found there may be still on the breeding grounds.

In figure 2 the lengths and weights of the suckers included in table 1 have been plotted and a curve sketched to show their relation. It is clear that there is a definite relation of such a sort that, after a weight of 4 or 5 ounces has been reached, length increases less rapidly than weight. Thus between the weights of 4 and 5 ounces the increase in length is about 0.75 inch, while between 14 and 15 ounces it appears to be scarcely 0.1 inch. At the 15-inch length the line is nearly horizontal. Our data are not enough to make it advisable to draw the length-weight curve mathematically or to determine its formula. (See Hecht, 1913.)

PIMEPHALES NOTATUS (Rafinesque), *blunt-nosed minnow*.—Ten specimens, 2½ to 2⅞ inches long and evidently adult, were taken in the seine at Bryant's dock (location 9

on the map) on September 20, 1911. One hundred and ninety-three individuals about 1¾ inches long were taken on stony shoals on the west side of the entrance to North Fishtail Bay on September 18 of the same year. With them were three *Notropis cayuga* and four *N. hudsonius*. A few were taken in August in company with large numbers of *N. hudsonius* on the sand shoals of South Fishtail Bay. In life they are distinguishable from *N. hudsonius* by the following field characters: (*a*) Darker color; (*b*) a peculiar jerky movement in progression. The fish do not move directly ahead, but by a flick of the tail and of the pectorals the head is jerked to one side and then to the other or several times to one side and several times to the other, so that the course is zigzag; (*c*) the body is semitranslucent, so that the vertebral column and the viscera may be seen faintly from the back; (*d*) the scales in front of the dorsal on the back are crowded so as to appear much smaller than the scales behind them.

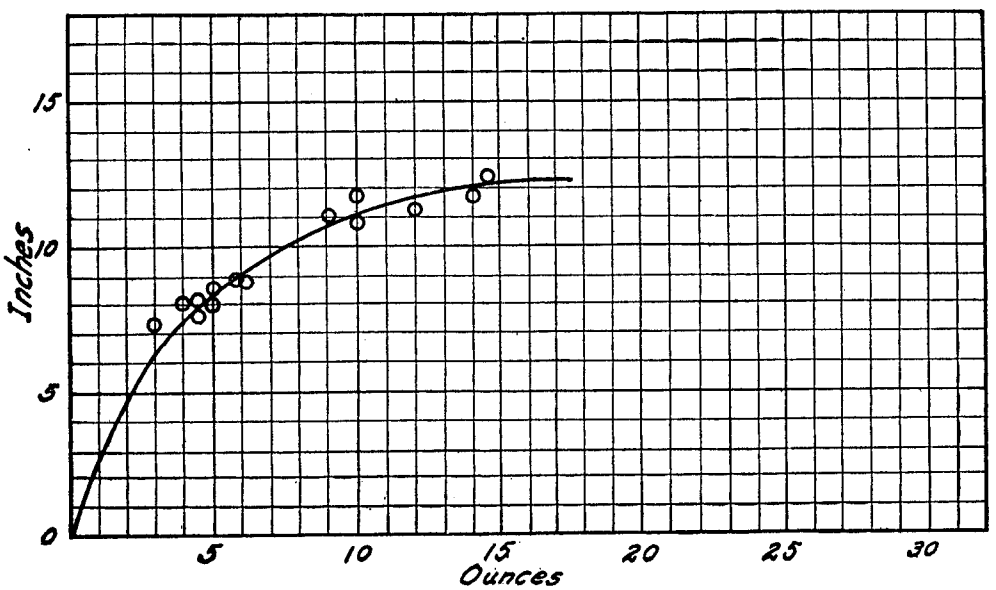

FIG. 2.—Graph showing the relation of length and weight for the 14 common suckers, *Catostomus commersonii*, included in table 1. Each space on the horizontal line represents 1 ounce; each space on the vertical line 1 inch. Curve drawn free-hand.

We have collected this species in numbers only on or very near stony shoals and in the neighborhood of protected bays. Stony shoals afford it breeding grounds, for it lays its eggs beneath flat stones and similar objects on the bottom, and the mucky bottom of protected bays affords it food, for it is a "mud eater." It has been taken but rarely and in small numbers on the sand shoals along the south and west shores of South Fishtail Bay, although frequent collections have been made there. These wave-swept shoals afford neither stones nor muck.

SEMOTILUS ATROMACULATUS (Mitchill), *horned dace*, or *creek chub*, has been taken only in the vicinity of Bryant's dock (locality 9). Here the adult was found in considerable numbers in company with *Pimephales notatus* and *N. hudsonius*. It is abundant in Maple River near the lake. Bryant's is a resort of fishermen. It is possible that the horned dace has been introduced here as a bait fish and has not extended its range to other

parts of the lake, but it is more likely that it has made its way thither from Maple River.

NOTROPIS CAYUGA Meek, *Cayuga minnow.*—Three specimens only have been taken in the lake at locality 11, on stony shoals at the west side of North Fishtail Bay at the entrance. The slopes bordering these shoals are sparsely grown with plants, and they are so much protected from wave action that there is a thin crust of algal marl uniting the superficial sand particles.

NOTROPIS HUDSONIUS (De Witt Clinton), *spot-tailed minnow,* and the common shiner are the most abundant of the Douglas Lake minnows and the most widely distributed. On July 29, 107 specimens of the spot-tailed minnow were taken with the seine on the sand shoals of South Fishtail Bay in about 2 feet of water. They were of nearly uniform length and averaged 2.8 inches; 64 were females and 24 males. These were immature fish and were in schools together with young of the yellow perch and common sucker. Two hundred and sixty-seven immature individuals were seined in the same place on September 1, 1911. On the 20th of July, 1912, 9 mature individuals $3\frac{1}{8}$ to $3\frac{7}{16}$ inches long were seined at Bryant's dock (locality 9), together with mature *Pimephales notatus* and *S. atromaculatus.* Mature individuals 4 to 6 inches long are found in many places in the lake where there is abundant vegetation on the slope. Here the fishermen seek them for bait and take them with the baited minnow hook by casting, as one casts for trout with the fly. They are taken in company with the common shiner and in about equal abundance. The fishermen locate these schools by the disturbance of the water's surface due to their rising, and often visit several patches of vegetation before they find them. Hence it appears that the fish may travel together in schools from one patch of vegetation to another.

The alimentary canal of one of the immature individuals taken on the sand shoal was found to be filled with Cladocera, apparently of the genus *Chydorus,* the form that makes up the bulk of the food of the young perch and suckers taken in the same habitat. The Cladocera were apparently as numerous as in the young perch, but there were no other Crustacea such as occur in the perch. The short, slender, close-set gill rakers with the narrow gill openings make an excellent apparatus for the capture of these small Crustacea. The roof and sides of the mouth and the tongue have many short papillæ set in curved longitudinal rows, and these may serve to hold the Crustacea while permitting water to pass backward. There are no records of the stomach contents of the adults of this species in Douglas Lake. Elsewhere (Forbes and Richardson, 1908) it is known to feed on insects, crustaceans, and vegetation.

NOTROPIS CORNUTUS (Mitchill), *common shiner,* is taken on the hook in the same manner as *N. hudsonius* and in company with it in patches of vegetation in nearly all parts of the lake. It is very abundant. Three taken in South Fishtail Bay in August, 1911, measured, respectively, 3.5, 3.75, and 4.06 inches in length. These were in fine condition, the mesentery heavily laden with fat. The contents of the alimentary canal were as follows for the three specimens:

1. About two-thirds Cladocera, apparently *Chydorus;* one-third insects, apparently larval.

2. Remains of insects and a small quantity of Cladocera.

3. Some fragments of broad, green leaves on which were bryozoan tubes; some *Gloitrichia:* a large number of detached bryozoan branches, some of them with stato-

blasts; an insect larva; a green gelatinous mass including Cladoceran shells and probably composed of partly digested algæ.

Forbes and Richardson (1908) say of the common shiner: "It is especially a minnow of creeks and the smaller rivers—our coefficients for which are 3 and 2.45, respectively—scarcely ever occurring in either lakes or the smaller streams. It shows also a marked preference for clear waters." Hankinson found this species in Walnut Lake, chiefly on shoals with "abundant luxuriant aquatic vegetation and black bottom soil." It was common on but one shoal. Its abundance and wide distribution in Douglas Lake are unusual. It occurs not only in the lake but is the commonest fish taken in the seine in Maple River. According to the writer's unpublished observations, the species breeds only in running water on gravel bottom. Maple River and Bessie Creek afford the conditions of its known breeding grounds. Moreover, the young fish have not been recognized with certainty in the lake, which adds to the probability that it does not breed there. It is more likely that the adults travel from the lake to the breeding grounds in Maple River and Bessie Creek and that when partly grown the young go from the breeding grounds as far as the eastern end of the lake, a distance along shore of about 6 miles. The breeding grounds of this most important bait fish of the lake should be located and preserved.

AMEIURUS NEBULOSUS (Le Sueur), *common bullhead*, does not appear to be abundant. In 1911 four were taken on the hook in the vegetation on the east shore of South Fishtail Bay. Three of them measured 9½ inches in length and the fourth 10¾ inches. The records for 1912 are given below:

TABLE II.—RECORDS OF AMEIURUS NEBULOSUS TAKEN IN DOUGLAS LAKE IN 1912.

No.	Weight.	Length.	Sex.	Water depth.	Apparatus.	Stomach contents.	Locality in fig. 1.	Date.
	Ounces.	*Inches.*		*Feet.*				
1	13	10.4	Female	5.5	Fyke	Bumblebee	4	Aug. 6
2	10	11	do	5.5	do	Small fish	4	Aug. 10
3	3	6	Male	12	Gill	Empty	7	Aug. 20

None were taken in gill nets set at greater depth than 12 feet, so that they are probably confined to the vegetation of the slope and to similar situations elsewhere. The largest specimen taken is 6 inches shorter than Hankinson's (1908) largest (Hankinson's measurements include the caudal fin), and 7 inches shorter than the Illinois maximum as recorded by Forbes and Richardson (1908). Examination of the contents of two stomachs shows nothing unusual except the inclusion of a bumblebee.

Young individuals of this species were taken in July at various points along the shore of North Fishtail Bay and in an adjacent beach pool. In 1909 swarms of young were seen in the same place together with the male. In August they had reached a length of an inch and a quarter.

UMBRA LIMI (Kirtland), *mud minnow*, has not been taken in the lake itself, but is abundant in the oxbow ponds that have been cut off from Maple River near the lake. It should occur in the mucky bays and estuaries of the lake itself.

ESOX LUCIUS (Linnæus), *common pike*, or *pickerel*, is the largest and one of the most abundant fish in the lake. The following table gives data concerning 22 individuals of this species.

TABLE III.—RECORDS OF ESOX LUCIUS TAKEN IN DOUGLAS LAKE.

No.	Weight.	Length.	Sex.	Water depth.	Apparatus.	Stomach contents.	Locality in fig. 1.	Date.
	Ounces.	*Inches.*		*Feet.*				
1	104	30.6	.....	15	Gill.....	2 4-inch perch.....	12	July 3, 1911
2	28	15	.....	25–30	.....do.....	2 perch, 4½ and 3⅜ inches long.	3	July 11, 1911
3	12	13.2	Male.....	4	.....do.....	Fish, not determined.....	3	Aug. 3, 1912
4	15	15.4	Female.....	12	.....do.....	Empty.....	3	Aug. 8, 1912
5	15	14.8	Male.....	12	.....do.....	Fish remains.....	5	Aug. 11, 1912
6	16	15.2	.....do.....	12	.....do.....	Fish.....	5	Aug. 12, 1912
7	21	16.4	.....do.....	12	.....do.....	Empty.....	5	Aug. 13, 1912
8	30	18	Female.....	26	.....do.....	.....do.....	4	Aug. 15, 1912
9	29.5	16.8	Male.....	26	.....do.....	.....do.....	6	Do.
10	26	17	.....do.....	26	.....do.....	.....do.....	6	Do.
11	10	12.8	Female.....	26	.....do.....	.....do.....	6	Do.
12	10	12.8	.....do.....	26	.....do.....	.....do.....	6	Do.
13	18	15.6	.....do.....	26	.....do.....	.....do.....	6	Aug. 16, 1912
14	24	17.6	.....do.....	26	.....do.....	.....do.....	6	Do.
15	24	17.6	.....do.....	13	.....do.....	2 fish.....	7	Aug. 20, 1912
16	29.5	17.2	.....do.....	13	.....do.....	.....do.....	7	Do.
17	10	13.6	.....do.....	25	.....do.....	Empty.....	7	Do.
18	10.5	13.6	Male.....	25	.....do.....	.....do.....	7	Do.
19	25	17.8	Female.....	25	.....do.....	.....do.....	7	Do.
20	27	18.?	Male.....	12	.....do.....	.....do.....	5	Aug. 14, 1912
21	12	13.2	.....do.....	12	.....do.....	.....do.....	5	
22	59	19.6	.....do.....	45	.....do.....	.....do.....	6	Aug. 18, 1912

This fish has been taken in all parts of the lake and at all depths between 4 and 45 feet. It appears not to go below the thermocline in midsummer, but at other seasons it is possible that, like the sucker, it goes into deeper water.

Seven of the 22 stomachs examined contained the remains of fish, while the rest were empty. There is no evidence that in midsummer the Douglas Lake pike takes other food than fish. It is clear that the spiny fin rays of such fish as perch do not keep them from the maw of the pike, for in two cases the stomach contents were perch about 4 inches long.

The pike tabulated range in weight from 10 to 104 ounces. We have taken individuals whose weight we estimated at 10 to 12 pounds and those of 18 pounds have been reported by fishermen. Of the 20 whose sex was determined, half were males. The average weight of males is 22.8 ounces, of females 19.6 ounces, but the number of fish used is too small to make the figures significant and includes a single male of 59 ounces.

A curve showing the relation of length to weight in these 22 fish is sketched in figure 3. The data are insufficient to show more than the general fact indicated for the sucker that there is a definite relation between length and weight of such a sort that, above 8 ounces, the length increases much less rapidly than the weight. The data represented by the curve, although meager, would be of considerable value if there were similar data from other lakes for comparison. It is probable that each species in a lake shows a length-weight curve peculiar to it. It is also probable that curves for the same species from different lakes might be characteristically different. The form of the curve for a single species from one locality might show to what extent the conditions of that locality are favorable to the species. Unfortunately the literature appears to contain no records full enough for comparison with those of Douglas Lake. Forbes and Richardson (1908) mention for the pike an average length of 36 inches and an average weight of 5 pounds. A curve for the Illinois pike, if it were to pass through the point thus

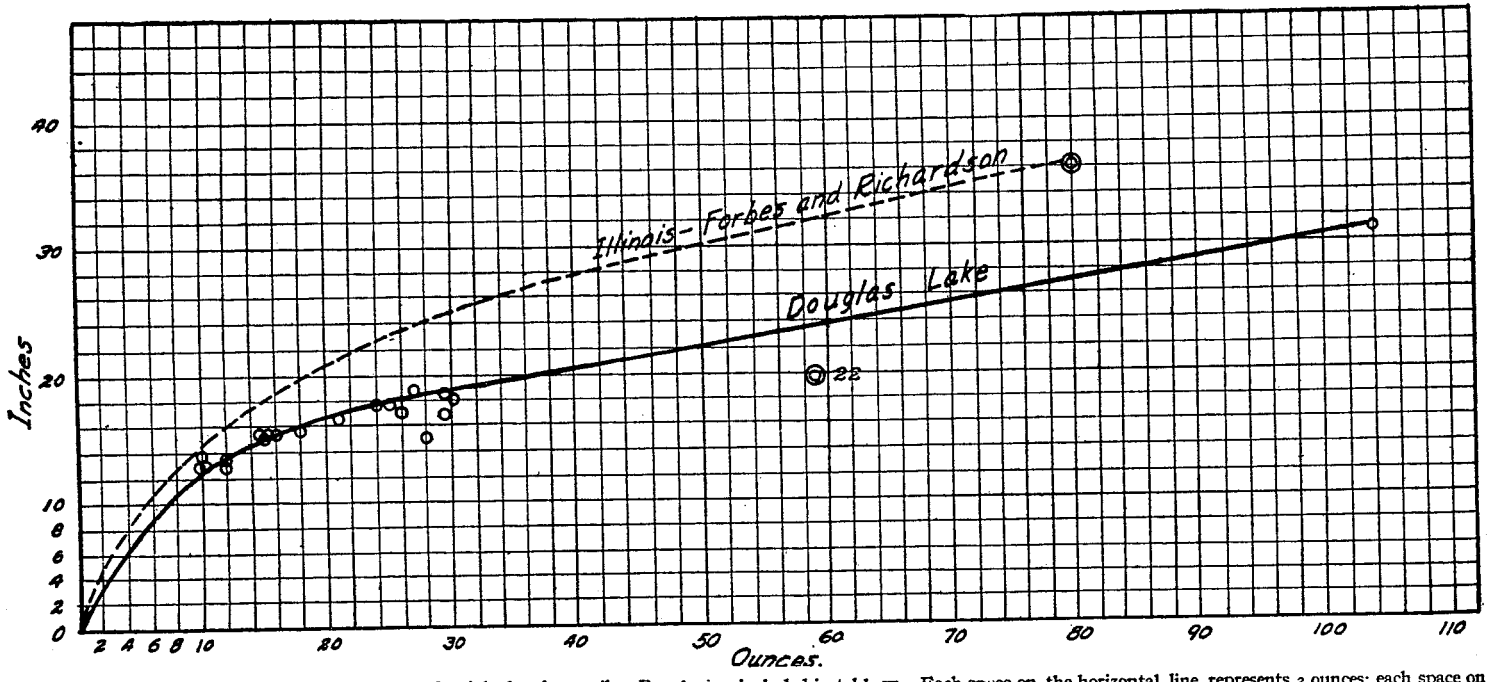

FIG. 3.—Graph showing the relation of length and weight for the 22 pike, *Esox lucius*, included in table III. Each space on the horizontal line represents 2 ounces; each space on the vertical line 2 inches. The individual numbered 22 had a spinal curvature, so that its length was less than the normal for a fish of its weight; the curve is therefore not drawn through it. Curve drawn free-hand. The broken line is a hypothetical curve for the pike of Illinois.

located for a single average fish, would be higher and of different form from that for the pike of Douglas Lake. Such a hypothetical curve is sketched in broken lines in figure 3 to show how curves for single species might be characteristic of localities. A comparison of our curve for the pike with that for the sucker shown in figure 2 shows that they differ. The sucker increases less rapidly in weight with increasing length than does the pike. A sucker of 12 inches weighs about 14 ounces; a pike of 12 inches, if our curve is correct, about 10 ounces. This is for Douglas Lake. With data enough for many species from many localities, one might be able to say, from a study of such curves, for what species of fish the conditions of each locality were most favorable. By defining or describing these conditions one might then possibly use them as a guide in the practical operations of fish culture.

It is interesting to note that pike 22 of our list had a large hump on the back due to curvature of the spine. If this was the result of an injury it had been inflicted so long before that no external scars remained. The deformity may even have been congenital. In spite of it the fish had thriven. The conditions of existence were not severe enough to eliminate it. Its position is shown at 22 on figure 3.

PERCOPSIS GUTTATUS Aggassiz, *trout perch*, is known only from the numerous specimens thrown up on the beach of South Fishtail Bay. It has not been taken in nets. In one specimen 2½ inches long the intestine contained the chitinous parts of an insect larva. This indicates that its habitat is the vegetation zone.

July 17, 1912, following a storm, many adults were picked up on the beach. Among these were females that gave up eggs freely on slight pressure. On the following day a search was made of the shoals in the hope of locating the breeding fish, but without result.

AMBLOPLITES RUPESTRIS (Rafinesque), *rock bass*.—The data collected concerning this fish are brought together in table IV.

TABLE IV.—RECORDS OF AMBLOPLITES RUPESTRIS TAKEN IN DOUGLAS LAKE IN 1912.

No.	Weight.	Length.	Sex.	Water depth.	Apparatus.	Stomach contents.	Locality in Fig. 1.	Date.
	Ounces.	*Inches.*		*Feet.*				
1	8	7.6	Female	5½	Fyke	1 small fish	4	Aug. 6
2	7	7.6	do	5½	do	do	4	Aug. 9
3	3½	5.7	do	5½	do	do	4	Do.
4	9	8	Male	5½	do	Crayfish, fish	4	Aug. 12
5	6½	7.2	do	5½	do	Not determined	4	Do.
6	1	4	Female	4½	do	Empty	2	July 26
7	2	5.2	do	4½	do	do	2	Aug. 21
8	1	4		4½	do	Not examined		Aug. 1
9	1¼	4.2		4½	do	do		Aug. 3
10	1	3.7		4½	do	do		Do.
11	2	4.8	Male	5½	do	Empty	4	Aug. 15
12	12	8.8	do	5½	do	do	4	Do.
13	13	9.6		5½	do	do	4	Do.
14	9	8		5½	do	do	4	Do.
15	2	4.8	Female	5½	Gill	Cambarus virilis; dragon fly larva.		Aug. 16
16	2	4.8	do	5½	do			Do.
17	2	5	Male	5½	do			Do.

Although the records show it from but two localities, it is taken wherever there is vegetation in the lake. We have taken it at no greater depth than 5½ feet, a depth which is usually reached a little beyond the edge of the terrace. It may go deeper. I believe it is sometimes taken at greater depth on the hook, but not beyond the vegetation.

The table shows six specimens with food in the alimentary canal, and we have records of four others. Five of the 10 fish whose stomach contents were studied contained fish, in one case a sunfish, in the other cases the contents were not determinable. Three contained the remains of insects, in one case a dragon fly larva. Two contained each a crayfish; in one this was determined as *Cambarus virilis*, a species which is common in the deeper water of the lake and which occurs also under logs and the like in shallow water. In respect to food, the rock bass of Douglas Lake agree with those of Walnut Lake and of Illinois waters.

Systematic descriptions give the length for the species as 8 to 10 inches, which agrees very well with the 9 to 13 inches of our adult specimens. The weight-length

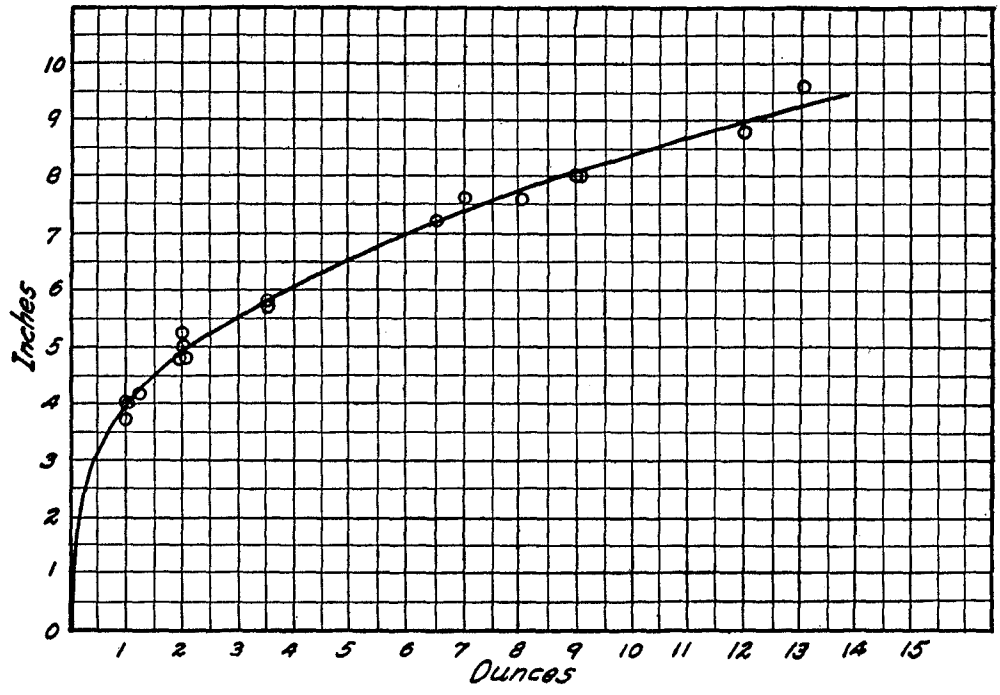

FIG. 4.—Graph showing the relation of length and weight in the 17 specimens of rock bass, *Ambloplites rupestris*, included in table IV. Each space on the horizontal line represents one-half ounce; each space on the vertical line one-half inch. Curve drawn free-hand.

curve is given in figure 4, but there are no similar data from other localities for comparison. There is nothing in our data to indicate that Douglas Lake is an unfavorable habitat for this species.

A rock bass 8½ inches long noted near the laboratory dock paid no attention to a baited hook until touched by it, but was then hooked. It proved to be blind, emaciated, with characteristically large head and without mesenterial fat. It was not too large for a good sized pike to swallow, and it is remarkable that it should have escaped death so long. The struggle for existence among the inhabitants of the lake had not been severe enough to eliminate either the blind rock bass or the crippled pike already referred to. Both were finally eliminated by man.

LEPOMIS PALLIDUS (Mitchill), *bluegill*.—Records for 8 specimens are given in table V.

TABLE V.—RECORDS OF LEPOMIS PALLIDUS TAKEN IN DOUGLAS LAKE.

No.	Weight.	Length.	Sex.	Water depth.	Apparatus.	Stomach contents.	Locality in Fig. 1.	Date.
	Ounces.	*Inches.*		*Feet.*				
1	11	8	Male	4·5	Fyke	Empty	2	July 26, 1912
2	6	6.8	do	4·5	do	do	2	Do.
3	1	3·6		4·5	do	do	4	Aug. 5, 1912
4	1·5	4·4		5·5	do	do	4	Aug. 6, 1912
5		5·9		3	Seine	Plant materials	5	Aug. 5, 1911
6		7·3		3	do	do	5	Sept. 5, 1911
7		4·5		3	do	Plants, mostly	5	Do.
8		3·8		3	do	Plants, insects	5	Do.

This species was taken only in shallow water and in vegetation. Although our records are from but three localities, the fish is taken on the hook wherever there is vegetation.

In size our specimens accord with the 5 to 8 inches of the systematic descriptions. The food of the 4 specimens examined is unusual and is therefore given in detail.

No. 5 had in its stomach 6 terminal buds and leaf whorls of *Elodea*, 2 terminal buds and leaf whorls of a *Chara*, numerous detached leaves of *Elodea*, and some brown, half-decayed fragments of vegetation, most of them long, like leaves of one of the Potamogetons. The intestine was crammed with partly digested plant fragments, on some of which were bryozoan statoblasts, and with numerous heads, wings, and legs of insects, apparently dipters, all imagoes. No. 6 contained in the stomach a mass of vegetation but no animal food. The vegetation was apparently water milfoil. The intestine held the same material in addition to one or two small insects. No. 7 had the stomach filled with unrecognizable plant fragments, on which were statoblasts. The intestine contained plant débris, together with two hydrachnids and two or three ostracods. In no. 8 the stomach was empty, but the intestine was filled with plant débris with numerous fragments of insects. Some of these appeared to be dipterous imagoes.

The fact that the plant fragments form so large a part of the food and that they consist in so many cases of succulent terminal buds and leaf whorls indicates that plant tissues form a normal part of the food and are not merely taken adventitiously with other food. Hankinson (1908) found only animal food in the stomachs of specimens examined by him, while Forbes and Richardson (1908) record the occurrence of 24 per cent of plant food in some of their specimens, a percentage much less than in our specimens.

EUPOMOTIS GIBBOSUS (Linnæus), *pumpkinseed*, is one of the commoner fishes in the vegetation of the lake. Our data concerning it are given in table VI.

TABLE VI.—RECORDS OF EUPOMOTIS GIBBOSUS TAKEN IN DOUGLAS LAKE.

No.	Weight.	Length.	Sex.	Water depth.	Apparatus.	Stomach contents.	Locality in Fig. 1.	Date.
	Ounces.	*Inches.*		*Feet.*				
1	4	6.6	Male	4·5	Fyke	Small shells	2	July 26, 1912
2	4	6.1	do	4·5	do	do	2	July 29, 1912
3	4	6.2		4·5	do	Not examined	4	Aug. 5, 1912
4	3	5·4	Female	5·5	do	Empty	6	Aug. 12, 1912
5	1½	7·8	Male	26	Gill	Minute shells, insects	6	Aug. 16, 1912
6								
7		6·5				Insect larvæ	5	Sept. 5, 1911
8		5·75				Chara, snails	5	Do.
9		5·5				Empty	5	Do.
10		5·25				do	5	Do.
11		5·6				Snails, insect larvæ	5	Do.
12		3·75				Crushed snails	5	Do.
13		3·5				Insect larva, ostracod shells, sand.		Do.

Like the bluegill, the pumpkinseed is found only in shallow water and among vegetation, but may occur wherever these are found in the lake.

The contents of the alimentary canal were examined in 9 individuals. In 6 small snails and their crushed shells were found. In 4 of the 6 the alimentary canal contained no other material than the snails; while in the fifth it contained in addition to the fragments of 3 small snails a quantity of *Chara* with orange fruits. The *Chara* may have been adventitious. One stomach contained insect larvæ exclusively; one contained insect larvæ in addition to snails; and a third insect larvæ, snails, and other material. Snails appear to be the most important element of the food and next to these insect larvæ, but exact percentages are not available. The snails found in no. 8, 11, and 12 were determined by Mr. H. B. Baker to belong to the species *Amnicola limosa* and *Planorbis bicarinata portagensis*. The former were adults from one-sixth to one-eighth inch long, while the latter were young individuals. Forbes and Richardson (1908) found that snails made up nearly half the food of 9 specimens examined by them, insects a fifth, and Crustacea a fifth. Hankinson (1908) examined 32 stomachs and found May-fly larvæ to be the favorite food, although Crustacea, snails, leeches, and other insects were included. The evidence on the whole indicates that snails are the most important element in the food. Fishes appear not to be taken.

Hankinson gives the length of 16 specimens and their average is 5.8 inches, including the caudal fin. The length without caudal fin as determined from figures forms 84 per cent of the total length, which makes the average of Hankinson's specimens 5.1 inches without caudal fin. Our Douglas Lake specimens average 5.2 inches. Judged by this standard, the conditions are about as favorable in the one lake as in the other.

The pumpkinseed appears to be more resistant to foul water than the bluegill. When numbers of each were placed together in a pail of water, all the bluegills were found dead after a time, while the pumpkinseeds were still active.

MICROPTERUS DOLOMIEU Lacépède, *small-mouthed black bass*.—The Douglas Lake data on this fish are given in table VII.

TABLE VII.—RECORDS OF MICROPTERUS DOLOMIEU TAKEN IN DOUGLAS LAKE.

No.	Weight.	Length.	Sex.	Water depth.	Apparatus.	Stomach contents.	Locality in Fig. 1.	Date.
	Ounces.	*Inches.*		*Feet.*				
1	5.5	.....	Female.....	4.5	Fyke.....	.....	2	July 26, 1912
2	1.5	5.1	Male.....	4.5	.....do.....	Empty.....	2	July 29, 1912
3	1.5	5.2	.....	4.5	.....do.....	.....do.....	2	July 21, 1912
4	6	8	Male.....	4.5	.....do.....	.....do.....	2	July 31, 1912
5	40.5	14	.....do.....	45	Gill.....	.....do.....	6	Aug. 15, 1912
6	56	15.6	.....do.....	45	.....do.....	2 fish, 1 crayfish.....	6	Aug. 19, 1912
7	34	12.8	.....do.....	35	.....do.....	Empty.....	7	Aug. 20, 1912
8	.....	12–14	.....	10–15?	Angle.....	.....do.....	10	Aug. 8, 1911
9	.....	12–14	.....	10–15?	.....do.....	Leopard frog.....	10	Do.
10	.....	12–14	.....	10–15?	.....do.....	Small crayfish.....	10	Do.
11	.....	12–14	.....	10–15?	.....do.....	Small crayfish, shiner, and frog.	10	Aug. 15, 1911
12	44	14.6	.....	10–15?	.....do.....	3½ inch crayfish.....	10	Aug. 21, 1911
13	.....	.....	.....	.....	.....do.....	Good-sized crayfish.....	10	Aug. 25, 1911

The small-mouthed black bass is found over a large part of the lake, but anglers seek it along the lakeward margin of the patches of aquatic vegetation, where it is most abundant. Our data show that in midsummer it ranges to a depth of 45 feet, that is, to

the thermocline. In this it agrees with the pike, the perch, and the sucker. It may go deeper at other seasons of the year, as the sucker appears to do.

Six of the specimens in our table contained food, and we have records of two others. Of these eight, six had eaten crayfish with or without other food; four had eaten only crayfish; one had eaten crayfish in addition to a fish; one, crayfish in addition to fish and a leopard frog. The seventh had eaten a leopard frog only. The eighth contained numerous specimens of the large cladoceran, *Leptodora hyalina*. These last could not be examined with the lens, but the naked eye left practically no doubt as to their identity. With the exception of no. 6, the fish whose stomachs contained either frogs or fish (no. 9 and 11) had been taken on hooks baited with these but not with crayfish. If we exclude the frogs and fish in no. 9 and 11, there remain seven small-mouthed bass, six of which had eaten crayfish, accompanied in one case by fish, while one had eaten only *Leptodora*. The crayfish were not identified, but that found in the bass taken at 45 feet was probably *Cambarus virilis*, which has been several times brought up in the gill net from that depth, and is the only species known to occur there. There can be no doubt that in midsummer the crayfish is the chief constituent of the food of the small-mouthed bass in Douglas Lake. Forbes and Richardson (1908) call attention to the fact that little is known of the food of this species. They examined the stomachs of three individuals and found their contents to consist "wholly of fishes and crayfishes, approximately a third of the first and two-thirds of the second."

The size and the weight of the individuals taken in Douglas Lake indicate that conditions there are favorable to this species. The clear water and sand bottom are well-known characteristics of its preferred habitat; the pebble-strewn shallows, especially those about the north end of Fairy Island, afford it ideal breeding grounds (Lydell, 1903; Reighard, 1906).

The young of this species, together with those of the large-mouthed black bass, are often seen on the sand shoals in late summer in pursuit of the mixed schools of young suckers, perch, and spot-tailed minnows, which are common there. These they drive toward shore into shallow water. The bass, which are then 2 or 3 inches long, are two or three times as deep bodied as their intended victims, so that they are unable to follow them into very shallow water.

MICROPTERUS SALMOIDES (Lacépède), *large-mouthed black bass*, is not common in Douglas Lake. It occurs in North Fishtail Bay, where the bottom is mucky in places and the vegetation abundant, and is less common in other localities. We have records of two taken in South Fishtail Bay. One measured 12 inches and weighed 30 ounces; the other measured 14.4 inches and weighed 40 ounces. One contained a perch and the other two fish and a crayfish.

Fishermen tell of catching a peculiar bass on the "middle ground" to the east of the north end of Fairy Island. The writer has never seen it. It is probably the large-mouthed bass.

The conditions in Douglas Lake can not be regarded as favorable to this species. There is little mucky bottom in the shallow water, and few places afford the thick growth of aquatic plants that the small-mouthed bass prefer for its breeding ground (Lydell, 1903; Reighard, 1906).

PERCA FLAVESCENS (Mitchell), *yellow perch*, is one of the most abundant fishes in the lake. Our data concerning it are given in table VIII.

TABLE VIII.—RECORDS OF PERCA FLAVESCENS TAKEN IN DOUGLAS LAKE.

No.	Weight.	Length.	Sex.	Water depth.	Apparatus.	Stomach contents.	Locality in Fig. 1.	Date.
	Ounces.	*Inches.*		*Feet.*				
1–71	[a] 0.5	[a] 4.4	14 males, 57 females.	5.5	Fyke	2 insects, 1 copepod, in 6 fish examined.	4	Aug. 6, 1912
72–77	[a] .75	[a] 4.6	All females	5.5	do	Empty	4	Aug. 7, 1912
78	3	7.2	Female	5.5	do	Small perch	4	Aug. 8, 1912
79	1.5	6	do	5.5	do	Empty	4	Do.
80–221	[a] .5	[a] 4	22 males, 120 females.	5.5	do	Of 20, 14 empty, 6 with insects.	4	Do.
222	5.5	8.4	Female	26	Gill	Empty	6	Aug. 16, 1912
223	5	8	do	41	do	do	6	Aug. 17, 1912
224	5	8	do	41	do	do	6	Do.
225	5	7	do	41	do	do	6	Do.
226	4	7.6	do	41	do	do	6	Do.
227	3	7.6	do	41	do	do	6	Do.
228	5	8.4	do	43	do	do	6	Do.
229	4	8	do	43	do	do	6	Do.
230	4	8	do	43	do	do	6	Aug. 18, 1912
231	4	8	do	43	do	do	6	Do.
232	6	7.6	do	12	do	*Cambarus virilis*	7	Aug. 20, 1912
233	7	8.4	do	12	do	Empty	7	Do.
234	4.5	8	do	12	do	do	7	Do.
235	4.5	8	do	12	do	do	7	Do.
236	3			6	do	Not examined	6	Aug. 3, 1912
237		5.8			Angle	Empty	5	Sept. 5, 1911
238		5.8			do	do	5	Do.
239		5.1			do	Small crayfish	5	Do.
240		4.25			do	Empty	5	Do.
241		4.25			do	1 crayfish, 1 dragon fly, nymph.	5	Do.
242		4.25			do	Small fish	5	Do.
243		4.25			do	Empty	5	Do.
244		4.25			do	do	5	Do.
245		4.6			do	Piece earthworm	5	Do.
246–66		3.75–5.5			do	1 with fish, 2 with insects	10	Sept. 9, 1911
267–8		7.5	Female		Gill	1 empty, 1 with insects	10	Do.
269		7	do	35–40	do	Fish muscle	10	Sept. 14, 1911
270		7.5	do	35–40	do	Not recognizable	10	Do.
271		7.75	do	35–40		do	10	Do.
272		7	do	35–40	Gill	Empty	10	Sept. 15, 1911
272a		7.25		26–30	do	do	10	Sept. 13, 1911
272b		7.75		26–30	do	do	10	Do.
273		8	Female	35–43	do	do	3	Sept. 16, 1911
274		7.25	do	35–43	do	do	3	Do.
275		7	do	35–43	do	1 2-inch crayfish	3	Do.
276		7	do	35–43	do	Empty	3	Do.

[a] Average.

Perch range in midsummer from a depth of a few inches to about 45 feet. They have not been found below the thermocline even in September. They occur in all parts of the lake.

Perch of about 2 inches long are common on the shoals in misdummer, but have not been found elsewhere. In our collections made with the hook from the aquatic vegetation there are no perch more than 6 inches long (no. 237 to 266, table VIII). Larger perch do not appear to occur there, else we should have taken them among the 29 fish from the vegetation. For the 221 perch taken in the fyke net in shallow water we have recorded for most of them only the average length in each lot. There is one fish slightly in excess of 7 inches. On the other hand, in deep water where we have used a gill net of 1-inch square mesh we have taken no perch under 7 inches and many of 8 inches and more (no. 223 to 235 and no. 267 to 276). As perch of 4 or 5 inches have a depth of an inch or more, a net of inch mesh should have taken them if they were present. The average of the fish taken with the hook in vegetation is 4.6 inches (no. 237 to 245), while the average of the 25 taken in the gill net is 7.7 inches. It appears, then, that in

midsummer perch of less than 2.26 inches are found on the shoals, those between 4 and 6 inches in the vegetation, and those of 7 inches or more in deeper water.

Nineteen of the perch in our table showed recognizable stomach contents. Of these, 11 contained insects only, 1 contained insects together with a crayfish, 3 contained crayfish only, and 4 contained fish only. The relative importance of the three kinds of food is perhaps indicated by the frequency of the occurrence of each, which is the ratio: Insects 3, fish 1, crayfish 1.

The largest perch in our record is 8.4 inches. They appear to be exceptionally slender, for while in systematic descriptions the depth is given as contained 3.3 to 3.8 times in the length, the depth in Douglas Lake specimens is contained about 4.3 times in the length. Forbes and Richardson (1908) say "the species may reach the length of a foot and a weight of more than 2 pounds, but does not commonly weigh much more than a pound." The heaviest Douglas Lake specimen weighed but 7 ounces. There are no data from other lakes for comparison with those from Douglas Lake, but Douglas Lake specimens strike one as being slender, short, and under weight.

After storms perch are found dead on the beach in great numbers. Protruding from the mouth of such a one is often seen the head of another that has been swallowed tail first. When pulled out the swallowed perch is, in many cases, found to be almost as large as that from which it was drawn, and is without doubt the cause of its death. Cannibalism of this sort is common in the writer's experience among young wall-eyed pike kept in confinement and insufficiently fed. It indicates starvation. The small size of the Douglas Lake perch, the high midsummer mortality, and the occurrence of the sort of cannibalism described, indicate that the conditions in the lake are not the most favorable for perch.

In August and September schools of young perch are conspicuous on the sand shoals of South Fishtail Bay and doubtless on the other shoals. One of these was seined, and of the 475 individuals captured 203 were found to be young perch from 1.85 to 2.25 inches long. The remainder of the school was made up of 267 spot-tailed minnows and 5 suckers of about the same length as the perch. The young perch are readily distinguished in the water from the other species in the school by the seven bars on the sides, which are more pronounced than is usual with larger perch. They have also a well-defined black, basal spot at the caudal margin of the first dorsal, which has a more pronounced black border than in the adult.

Placed in an aquarium, the young fish may be seen to feed on plankton. When the fish are placed in formalin immediately after capture, twisted cords of brown fecal matter are soon found hanging from the vent. These have on the surface a smooth coherent pellicle consisting apparently of granular mucus. Under the needle the cord breaks readily into ovoid masses of equal size each of which is seen to consist, within the pellicle, of many tests of microcrustacea. The most numerous individuals were those of a *Chydorus*-like cladoceran, but *Simocephalus* and *Daphnia* were also present in numbers and there were a few copepods. These fecal cords varied in length from ½ inch to ⅝ inch. By counting the number of tests in one of the 40 ovoid masses into which a ⅝-inch piece broke, the number in the whole piece was estimated at 800. There were no remains of insect larvæ. The 203 young perch had thus consumed about 162,400 Crustacea, whose remains were found in the fecal cord. The stomach and intestine of one

individual opened was estimated to contain about four times as many Crustacea as the fecal cord, whose contents therefore represent one-fifth of the food contained in the alimentary canal at the time of capture. The total number of Crustacea in the alimentary canals of the 203 young perch at the time of capture was therefore in the neighborhood of 812,000. This is not the daily consumption, but represents rather the food taken within a comparatively short time. If one should collect the fecal cords passed by a known number of perch under normal conditions in unit time, there might be obtained a measure of the total daily consumption of microcrustacea per individual. The rate at which food consumption increases and the relation of this increase to the rate of growth might also be thus determined. The whole branchial apparatus of the young perch with its slender, close-set gill rakers forms an excellent instrument for the capture of microcrustacea.

By an examination of the gonads, the sex of 237 of the perch included in our table was determined. Thirty-six were males and 201 females, a ratio of about 1 to 6. In two instances our records (table viii, no. 1 to 71 and 80 to 221) show a considerable number of perch taken at one time and place. These two lots include 36 males and 177 females, a ratio of about 1 to 5. The remaining captures consist of from one to three fish, and in each case in which the sex is recorded the fish are females. The record shows that all the fish under consideration were taken with the gill and fyke nets. It does not show the relative size of males and females. If, as is possible, the males are smaller than the females, the smaller males may pass through the meshes of the nets and the sex ratio found in our collections may be due to the selective action of the nets used. One must be sure that the apparatus does not act selectively before a positive statement can be made as to the sex ratio.

The young perch found on the shoals secure an abundance of food, while the shallow water affords them a refuge from their enemies. When they have reached a length somewhere between 2¼ and 4 inches they seek larger food among the aquatic vegetation, which at the same time affords them a certain protection from their enemies. Here they remain until they are approaching 6 inches in length. They are then able to leave the aquatic vegetation and wander into deeper water, for their size affords them some protection from pike and bass. They now descend as far as the thermocline and make forays into shallow water beyond the vegetation.

PERCINA CAPRODES (Rafinesque), *log perch.*—Mr. H. V. Heimburger, a research worker at the biological station, reported that he had seen several individuals of this species in water about 3 feet deep at the edge of the vegetation in North Fishtail Bay. In August we have seined them in South Fishtail Bay on the sand bottom near vegetation in 3 feet of water, but they have not been taken in any of the other apparatus used by us.

We have no records of the stomach contents. Forbes and Richardson (1908) say: "A third of the food of 11 specimens was found by us to consist of crustaceans (mainly Entomostraca) and the remainder of insects, the latter chiefly *Chironomus* larvæ, larvæ of day flies and water bugs (*Corixa*)." Their habitat, as judged by the food, is probably the bottom in or near patches of vegetation.

Between June 28 and July 8, 1912, between 100 and 200 individuals were breeding on the sand shoals at the south end of South Fishtail Bay in water from a few inches to

2 feet deep. The eggs were laid in the sand and were fed upon by the log perch themselves as well as by suckers. A preliminary note on the breeding habits has been published (Reighard, 1913). A detailed account will be published later.

BOLEOSOMA NIGRUM (Rafinesque), *johnny darter*, is common in Douglas Lake. Its distribution in the lake and the factors controlling it are the subjects of a paper by Mr. H. V. Heimburger, now in manuscript (see also Heimburger, 1913), from which the following statements are extracted:

It occurs commonly in water of about 18 inches depth, on bottom which contains some muck and in the neighborhood of aquatic plants. The food of the adult consists, in midsummer, chiefly of larvæ of midges, but partly of Entomostraca. The midges lay their eggs on the floating leaves of aquatic plants and their larvæ are found in muck near by. It is well known that the eggs of this species are laid under stones, sticks, mussel shells, and the like in shallow water and are guarded by the male fish. Mr. Heimburger concludes that the localities in which the johnny darter occurs in Douglas Lake present "several features in common: (1) Mussel shells, sticks or stones are found on the bottom; (2) quiet water protected from prevailing winds so that the bottom is not subject to violent wave action; (3) a thin deposit of muck in the shallows, with patches of clear sand exposed; deeper muck deposit is found in the deeper water of the locality, but a deep muck deposit is not found in the shallow water; (4) masses of *Potamogeton*, etc., are found near the habitats where both adults and young are found. These factors are seen to be related very definitely to the food or breeding habits of *Boleosoma* and may therefore be regarded as factors determining the local distribution of this species."

ETHEOSTOMA IOWÆ Jordan and Meek is rare in Douglas Lake. Two specimens were taken in the minnow seine along with 75 johnny darters at the west side of the entrance to North Fishtail Bay in September, 1911. One was taken on sand bottom at the mouth of Bessie Creek. The species is recorded by Hankinson (1908) from Walnut Lake. The two Michigan records appear to mark the eastern limit of its range, which is the Mississippi Valley and northward.

COTTUS ICTALOPS (Rafinesque), *miller's thumb*.—This species is rare in Douglas Lake, and our records indicate that it is confined to the stony shoals. Its eggs are known to be laid under stones and other objects lying on the bottom and to be guarded by the male fish. We have found it in the lake only in localities which afford it nesting sites. These are Grapevine Point and the west side of North Fishtail Bay. In the latter locality it is recorded as abundant under stones. We have taken it also in Maple River.

LOTA MACULOSA (Le Sueur), *burbot*.—We have taken a young individual about 2½ inches long at the mouth of Carp Creek, where it enters Burts Lake. It was taken with *Umbra limi*, near dense masses of aquatic vegetation. Prof. N. H. Stewart is reported to have taken two very large specimens in a gill net in Douglas Lake in the summer of 1910.

FISH COMMUNITIES OF DOUGLAS LAKE.

In the second part of this paper there is suggested a classification of the fish habitats of the lake. The fish found within each of these habitats in midsummer might now be considered without reference to other habitats. But since fish may pass from one habitat to another with increase of size and change of food, it seems best to consider

communities of fish primarily with reference to their origin and interrelations. By community is here meant no more than a group of species or individuals whose members are regularly found together for a longer or shorter time. No precise use of the concept in its relation to habitat is attempted. Thus, the stony-shoal habitat harbors two communities, one of which, that of the young fishes, is more characteristic of sandy shoals. To attempt greater accuracy at the present time would be misleading. Although it seems best to treat communities of fish rather than the fish of a habitat, it is most convenient to use for most of these communities the names of the habitats in which they are best developed.

THE COMMUNITY OF YOUNG FISHES.

On the sand shoals are found mixed schools of young perch, spot-tailed minnows, and suckers, all about 2 inches long in late summer. These schools doubtless vary in the proportion of their constituents. Our only record gives perch 203, spot-tailed minnows 237, suckers 5; but the suckers are often seen to make up a larger percentage of the whole. On the stony shoals there are added to these schools many young blunt-nosed minnows, distinguishable from the other fish of the school by the jerky mode of swimming and by other field characters already described. What follows refers to the schools on the sand shoals, but would presumably answer as well for those on the stony shoals. Perch are known to spawn in the spring. In the hatchery ponds at Mill Creek, Mich., Mr. Dwight Lydell reports to me that they hang their purse-like masses of eggs on aquatic plants, brush, and the like. Abbott (1878) found them spawning on gravel amongst vegetation. H. M. Smith (1907) gives a similar account, and also finds the egg masses floating. I have found the floating egg masses in Saginaw Bay, Mich. Their spawning grounds in Douglas Lake are unknown, but from them the young perch must make their way to the shoals. Suckers (see under that species) are not known to spawn except in running water on gravel bottom. Presumably those of Douglas Lake spawn in such situations in Maple River and Bessie Creek, and the young fish make their way along shore to the shoals. The breeding habits of the spot-tailed minnow are unknown. From two or perhaps three different breeding grounds the young fish forming the communities under discussion reach the sand shoals and finally live together there in schools through the summer. The schools move about over the sand shoals, engaged now in feeding, now in fleeing from enemies. If the water is quiet, they approach the shore; as it becomes rougher they pass toward the lakeward edge of the shoal and are lost to sight. They may then enter the vegetation, but as this harbors many enemies it is more probable that they avoid it. All three species feed on the microcrustacea of the plankton, which at this season are extremely abundant. The total daily consumption of the whole school referred to above must reach many millions. Since the alimentary canals of the young fish are at all times crammed, there appears to be enough food for all, so that the fish are not in competition with each other.

While the schools of young fishes are on the sand shoals they are often pursued by young black bass about 3 inches long, small-mouthed and large-mouthed. The water on the shoals is not obstructed by vegetation, so that the stalking bass are plainly visible to their prey which attempt to escape the rushes of the bass by short rapid flights,

that may end in leaps into the air. Often the flight is toward shore into water so shallow that the deep-bodied bass are unable to follow.

Two influences seem to keep the young fish on the sand shoals. The first is abundance of plankton Crustacea, which at this time make up the whole food of the three species. Since these Crustacea no doubt have a uniform horizontal distribution, as in other lakes, their abundance merely *permits* the shoals to be used; it does not restrict the fish to them. The second influence, that which determines the use of the shoals, is the relative freedom that they afford from attacks of enemies. This freedom afforded by the mere presence of the fish on the shoals is increased by their habit of schooling. The coming together of three or four species to form a large school lessens the loss to each species and the risk to each individual. This follows in part from the fact that, if the toll taken by enemies remains constant, a large school loses a smaller percentage of its constituents than a small school. It follows also because in a large school there are more eyes on the watch for enemies, and therefore more chance that any individual will be warned by the flight movements of comrades, and thus be enabled to escape. Both the habit of schooling and that of frequenting the shoals are measures of protection, most effective when coincident, for the habit of schooling is most effective in open water, which affords no lurking place for the enemy, and in shallow water in which an enemy's approach is limited to the lakeward side. It would probably be ineffective or harmful in vegetation. That the occurrence of young fish on the shoals may be further influenced by temperature is indicated by the observations of Michael (Hankinson, 1908, p. 202–204).

By early September the young perch have reached a length of somewhat more than 2 inches. The suckers and spot-tailed minnows are of the same size. A year later the young perch have reached a length of about 4 inches, have changed their food habits, and are found in the vegetation. They have left the shoals and the community of young fishes and have now become for a time a part of the vegetation community. They do not return to the shoals, but their place there is taken by young fish of the year. The migration from the shoals probably takes place in late fall or early winter, so soon as low temperature or ice makes them uninhabitable, and when the perch are probably less than 3 inches long. The spot-tailed minnows also pass from the shoals into the vegetation and are thenceforth a part of its community. The young suckers, too, leave the shoals, and our next knowledge of them is when they are about 7 inches long. They are then ranging far beyond the vegetation into deeper water. Whether they are confined to the vegetation when between 2 or 3 and 7 inches in length we do not know. It is clear that the communities of young fishes are temporary.

The community of young fishes is found on the stony shoals as well as on the sand shoals, but on the stony shoals there is added to it the blunt-nosed minnow. The adult males of this species burrow beneath stones or other objects on the bottom, and thus form nests (Hankinson, 1908). The eggs are attached to the lower sides of the stones. The young fish are added to the community last described. Nothing is known of their food in Douglas Lake. It is presumably plankton Crustacea, the same as that of the other young fishes of the community, and they are doubtless held to the community by the same factors. The adults have been found only on or near stony shoals.

THE STONY-SHOAL COMMUNITY.

It has been pointed out that the presence of stones adds to the community of young fishes found on the stony shoals the blunt-nosed minnow. This form when breeding may be regarded as a member of the stony-shoal community. This community would then consist of three fishes which lay their eggs beneath stones—the blunt-nosed minnow, the miller's thumb, and the johnny darter. The miller's thumb makes excavations beneath stones and attaches its eggs in a mass to their lower sides (Hankinson, 1908). This has not been observed in Douglas Lake, but the fish themselves have been found beneath stones on the stony shoals and nowhere else. The johnny darter also lays its eggs on the lower sides of stones. It has rarely been found in Douglas Lake or elsewhere than on stony shoals or adjacent to them.

With the three fishes which form this community is found a small crayfish, *Cambarus propinquus* Girard. It forms burrows beneath stones, and these may be recognized by the little piles of fresh sand at their mouths.

The stony-shoal community, unlike that of young fishes, is composed of both young fish and adults and is probably permanent. What happens to its members when the shoals are ice covered we do not know.

The food of the young johnny darter is known to consist of Entomostraca, while that of the adult is chiefly midge larvæ with an admixture of Entomostraca. (Heimburger, 1913.) The blunt-nosed minnow takes a varied food, which, according to Hankinson (1908), consists chiefly of small organisms taken from the bottom, from water plants, and from the water. It appears that midges in various stages of development formed the chief food of this species in April and May. Besides midges, filamentous algæ, desmids, entomostracans, and in one case beetles, were found in the stomach. The food of the miller's thumb in Illinois was found to contain about 25 per cent of small fishes, 40 per cent aquatic larvæ, and the rest mostly Crustacea (*Asellus*). (Forbes and Richardson, 1908.) In Douglas Lake the blunt-nosed minnow and the johnny darter take similar food and find it in the same place, yet the blunt-nosed minnow has a somewhat larger choice, and the two forms are not altogether in competition. The miller's thumb presumably takes its supply of fish from among the blunt-nosed minnows and johnny darters, while the crayfish afford it Crustacea.

THE VEGETATION COMMUNITY.

The vegetation community includes the following forms:

Ameiurus nebulosus, bullhead.
Notropis hudsonius, spot-tailed minnow.
Notropis cornutus, common shiner.
Percopsis guttatus, trout perch.
Ambloplites rupestris, rock bass.
Lepomis pallidus, bluegill.
Eupomotis gibbosus, common sunfish.
Micropterus salmoides, large-mouthed black bass.
Perca flavescens, yellow perch, 4 to 6 inches long.
Percina caprodes, log perch.

Of these 10 species 8 have been found only in the vegetation, although, as pointed out elsewhere, some of them enter other habitats at the breeding season or when young. The

inclusion of *Percopsis guttatus* and *Percina caprodes* in the list is provisional. We have found insect remains in the stomach of the former, while Forbes and Richardson (1908) find the latter to feed on entomostracans and insects, and we found it always on the bottom near vegetation. What we know of the food of both forms, therefore, suggests the vegetation habitat. The small-mouthed bass, the sucker, and the pike are found in the vegetation habitat, but chiefly about its edges, while they are characteristic of the deep-water community in which we have placed them. The whereabouts of the young of these forms is unknown to us, but there is little doubt that they will be found in the vegetation. Here also we are likely to find the young of *Lota maculosa*.

With the possible exception of the log perch and the trout perch, the forms listed above are confined to the vegetation. We do not ordinarily find them elsewhere. They invade the shoals only, so far as known, when they afford them shelter similar to that to be had amongst vegetation. From the laboratory on South Fishtail Bay a dock consisting of planks supported on spiles extends from the shore to the seaward edge of the terrace, which is here an unprotected sand shoal. Beneath this numerous fishes found shelter—small black bass, both large and small mouthed; bluegills, rock bass, adult spot-tailed minnows, and small perch. To this retreat they had been able to make their way readily from the adjacent vegetation, and from it they were able to harry the schools of young fishes on the shoals. Such shelters were no doubt formerly afforded by the trunks and branches of fallen trees. With the destruction of the forests, fallen trees have become rare along the shore and are removed by those who use the shore as a roadway. When they were more abundant, fish must have been more plentiful on the shoals, for they were then able to find there the shelter conditions of the vegetation.

Short forays into deeper water in search of food are probably made by several of the fishes of the vegetation community. By the extension of these the 6-inch perch no doubt attain in time to a membership in the deep-water community. The breeding season finds certain of the vegetation fishes outside of the vegetation. The log perch then betakes itself to the sand shoals and is there closely associated with the adult suckers, which come to the shoals to feed on its eggs. The common shiner and the small-mouthed bass must also leave the vegetation to breed.

That the larger small-mouthed bass and the pike make raids from deeper water into the vegetation in pursuit of prey we can not doubt, for they are captured at its borders. Moreover, in the stomachs of the pike are found 4-inch perch, which are not known to occur outside the vegetation.

Three factors suggest themselves as determining the make-up of the vegetation community—food, shelter from enemies, and breeding habits. All 10 forms, of course, find their food within the vegetation. Three members of the community—the bullhead, the rock bass, and the large-mouthed bass—reach a considerable size, feed in part on fishes, and might obtain these outside the vegetation. But, of these, the bullhead is largely nocturnal or crepuscular and is slow of movement. It tends to lie in wait for its prey rather than to seek it actively. The conditions within the vegetation zone are favorable for this method of getting food. The rock bass and the large-mouthed bass include a considerable proportion of small fishes in their diet, and these are most abundant in the vegetation. The remaining forms feed on invertebrates, largely

insects, and find these most plentiful within the vegetation areas. Here the common sunfish finds snails and the bluegill plant tissues. The two species of *Notropis* might feed on plankton outside the vegetation, while the bullhead, rock bass, and large-mouthed bass might, like the pike and small-mouthed bass, obtain fish without restricting themselves to the vegetation. Food alone does not appear to limit these forms to the vegetation.

In addition to food, the seven smaller forms (excluding *Ameiurus*, *Ambloplites*, and *Micropterus*) find within the vegetation a refuge from certain enemies, chiefly pike and small-mouthed bass. Within its mazes rapid flight is much easier for a small form than rapid pursuit by a larger enemy.

The breeding habits of one of the forms in our list (the spot-tailed minnow) is unknown. The writer is familiar with the breeding habits of the remaining forms, and the papers of Hankinson (1908) and Shelford (1911) contain references to some of them. Two species (the log perch and the common shiner) breed on plant-free sand or gravel. A third, *Percopsis*, according to the unpublished observations of Doctors A. H. Wright and A. A. Allen, of Cornell University, to which reference is made by their permission, breeds "in swiftly running water and over a stony bottom." The common sunfish also may breed on gravel bottom. Four species (bullhead, rock bass, bluegill, and large-mouthed bass) lay their eggs, by preference, in nests made by exposing the fibrous roots of water plants. The bottom material removed in making the nests may be sand, marl, muck, or mud, but the essential of the nests is the rootlets which form its bottom. The eggs of the sunfish, rock bass, and bluegill adhere to the rootlets. Those of the bullhead lie free, united in masses. The bullhead, since its eggs are not adhesive, may make its nest in situations in which rootlets do not occur, but in the writer's experience these nests are always in vegetation. The common sunfish also makes a nest, the bottom of which is sometimes gravel but more often rootlets. The perch, too, commonly deposits its masses of eggs amongst vegetation, although when adult it wanders far beyond the vegetation. Thus there are 6 of the 10 forms that require vegetation as a part of their breeding environment (*Ameiurus*, usually; *Ambloplites; Lepomis*, usually; *Eupomotis*, usually; *M. salmoides; Perca*). In some of these forms the relation of the breeding habits to vegetation is not at first apparent. Thus *Lepomis pallidus* is often found breeding where there seems to be no vegetation, but in such cases closer inspection will show that its eggs are laid on the rootlets of bulrushes which are so near the denser vegetation as to afford the fish a ready retreat into it. The same is often true of the large-mouthed bass and the common sunfish.

Of the 10 forms included in our vegetation community we may repeat that all obtain food in the vegetation. Although some of them might obtain it elsewhere it is doubtful whether they could obtain it as well. At least four of these probably need the vegetation for protection (the two species of *Notropis*, *Percopsis guttatus*, and *Percina caprodes*). This need is scarcely less in the bluegill, the sunfish, and the perch, and in the young of the other forms. The two species of *Notropis* are not bottom forms. While they might obtain plankton food outside the vegetation, they would be very conspicuous there and could scarcely long escape their enemies. *Percopsis* and *Percina* are bottom forms and would more readily escape detection outside the vegetation. It is possible that they may wander to some distance from it. The relation of these 10 forms to the vegetation is summarized in table IX.

The cross in the table indicates that the species avails itself of the factor indicated; the zero indicates that it does not, or, in the case of the column headed "Protection," that it does not need to. Adult *Ameiurus*, *Ambloplites*, and *Micropterus* are larger than the perch which exist outside the vegetation. They must be held to it by some other factor than the need of protection. This may be food or some factor not included in our table. Since some of the species may obtain their food outside the vegetation, the data in our table indicate *that no one of the factors considered necessitates the occurrence together of the species which make up the vegetation community.*

TABLE IX.—SHOWING THE RELATION OF THE SPECIES OF FISHES INCLUDED IN THE VEGETATION COMMUNITY TO THE THREE FACTORS OF FOOD, PROTECTION, AND BREEDING AFFORDED BY THE VEGETATION.

Species.	Food.	Protection.	Breeding.	Species.	Food.	Protection.	Breeding.
Ameiurus nebulosus	×	o	×	Lepomis pallidus	×	×	×
Notropis hudsonius	×	×	(a)	Eupomotis gibbosus	×	×	×
Notropis cornutus	×	×	o	Micropterus salmoides	×	o	×
Percopsis guttatus	×	×	o	Perca flavescens, 4–6 inches	×	×	×
Ambloplites rupestris	×	o	×	Percina caprodes	×	×	o

[a] Unknown.

THE DEEP-WATER COMMUNITY.

In the deeper water near the bottom, down to about 45 feet, the following forms have been taken in midsummer:

Catostomus commersonii (common sucker), from 7 to 12 inches long, approximately.
Esox lucius (pike), between 12 and 30 inches long.
Micropterus dolomieu (small-mouthed black bass), between 12 and 16 inches long.
Perca flavescens (yellow perch), 8½ inches long.
Lota maculosa (burbot), large.

All these fishes wander far from the vegetation. The small-mouthed black bass feeds chiefly on crayfish. Presumably the larger pike prey on the other fishes of the community, but our records do not show that any pike had eaten fishes more than 4½ inches long. These were perch of a size found only among vegetation and were doubtless taken by the pike at its borders. It seems probable that the five fishes of this community are protected from one another in part by their size, for the individuals of each kind are usually too large to be eaten by the others except by the largest pike or burbot. The perch remain in the aquatic vegetation until they are about 6 inches long and large enough to enter the deep-water community.

The fishes of the deep-water community, except possibly the burbot, are much about the borders of the patches of vegetation and more or less within these patches. Here the pike (no. 1, 2, 3, 5, 6, 15, 16, table III) obtains the smaller perch and probably the other fishes of the plant zone. To a lesser degree, the small-mouthed black bass may obtain fish from the same source; at any rate it is commonly taken on the lakeward margin of the plant zone on hooks baited with shiners or spot-tailed minnows. The sucker is also known to enter the patches of aquatic plants. The characteristic feature of the deep-water community is then that its members occur near the bottom in deeper water outside the patches of aquatic plants, not that they may not also occur

within these patches. In this they differ from the rock bass, the small-mouthed bass, and the bullhead, which have been taken only in or near vegetation and in shallow water, although their size would apparently enable them to enter the deep-water community.

In addition to the five species listed, one other, the cisco, or lake herring, must occur at some level considerably above the bottom. This may be inferred from the fact that it has never been taken by us in fine-meshed gill nets set on the bottom in either shallow or deep water. It is probable that it should be regarded as a member of a nekton community characteristic of a mid-water habitat. No other fishes are known to be associated with it.

RELATION OF DOUGLAS LAKE SPECIES TO THOSE OF OTHER WATERS.

Douglas Lake has large areas of bare sand or gravel bottom, comparatively clear water, kept well agitated by the wind, and a relatively sparse growth of vegetation. It would be of interest to learn: (1) Whether its fishes give preference in other regions to the conditions that they find in Douglas Lake, and (2) whether their distribution over the continent is such as to afford these conditions. Forbes and Richardson (1908) give the only data known to me on the habitat preferences of American fresh-water fishes. For many species they indicate by coefficients or percentages the kind of water preferred (whether large rivers, small rivers, creeks, lakes, or ponds), the kind of bottom (mud, rock and sand, mud and sand), and the amount of current (swift to moderate, slow to stagnant, variable). I am unable to interpret these data in such a way as to make them available for a detailed comparison of the habitat preferences of the fishes of Douglas Lake with those of Illinois, and therefore restrict myself to noting two points:

There is no mud bottom in Douglas Lake, none at least in its shallower parts. The bottom is sand or gravel, with an overlying stratum of muck in the deeper water and in protected situations in shallow water. None of the species occurring in the lake are among those given by Forbes and Richardson as preferring mud bottom in other waters, and but two species (*Ameiurus nebulosus*, and *Umbra limi*) are commonly found on such bottom in other waters. The other Douglas Lake species, in so far as their preferences are indicated for Illinois, are found with greatest frequency on a bottom which includes rock or sand or both.

Among the fishes in our list the following are found by Forbes and Richardson to show a preference for small rivers or creeks: *Catostomus commersonii*, *Semotilus atromaculatus*, *Notropis cornutus*, *Ambloplites rupestris*, *Micropterus dolomieu*, *Percina caprodes*, *Boleosoma nigrum*. Suckers, rock bass, and small-mouthed bass occur often in lakes, but the horned dace and the common shiner are rare in lakes. Forbes and Richardson give the water preference of the horned dace as large rivers 1.67, creeks 3.77, lowland lakes 0.11. The species is of local occurrence in Douglas Lake and is possibly introduced. For the common shiner the Illinois preferences are large rivers 0.11, small rivers 2.45, creeks 3.00, lowland lakes 0.02, upland lakes 0.20. The species is one of the most widely distributed and abundant of the Douglas Lake fishes. Its abundance, together with the presence of the other species showing preference for small rivers and creeks, indicates that in the character of its bottom, the movement of its waters and the sparseness of its vegetation Douglas Lake affords the small-river-creek conditions preferred by these species.

For the purpose of discussing the general distribution of the fishes of Illinois, Forbes and Richardson have divided the region over which they occur into 12 districts and have tabulated the distribution of each species in these districts. The number of Douglas Lake species found within each of these districts is shown in table X, arranged in numerical order.

TABLE X.—SHOWING NUMBER OF DOUGLAS LAKE FISHES FOUND IN OTHER REGIONS.

Great Lakes basin	22
Upper Mississippi and Missouri Valleys	21
Lower Mississippi and Ohio Valleys	20
Quebec and New England	19
North Atlantic, New England to Chesapeake Bay	17
South Atlantic, Chesapeake Bay to Florida	13
Hudson River	12
Far north, north of Mississippi drainage, between Rocky Mountains and Lake Superior drainage	12
East Gulf district, to Mississippi drainage on west	10
West Gulf district, westward from Mississippi drainage, including Rio Grande	4
Florida Peninsula	3
Far northwest, west to Rocky Mountains	1

It is clear from the table that the Douglas Lake species are northern and northeastern rather than southern or southwestern in their range. A single species, the pike, crosses the Rocky Mountains into the far northwest, but the species is of common occurrence in the Northern Hemisphere. Three species, the large-mouthed bass, the bluegill, and the bullhead, occur in Florida, while four species are found in the west Gulf and Rio Grande region. All the Douglas Lake species, with the exception of the cisco, occur in the upper Mississippi and Missouri Valleys. In the lower Mississippi and Ohio Valleys all are found, with the exception of the cisco and *Etheostoma iowæ*. In Quebec and New England *Etheostoma iowæ*, *Notropis cayuga*, and the bluegill are lacking. These three, with the cisco and the miller's thumb, are lacking in the north Atlantic district, leaving 17 species. This number is reduced to 13 in the south Atlantic district, 10 in the east Gulf, and 4 in the west Gulf district.

In general, more Douglas Lake species are to be found in clear, rock and sand-bottomed, northern waters than in the more turbid southern and southwestern waters. Forbes and Richardson (1908) publish a list of 34 Illinois species that avoid the turbid waters of the lower Illinoisan glaciation. Ten of these are also Douglas Lake species. They give also a list of 37 species that tolerate the lower Illinoisan glaciation. In this list are but two Douglas Lake species.

The fishes of Douglas Lake appear, then, to give preference in Illinois, and presumably elsewhere, to those conditions of water and bottom that are available to them in Douglas Lake and to be distributed over the continent in districts in which such conditions are found.

SUMMARY.

1. Four fish habitats are provisionally recognized in Douglas Lake—the barren sand-shoal, the barren stony-shoal, the vegetation, and the deep-water. Each is defined (pp. 221, 222).

2. Twenty-two species of fish are listed from Douglas Lake, and detailed data on their occurrence, weight, length, food, and interrelations are tabulated and discussed (p. 229).

3. Four fish communities are provisionally recognized in Douglas Lake in midsummer—the community of young fishes, the stony-shoal community, the vegetation community, and the deep-water community (p. 239–246).

4. The community of young fishes is characteristic of the shoals. On the sand shoals it consists of perch (*Perca flavescens*), spot-tailed minnows (*Notropis hudsonius*), and suckers (*Catostomus commersonii*), all in schools together and all about 2 inches long in late August. On the stony shoals, young blunt-nosed minnows (*Pimephales notatus*) may be added to these schools (p. 241).

5. All the members of the young-fish community feed exclusively on plankton Crustacea (p. 241).

6. The occurrence of young fish of several species (*a*) in large schools and (*b*) on open shoals are conditions which, when they occur together, favor escape from predatory enemies. It is held to be the presence of such enemies that keeps the members of the young-fish community together and on the shoals (p. 241).

7. The community of young fishes is temporary. Before their second season its members forsake the shoals (p. 241).

8. The stony-shoal community consists of the young and adults of three species which lay their eggs beneath stones—the blunt-nosed minnow (*Pimephales notatus*), the johnny darter (*Boleosoma nigrum*), and the miller's thumb (*Cottus ictalops*). With these is associated a small crayfish (*Cambarus propinquus* Girard) (p. 242).

9. The factor which holds the members of the stony-shoal community together is the presence of stones or other similar objects which afford the conditions necessary for breeding (p. 242).

10. The stony-shoal community is permanent, except as it may be interfered with by winter conditions (p. 242).

11. The vegetation community consists of 10 species which, with one exception, are unknown except in or very near vegetation (p. 242).

12. The occurrence together of the members of the vegetation community is not attributed to a single factor, but to two or more factors, of which food, protection, and breeding conditions are specified (p. 243).

13. All members of the vegetation community find their food in the vegetation; in addition seven of them find there probably necessary protection, and six find in connection with vegetation their usual breeding conditions (p. 243).

14. The deep-water community consists of four or five species—the common sucker (*Catostomus commersonii*), the pike (*Esox lucius*),the small-mouthed black bass (*Micropterus salmoides*), the burbot (*Lota maculosa*). All of these occur near the bottom in deep water outside vegetation, although they may also penetrate vegetation and invade shallow water (p. 245).

15. The members of the deep-water community obtain their food in the deeper water and about vegetation. Their size is held to enable them to leave the vegetation, since by it each species is in some degree protected from enemies (p. 245).

16. The species of fishes found in Douglas Lake give preference in Illinois (Forbes and Richardson) to those conditions of water and bottom that are available to them in Douglas Lake (p. 246, 247).

17. The fishes of Douglas Lake are distributed over the continent in those districts in which the conditions available to them in Douglas Lake occur (p. 247).

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THE POTAMOGETONS IN RELATION TO POND CULTURE

By Emmeline Moore

Contribution from the Department of Limnology, Cornell University

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THE POTAMOGETONS IN RELATION TO POND CULTURE.

By EMMELINE MOORE,
Contribution from the Department of Limnology, Cornell University.

INTRODUCTION.

The cultivation of lakes, ponds, and streams follows as a natural consequence the biological investigation of the aquatic life within them. Herbivores and carnivores live their life in the water, and if we ponder over their means of sustenance we are struck by the fact that the natural food supply has rarely been augmented by cultural methods.

"The larger aquatic plants," says Pond (1903), "form a link in the chain of nutritive relations that stretches from the water and soil to the higher fishes." If such is the importance of these plants, the great mass of vegetation which comes to maturity each season is a national asset. Yet the annual yield has never been estimated or given a place in the Government crop reports.

Aquatic plants have contested for possession of the waters much as the grasses have contended for supremacy on land, until it may be said that the dominant forage crop of our lakes, ponds, and streams is to be found among the pondweeds, the Potamogetons. Variety in form, adaptability to environment, and diversity in range have all contributed their share in giving prominence to this group and in furthering a natural resource whose propagation and control are vital factors in the economic relations of the life of inland waters.

The object of this investigation is to present such observations and experiments on the natural and artificial propagation of the Potamogetons as will render cultural methods economical and practical.

The work herein recorded was carried on at Cornell University under the direction of Dr. James G. Needham, to whom I wish to express my grateful thanks for help and suggestion.

HISTORICAL.

The cultivation of aquatic plants was an ancient occupation, one which concerned itself with the beautification of pools and fountains. In modern times, too, aquatic plants have been used in variety and profusion in the ornamentation of artificial or natural ponds. But the cultivation of aquatics from an economic standpoint is a new idea, so new, in fact, that data regarding it are just beginning to appear in bulletin form in the Government compilations of scattered and isolated experiments. In the bulletins plants of the genus Potamogeton have received the larger measure of notice because observations on the feeding habits of animals associated with them point to the important rôle of these plants in the economy of nature.

Further contribution to the present status of the Potamogetons incorporates of necessity a considerable body of observation pertaining to the systematic, morphological, and biological aspects of this group, and renders it highly desirable to set forth the historical background of each of these three phases of the subject.

JOHN GERARDE, 1633.

A beginning in the classification of the Potamogetons was made by the old herbalists, medical men, who found it necessary to study plants in detail in order to discriminate the kinds employed for different purposes. The special virtue in Potamogetons, for example, resided in the leaves, which were applied to reduce inflammation. In the herbal of John Gerarde the group Potamogeton (*Potamogeiton* in the old spelling and pondweed or water spike in the common parlance of the time) consisted of four species—a broad-leaved pondweed, a narrow-leaved pondweed, a small pondweed, and a long sharp-leaved pondweed. There was a figure of the entire plant accompanied by the Latin and English name. Then followed the "description, place, time, names, nature, and virtues agreeing with the best received opinions." A "fennel-leaved water milfoile" illustrated by a figure easily recognized as our fennel-leaved pondweed, *Potamogeton pectinatus*, was given a place among the Myriophyllums. Such was one of the earliest attempts to classify the group.

CHAMISSO AND SCHLECHTENDAL, 1827.

The first important monograph of the Potamogetons was the work of Chamisso and Schlechtendal, who, in Linnea, volume 2, 1827, systematized the results of scientific observation during the latter part of the eighteenth and the beginning of the nineteenth centuries. Under the family name of Alismaceæ 21 species were described and illustrated by drawings of fruit and leaf, including among them many of the common and widely distributed species of to-day. Several other Potamogetons were listed as uncertain in position and difficult to classify, a condition which holds as true to-day as then, when Chamisso and Schlechtendal struggled to bring order out of chaos in this puzzling group and recorded this pertinent observation: "Species Potamogetonum habitum mutantes in alias sæpe transire videntur, alienæque speciei habitum mentientes scrutatorum irrident," which translated is, "Species of Potamogeton changing their habit seem often to pass into others, and feigning the habit of other species baffle research."

REICHENBACH, 1845.

Reichenbach's monograph of the Potamogetons, in his Icones Floræ Germanicæ et Helveticæ, followed in 1845. More intensive in scope than any preceding work, it marked a distinct advance both in the method of description and in the matter of illustration. Several reproductions, especially of flower and fruit, which were drawn with great clearness and accuracy, have found their way in the latest authoritative works on the subject. In this monograph the author introduced the figure of the so-called "bur," the vegetative propagative body of *P. crispus* Linnæus, though he apparently did not recognize its significance in the rapid propagation of this species. It is interesting to note that the figure is inserted without further description or comment. Moreover, it is erroneously drawn, and the error has been copied time without end.

IRMISCH, 1851.

Thilo Irmisch, in a published note in Flora, 1851, first recognized the presence of tubers on *P. pectinatus*.

AGARDH, 1852.

A year later J. C. Agardh, in Verhandlungen der K. Schwedischen Akademie der Wissenschaften, recorded several observations on the tubers of this species of Potamogeton.

CLOS, D., 1856.

D. Clos was the first to publish an account of the origin of the "bur" of *crispus*, though his observations are incomplete regarding both their development and their germination.

IRMISCH, 1858.

In a remarkable monograph by Irmisch, Über einige Arten aus der naturlichen Pflanzenfamilie der Potamogeton, the history of the development of the tuberous growths on *P. pectinatus* is recorded and their morphological and anatomical structure described. The author states that, at the end of the vegeta-

tive period in the fall, the shoots of recent formation have a singular appearance, the last two thin internodes bearing tubers at the end. At first the tuberous end resembles a conical terminal shoot or bud surrounded by scales. Internodes make their appearance and soon become thickened; eventually the scales split and disclose a tuber of two swollen internodes. Simultaneously a slender bud forms at the distal end of the tuber, and axial outgrowths develop from the sides that bespeak the shoots of ordinary branches. These axial shoots in turn develop swollen internodes which follow two thinner ones as in the preceding case and produce a series of tubers dichasial in form. The excellent series of drawings by means of which the author depicts the transition from internode to tuber leaves nothing to be desired in the morphological interpretation of them. They are clearly two modified internodes.

Tuber-bearing shoots grow out of the upper leaf axils also, and follow the usual development of generations of internodes with leafy shoots, besides the tuber-bearing ones in two or three series. The anatomical structure of the tuber resembles that of the stem excepting that all tissue not fibro-vascular is filled with starch. The observations on *P. obtusifolius* are incomplete, but the presence of winter buds is noted. For *P. natans* and *P. lucens* the morphology of the rootstock, stem, and shoot is completely determined, and the details are clearly shown in the drawings. The method of branching is fundamentally the same in the two species. In brief, the growing tips of the rootstocks branch dichotomously, giving an erect axis and a horizontal one. Each generation of the developing rootstock brings forth two horizontal internodes and a bud which is the incipient erect axis. The terminal bud at the end of the horizontal axis reproduces this condition as long as the plant lives. In the development of the erect shoot, the scales, usually three in number, grade into stipular sheath, phyllodes, and foliage leaves. A two-fifths arrangement of leaves is noted and the shoots follow the same order. The winter condition of *P. lucens* consists of rootstocks by means of which the plant propagates itself rapidly in the spring. The internodes of these rootstocks are shorter and thicker than the ordinary ones and are borne in a succession of three or more with terminal and axillary buds containing the incipient axes of the horizontal and erect shoots.

Irmisch made observations also on *P. crispus*, investigating especially the "burs" or propagative shoots, although this work was anticipated in part by D. Clos in his Mode de Propagation particulier au *Potamogeton crispus* L. Irmisch, however, found two forms of the bur of *crispus*, the slender spicular bur as well as the stout, horny, denticulate one observed by Clos. The former bur he observed growing in the axils of detached shoots in late autumn and afterwards breaking away from the axils and settling in the mud. The origin of the latter form he did not observe, but he found it in the muddy bottoms of ponds in great abundance. These "burs" or modified twigs, as Irmisch sometimes called them, he considered important examples of propagative structures.

In connection with these plants, Irmisch first pointed out the "Scheiden-Schüppchen, squamulæ intravaginales," scale-like structures developed at the leaf bases, having as a possible function the production of slime or mucilage for the protection of young and slender shoots.

This monograph is of special importance in presenting the morphological data of a few species of Potamogeton. From time to time further contributions have been made to the subject by other investigators in the field, but this still remains the greatest work of its kind.

As a result of these studies on the tubers of *P. pectinatus*, the rootstocks of *P. lucens*, and the burs of *P. crispus*, Irmisch came to appreciate the advantage of artificial propagation in this group and remarked in conclusion, in an observation that is prophetic of present day interest, "That many of the Potamogetons, as well as other aquatic plants, possess in a singular way that possibility of domestication which has given us the tame animals from the wild ones."

ROBBINS, 1867.

Thus far the work of the Potamogetons was confined principally to European species. In 1867, however, the American species were reduced to something like a complete intelligible systematic shape by Dr. G. W. Robbins, whose descriptions, as far as they came within the range, were incorporated in Gray's Manual, edition 5. Later descriptions of the western species were published as they became known.

MORONG, 1893.

The greatest contribution to the literature on the North American species of Potamogeton is by Thomas Morong in his Naiadaceæ of North America, a monograph which includes 37 North American species, 14 of which are confined to this country. Many of these species were studied through succes-

sive seasons of the year and a considerable body of knowledge pertaining to the development of the plants was accumulated. It is recorded that 17 of the described species are propagated vegetatively by one or more of the following structures: Rootstocks, tubers, winter buds, and stems.

SAUVAGEAU, 1894.

The work of Sauvageau is particularly a contribution to the biology of the Potamogetons. While there are additions in morphology and anatomy extending the observations of Irmisch to other members of the genus, the most noteworthy investigations pertain to the origin and the development of those vegetative structures which greatly facilitate the multiplication of species during the vegetative period.

Sauvageau devotes a special memoir to *P. crispus*. He observed both forms of the so-called "burs" of this species, the slender spicular one and the more common denticulate one, noting their origin, growth, and germination.

Experiments conducted in aquaria show that detached fragments of stems of various forms as *P. lucens*, *P. densus*, *P. perfoliatus*, and *P. crispus* develop roots, shoots, and buds, and that such detached parts of plants constitute a rapid means of propagation. Investigation of the growth habit of *P. natans* discloses a condition in marked contrast to the above-mentioned species. No special propagative bodies exist, but the species perpetuates itself by the continuance of the rhizome anchored in the mud, a rhizome which maintains itself through the winter rest period with the submersed shoots in various stages of growth.

Experiments on seed germination indicate a latent period of considerable variability. In *P. crispus* germination occurs within a year; in *P. natans* in from three to four years.

FRYER, 1900.

The first two installments of a fine quarto work, The Potamogetons of the British Isles, by Alfred Fryer, appeared in 1900. The monograph includes the varying forms and states as well as the recognized species, with accompanying plates, by the artist, Robert Morgan, who has reproduced the plants in color with singular beauty and accuracy. Unfortunately for science, the author's death occurred before this important work was finished.

Fryer had an intimate acquaintance with the Potamogetons and their habits. He grew many specimens in tanks in his garden, watching developments there and in their native haunts at different times of the year. He grew Potamogetons in order better to classify them, for he recognized the necessity of having a long series of specimens of the same form. "One set," as he says, "would contain a series of forms from *lucens* to *heterophyllus* without a single gap. This would show the way in which two quite distinct species pass from one to the other without a missing link." As a result of these observations a long and valuable series of communications on the genus, under the title "Notes on Pondweeds," appeared in the Journal of Botany from 1883 to 1899.

BENNETT, 1880–1914.

In the Journal of Botany Mr. Arthur Bennett's "Notes on Pondweeds" have appeared regularly from 1880 to the present time. He has become the acknowledged authority on the classification of the genus.

PIETERS, 1902.

In a Contribution to the Biology of the Great Lakes, Mr. A. J. Pieters notes the distribution of aquatic plants, describes the forms occurring in diverse situations, presents details of structure, and records various methods of vegetative reproduction. The Potamogetons, he observes, form a conspicuous feature of the aquatic vegetation, predominating, as a rule, in aquatic associations or flourishing in isolated patches. *P. heterophyllus*, he says, exemplifies the latter condition in that it thrives in a surf-beaten sandbar, where its runners ramify in all directions among the stones and pebbles, and its roots penetrate the underlying clay. Details of structure which are figured for *P. americanus* suggest the special adaptation of a thin, broad-leaved form, whose leaves are submerged, for withstanding diminution of light and rapid motion of water. The so-called hibernacula, or winter buds, represent the more familiar forms of vegetative reproduction observed by the author.

POND, 1903.

Further contributions to the biological literature of aquatic plants have been made by R. H. Pond. Two papers are presented on this subject. In the first, The Biological Relation of Aquatic Plants to the

Substratum, the author showed that rooted aquatics depend on the soil substratum for the supply of nitrates. In conducting the experiments various aquatic plants were used, among which were *P. perfoliatus* and *P. obtusifolius*. It was found that both of these plants are dependent on the soil substratum for optimum growth, though the cuttings which were employed behaved differently in manner of growth: *P. perfoliatus* showed an increase of growth through the development of new rhizomes; *P. obtusifolius* manifested it in a continuation of the branches already present. The behavior of *P. perfoliatus* is in accord with the observations of Sauvageau in his experiments on the propagation of Potamogetons by fragments of stems.

The second paper of the author, The Larger Aquatic Vegetation, to appear in Ward's American Fresh-water Biology, supplements the work of the first by additional observations, discussions, and generalizations. From his observations on the substratum of the larger aquatics it appears that they may be found growing on gravelly, sandy, or loamy soil, the loamy soil supporting the greatest variety of species. Direct experiments on this point, with the natural conditions reproduced as nearly as possible, bear out this observation. The author states, moreover, that the character of the soil is so important a factor that it is possible to predict the nature of the bottom from the species that are found growing in it. For example, "Among the islands of western Lake Erie *Potamogeton heterophyllus* is common on the reefs and pebbly shores, but it is not noticeable in the coves where a good soil substratum exists, and so prominent is it in the former places that its presence may be considered characteristic of the flora."

JEPSON, 1905.

In a popular article in the Sunset Magazine for February, 1905, Prof. W. L. Jepson has set forth the possibilities of the marshes as a feeding ground for ducks. He has taken as a concrete illustration the Suisun Marshes in California, marshes which abound in the fennel-leaved pondweed, *P. pectinatus*, and which afford natural feeding grounds for the various kinds of wild ducks, more particularly the canvasbacks. The canvasback and the broadbill, both diving ducks which visit these marshes, devour greedily the tubers that are developed in abundance on the rootstocks and upper portions of the stems of this Potamogeton in the autumn. It is claimed that these tubers give the fine nutty flavor to the canvasback at this season of the year. Other ducks, nondiving species, feed on the tender rootstocks and leafy stems which are brought to the surface in the feeding operations.

ASCHERSON AND GRAEBNER, 1907.

Ascherson and Graebner have published the last important monograph on the group, the Potamogetonaceæ, in Das Pflanzenreich. In this work the whole number of the described species has reached 87. Of these North America has 38, 14 of which are exclusively American. The numerous forms and varieties that are listed, though some common ones are omitted, illustrate how difficult the problem of classifying the Potamogetons still remains. In addition to the literature on classification the authors have assembled much important data on the anatomy and morphology of the group from foreign sources not generally accessible. Under the caption, Überwinterungsformen und Vegetative Vermehrung bei Potamogeton, the following propagative structures are figured: The slender, spicular bur of *P. crispus;* the large denticulate bur of *P. crispus;* the tuberous rhizome of *P. lucens;* the tubers of *P. pectinatus;* and the winter bud of *P. obtusifolius*. All except the first are reproductions of Irmisch's celebrated monograph.

MCATEE, 1911.

In a bulletin of the Biological Survey entitled Three Important Wild Duck Foods, Mr. McAtee has assembled for Government publication important data regarding these foods, in the hope that they may become more widely known and propagated for the preservation of wild ducks. Analyses of the food content in the stomachs of the more important species of the game ducks show that the pondweeds, the Potamogetons, are a favorite plant food. The ducks which apparently show a special fondness for it are the canvasback, the redhead, the scaup, and others, the first of which takes a very large proportion of the Potamogeton, the amount being nearly 50 per cent of the food eaten.

The best known duck food among the Potamogetons is *P. pectinatus*, of which the seeds, the tender rootstocks, and the tubers are eaten. It is general in distribution, thriving in fresh, brackish, or salt water. This and other widely distributed species are figured, and suggestions on how, when, and where to plant them are given.

MICKLE, 1912.

A Canadian bulletin, The Possibilities of Northern Ontario as a Breeding Ground for Ducks, by G. R. Mickle, is an investigation of the shoal waters of that Province with a view to their utilization for the propagation of wild game. In the preliminary survey the approximate amount of shoal waters is estimated to be 2,800,000 acres, on which various edible water plants grow. But it is hoped that the natural supply may be augmented considerably by transplanting such of the larger aquatics as will contribute especially to the food of wild ducks. Among the valuable water plants suitable for transplanting, the author names several species of Potamogeton, *P. natans*, because of its abundant seed habit, *P. perfoliatus* and *P. crispus* because of their edible leaves.

MICKLE AND THOMPSON, 1913.

A second Canadian bulletin by G. R. Mickle, written in collaboration with R. B. Thompson, supplements the work already done in this line. A table giving the estimated percentages of the various constituents of duck food shows that both *P. heterophyllus* and *P. perfoliatus* form an important food constituent in the diet of wild ducks.

It will be seen from the foregoing résumé of the literature on the genus Potamogeton, that an important extension of the subject is in the field of biologic research, an aspect of the study which regards also the economic significance of the group. It is apparent, too, that this field of research concerns itself primarily with the propagation of Potamogeton by such structures as tend readily and effectually to distribute the group, viz, by burs, tubers, rootstocks, and winter buds. These have been described generally in Europe and in America, and one may consider their production a natural phenomenon.

SPECIES OF POTAMOGETON INVESTIGATED.

The species which are included in this investigation have been selected from the more or less common forms growing in the lakes and ponds at Ithaca, N. Y., and vicinity (Spencer and North Fairhaven). And these species have been chosen because they offer variety in habitat and in methods of propagation, and because they serve an important rôle in the economic relations of aquatic life, affording food, shelter, and support to many forms of animals which exist among them. The list of species follows:

P. americanus C. and S.
P. amplifolius Tuckerm.
P. heterophyllus forma terréstris Schlechtd.
P. perfoliatus L.
P. crispus L.
P. zosterifolius Schumacher.
P. obtusifolius M. and K.
P. filiformis Pers.
P. pectinatus L.
P. Robbinsii Oakes.

These Potamogetons were studied from September, 1912, to June 1914, in their natural habitats and in aquaria. Entire plants were thus observed throughout the period of development of those structures which are valuable in the vegetative propagation of the species. From time to time collections were made of entire plants with their subterranean systems intact. In shallow waters the plants were uprooted by hand, but in the deeper waters they were obtained by means of a rake or a grapple thrown over the side or the stern of a rowboat. No collections were made in midwinter, i. e., from the latter part of December to the middle of February, when the frozen condition of the lakes and streams rendered it impracticable. *P. crispus* is an exception, since it was collected from spring pools at all times of the year.

These studies have afforded an opportunity to observe the animals that are intimately associated with the Potamogetons. Such have been noted, especially those forms which depend upon these plants for food, support, or shelter.

GENERAL SURVEY OF LIFE CONDITIONS OF THE SPECIES INVESTIGATED.

POTAMOGETON AMERICANUS.

This species, which has been grown from seed and cultivated through two successive seasons, will receive more specific treatment later under the caption "Natural and artificial propagation." In its natural habitat this plant has been observed growing near the mouth of Fall Creek, a tributary of Lake Cayuga, and in a near-by cove of the lake, at varying depths of 3 to 4 feet. It has been observed also at Spencer Lake, at about the same depth but in much swifter water. In the latter situation the blades of the leaves are conspicuously attenuated. According to Fryer (1900), who has observed this plant in various localities, it is a plant of upland streams and rivers rather than of stagnant waters.

By uprooting the entire plant in the growing season, it is found that the stem springs from a rootstock that is deeply anchored in the mud, where new shoots radiate horizontally from the established parts of the plant. During the summer these young rootstocks produce large buds at their tips (fig. 6). After the plant dies down, which may occur as early as August, the subterranean system remains intact for several weeks. The new rootstocks, however, carrying the buds at the tips, become eventually detached through the disorganization of the parent stem and in time die away, leaving but little beyond the isolated buds to perpetuate the plant the following spring. Such buds, since they remain in a quiescent state during the winter, may be called winter buds or hibernacula, a term applied to structures of a similar nature and function. Mr. A. J. Pieters (1901) doubtless referred to propagative structures of this kind when he recorded for *P. americanus* (*P. lonchites*) "extensive runners bearing buds at their ends," though no figures are given and no further observations are noted.

Fryer (1888) mentions an autumnal state of *P. americanus* (*P. fluitans*) in which the leaves are all narrowly linear or grasslike. These later growths, he says, are developed in the axils of old leaves during the natural decay of the lower part of the stem. They are ultimately set free as fascicles of narrow leaves which, after rootlets are formed at the base of the new growth, sink to the bottom and continue the life of the species. Such structures, which would be analogous to the winter buds of *P. obtusifolius* and *P. zosterifolius*, have not been observed in *P. americanus* under investigation, though they may have been overlooked in the changes of water level during the autumn.

POTAMOGETON AMPLIFOLIUS.

This is an American species distributed quite generally throughout the continent. It forms large patches in the open vegetation but thrives also in close association with *P. Robbinsii, Heteranthera dubia, Ceratophyllum demersum, Elodea canadensis*, and other plants of aquatic meadows. As a forage plant it may be regarded as one of the best, growing continuously from early spring to late fall or early winter and producing an abundant herbage by reason of its numerous large leaves. The rankest growths have been found in the more quiet waters of Lake Cayuga and "The Pond" at North Fairhaven, at depths of 5 to 7 feet, in a substratum of mud rich in vegetable mold. Propagation is rapid. The dense patches of stems, more or less unbranched, arise in great numbers from an intricately developed subterranean system (fig. 7). This

extensive ramification of underground stems, richly provisioned with starch, remains more or less intact during the winter, carrying at alternate nodes undeveloped shoots which quickly establish new extensions of the plant in the spring. Another means of vegetative propagation is found in the detached tips of branches which, after separation from the decaying parent stem in the fall or spring, sink to the bottom and become new centers of growth. New shoots also develop at the nodes of decaying stems and, on separation, sink to the bottom and take root as in the case of detached tips and stems. Besides these vegetative means of growth this Potamogeton produces an abundance of seeds.

POTAMOGETON HETEROPHYLLUS.

Various forms of this species occur throughout almost all of North America except the extreme north. One of the numerous forms, *forma terréstris* Schlectd., is represented in this investigation and all data herein recorded pertain to this plant. It is a so-called land form of Potamogeton and briefly characterized in Gray, seventh edition, as "freely creeping in exsiccated places, producing numerous branches which bear tufts of oblong or oval coriaceous leaves but no fruit." This plant, which grows in the open air after being left entirely uncovered by water, has been observed in two places along the shores of Lake Cayuga—one in a railroad pool 2 miles east of Ludlowville and the other on a sandbar at Myers Point. In each of these places it is interesting to note that gradations in habit accompany the varying changes in habitat. The railroad pool is a particularly favorable spot for the growth of this Potamogeton. It is an artificial pond which has been developed by building a railroad embankment near the foot of the bluff bordering the lake. In consequence, a long, trough-like depression exists between the bluff on one side and the railroad embankment on the other, with water from the lake seeping through and maintaining itself at about the level of the lake. It is a situation especially favorable to the growth of this plant, because the annual withdrawal of the water is gradual, following the natural lowering of the lake level during the summer months. The bottom of the pool is covered with black mud, largely marl in composition, a foot or more in thickness, over which water may rise to the height of 12 to 16 inches. During high-water level in the spring, this Potamogeton grows submerged in the pond with its tuberous rootstalks anchored in the mud (fig. 8). Upon the withdrawal of the water, following the lowering of the lake level in the summer, drought conditions prevail, and then the submerged leafy stems give place to the land forms. Upon the approach of drought conditions the previously submerged leaves die and from the main rootstock or from those arising from the axils of the lower leaves (upper in some cases, Bennett, 1880) runners extend horizontally to a depth of 2 to 6 inches below the surface of the mud. From the fertile nodes of these runners erect axes arise, bearing tufts or rosettes of leaves which cover the ground in great numbers and compete with mosses and small forms of sedges, carpeting the surface of the mud. The leaves of the rosette (fig. 12) are unlike the elongated, membranous, submerged ones. They are more rounded in form and coriaceous in texture, and by the presence of stomates on the upper surface of the lamina, they are enabled to function as ordinary leaves.

During the season of 1913 the plants which flourished in a submerged condition during the month of May gradually changed their habitat upon the withdrawal of the water during June and became land forms by the first of July. At this time the tuberous rootstocks which perpetuate the plant vegetatively were well developed, and waited only the final stages in the curing process to become the perfected vegetative structures which tide this species over the unfavorable season of growth.

On the sand bar at Myers Point, the other station where this Potamogeton thrives, the life conditions are not so sharply marked by the complete withdrawal of the water during the dry season, and the various stages exhibited in the transmutation from aquatic to land forms were easily observed. In water about 10 inches deep the continuously submerged plants developed low bushy stems, with a few coriaceous leaves at the top. In shallower water the plants behaved in the same way, producing bushy, stunted-looking stems, which finally graded into land form with leaves in tufts or rosettes resting on the exposed surface of the sand bar. The rootstocks, which were twisted and contorted in their effort to become established in the pebbly and gravelly sand bar, were buried from 2 to 4 inches beneath the surface in the rich, black soil of the bar. All of the internodes of these subterranean stems were more or less thickened and often attained a length of 8 to 14 inches.

No fruiting plants were found, and this observation is in accordance with the generally accepted opinion that this form of *heterophyllus* is propagated entirely by vegetative means. Observations on the artificial propagation of this species are recorded in a later chapter of this paper.

POTAMOGETON PERFOLIATUS.

The leaves of this plant afford valuable forage material, though the season of growth is comparatively short, the plants appearing late in the spring and dying quite early in the autumn. In the environs of Ithaca this species flourishes in quiet waters either in a substratum of sand at the relatively shallow depths of 2 to 3 feet, or in "aquatic meadows" in a substratum of mud at depths of 3 to 5 feet. The observations of Pieters (1901), in "The Plants of Lake Clair," and of Thompson (1897), in "The Biological Examination of Lake Michigan" extend the range of depth at which this species exists to 12 feet. During the growing season the vigorous undergrownd stems increase rapidly the output of forage material, since a single subterranean system produces a large number of erect, much branched, leafy stems. The experiments of Pond (1903) and Sauvageau (1894) and the observations of R. B. Thompson (1913) afford evidence of other means whereby the rapid extension of this plant takes place. In accordance with their observations, young branches, which are easily detachable, float away and rapidly become new centers of growth. In winter the vigorous and abundant subterranean system decays, leaving only the terminal shoots of two or three nodes (Fryer, 1900) to continue the plant the following spring. This plant, therefore, has three important means of vegetative propagation: By readily detached leafy stems, and by extensions of the subterranean system, both of which operate to multiply the plants during the growing season; and by the terminal portions of rootstocks which, remaining in a quiescent state during the winter, establish new plants in the spring.

POTAMOGETON CRISPUS.

This species, a native of Europe, was recorded in this country by Pursh as early as 1814 (Arthur Bennett, 1901). Since that time it has become established over an extensive area because of the remarkable facility for multiplying itself vegetatively. It is the most abundant Potamogeton in the vicinity of Ithaca, where it flourishes in various habitats—in deep or shallow water, in sand or mud bottoms, and in stagnant pools or flowing streams. It is singularly adaptive in each situation. It has been collected with *P. pectinatus* growing at depths of 8 feet, in which habitat the internodes are extremely elongate; it has been found in pools where the substratum is an accumulation of débris from ash heaps and dumping grounds; and it is not uncommon in the swifter parts of streams and along the lake shore in sandy situations where the substratum is thrown into ripples by wave and current action. In the latter situation it has always possessed short, stocky stems and a general dwarfish appearance.

P. crispus grows the year round and spreads with great rapidity. It is propagated primarily by "burs," peculiarly distinctive structures to which there is nothing quite comparable in our native species. Morphologically they are branches, but in the stage most frequently seen they are scarcely recognizable as such members of the plant structure. They have a horny look and a reddish color. The shortened internodes and thickened persistent leaf bases combine to give the characteristic bur-like appearance (fig. 22).

POTAMOGETON ZOSTERIFOLIUS.

This flat, grass-like species of Potamogeton is not largely foraged upon by aquatic herbivores, yet it appears in greater or less abundance in most ponds and lakes and doubtless serves an important rôle in the economy of life by furnishing support and shelter to the countless small forms which have been found upon it.

P. zosterifolius is among the earliest of the Potamogetons to appear in the spring, as well as among the first of them to disappear in the autumn. It flourishes in a substratum of mud in still or running waters, and while it is not adapted to possess the soil so completely as *P. crispus*, nevertheless it has effective means of perpetuating itself. Mr. A. J. Pieters (1901) remarks that this species, which he has observed growing in abundance in Lake Erie, may be losing the power to produce seeds. Indeed, during the past season few plants matured seeds in the several regions where they were observed, but all developed winter buds in great abundance (fig. 33).

Large quantities of vegetation, that is, the accumulation of the varied and abundant mass that still exists in the autumn, have been hauled up to the surface for examination, and it was both surprising and astonishing to see the vast number of winter buds of this Potamogeton that were entangled among the stems of other plants. It suggests to an extent how well this species accommodates itself to its surroundings. It never forms dense patches of growth, but it often occurs with aquatic plants that form them more or less densely. By virtue of its slender, grasslike habit, it occupies the interstices of the more rank aquatic flora, and it occupies these spaces as simple individual plants, not as erect axes of a complete and intricate subterranean system. The plants are anchored to the substratum by the roots only, which develop from the winter bud, and because of this loose hold in the soil they are readily pulled up. The large number of

plants which have been uprooted appeared always to possess a comparatively simple, erect stem which developed from a winter bud without the ramifications of rootstock which are characteristic of other species of Potamogeton not grasslike in habit.

POTAMOGETON OBTUSIFOLIUS.

This species is apparently an important aquatic forage plant, for its delicate leaves show abundant evidence of larval depredations throughout the growing season. It is somewhat grasslike, yet less stiff and harsh than the preceding species. It is a rare Potamogeton in the flora and has been observed in one place only, Spencer Lake, where it is found in a muddy substratum in shallow waters of more or less swiftness. The plant has a bushy habit of growth, branching widely toward the summit, a habit which tends to produce dense patches of these plants. At one place in the station it grows in such dense masses as to choke up the mouth of a small stream entering the lake.

The plants are late in appearing among the other aquatic forms in the spring, lagging behind *P. zosterifolius* a month or more. The bushy habit of the plant begins to show itself early in the summer, when branches arising near the base of the plant ramify toward the top until the characteristic bushy habit is attained. Fruit is produced abundantly, but doubtless an equally important structure in the reproduction and distribution of the species is to be found in the large winter buds. These appear on the much-branched stems in great numbers and differ in no essential respect from those of *P. zosterifolius* except that they are much less stiff. As in the above-mentioned species, they fall away from the parent plant when mature and sink to the bottom. Like *P. zosterifolius*, too, there is characteristic simplicity in the underground system. The mature plants which have been collected show no tendency to produce ramifications in the substratum, nor any indication of a perennial habit, but the plants become readily propagated vegetatively by means of winter buds or hibernacula.

POTAMOGETON FILIFORMIS.

A habit sketch of this plant is shown in figure 36. Morong (1893) states that this is a rare species in the United States. One collection only was obtained. The specimens were found early in July near Canoga on Lake Cayuga, where the plant flourished in shallow water and among calcareous rocks along the shore. The plants were short and bushy in habit and bore abundant fruit. In all cases the erect axes developed from a tuberous rootstock which, judging from the numerous erect shoots that grew therefrom, is the common method of vegetative propagation in this species. The tubers (fig. 37) occurred in series of 3 to 5 on the rootstock. Although no opportunity was afforded for studying this plant during the successive seasons, it is deemed worth while to record the observations of one collection of plants, since this species of Potamogeton is unique in its habitat and promising in the possibility of seed and tuber production.

POTAMOGETON PECTINATUS.

This species possesses many important characteristics which recommend it to the culturist of aquatic plants. It is one of the most abundant and widespread of the Potamogetons. *P. pectinatus* is regularly found in quiet waters, though it has a variable habitat in other respects, occurring in a substratum that is sandy or muddy and in waters

that are deep or shallow, fresh, salt, or brackish. It is also extremely variable in growth habit. Two of its remarkable forms which occur in Lake Cayuga and its environs and which Dudley (1886) describes as a slender form[a] and a gigantic form[b] are included in the present investigation of this species.

P. pectinatus, the species which is common everywhere, is among the first of the Potamogetons to sprout in the spring, making its appearance early in April. Of such plants which appeared early in the season, over a hundred individual specimens were uprooted to determine the agent of propagation. In all cases these plants developed from tubers which were buried in the mud or sand. Figure 38 shows the general habit of growth from these reproductive structures. The new plant quickly establishes itself by developing simultaneously with shoot formation an extensive subterranean system of stems, which in turn send up leafy shoots in great numbers. By this ramification of the underground stems, *P. pectinatus* encroaches upon the soil so effectively as to produce dense patches of growth, to the exclusion, in some cases, of other species of aquatics. The plants bear fruit more or less abundantly, but, in general, tuber formation doubtless equals or surpasses seed production.

Tubers of various size occur, the size being dependent, more or less, on the nature of the environment. The largest and finest specimens were found at North Fairhaven in the quiet waters of Sterling Creek, where *P. pectinatus* forms a part of an equatic meadow renowned for its luxuriance of vegetation. These large tuber-bearing plants grow in the rich, mucky substratum at a depth of 6 to 10 feet in association with *Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Utricularia vulgaris* var. *Americana*, *Nymphea advena* and other Potamogetons, such as *amplifolius* and *zosterifolius*. In this situation the plants are rapidly propagated from the tubers. On June 21 several specimens were collected which illustrate the complete cycle of tuber formation. Plants retained intact the old tubers, the new shoot—a tall, leafy, erect axis bearing in some cases a floral spike—and the new rootstocks bearing tubers. On many plants in this most favorable environment the tubers were greatly in excess of the matured fruits, and often the only reproductive structures. The plant dies down early in autumn. In October attempts were made to collect underground stems to determine, if possible, a perennial habit in this region. Only portions of the rootstock were secured, but in every instance disorganization had progressed to a considerable extent. The appearance of the tuber in the spring, when many of the plants were uprooted and observed with shoots growing from them, indicates a complete and natural separation from the parent stem, probably in the autumn. It may be inferred, then, that the tubers are the only vegetative structures that do survive the unfavorable growing season.

The slender form described by Dudley (1886) was found still occupying the same region in Cayuga Lake where it was observed by him many years ago. The plants

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[a] 1007. var. ——(?) with slender elongated stems (1 to 1¼ meters); nodes remote, as are the whorls of the spike, whose peduncle is usually over one-fourth meter long. Leaves few and slender, plants sometimes proliferous. Near the lighthouse, Cayuga L. Dr. Robbins "found no parallel for this remarkable form," in his own observations. Dudley, William R.: The Cayuga Flora. Bull. Cornell Univ. (Science), p. 107.

[b] 1008. var. ——(?) a gigantic form growing in deep water northwest and northeast of the lighthouse, Cayuga L. Not yet found in flower or fruit, though examined more or less frequently during 10 years past. It is frequently proliferous, especially if detached. It grows in banks, the plumelike bushy tops reaching the surface of the water. The leaves and sheaths are similar to *P. pectinatus*, except in length. Dr. Robbins remarked that he had "nothing that comes near to it in length of leaves—usque ad 10." Stipules are usually much shorter than in *P. pectinatus*. Specimens were obtained in 1874 from 4 to 5½ meters long. This form was also noticed by Mr. H. B. Lord, probably somewhat earlier than 1874. Loc. cit.

grew in banks in sand and silt bottoms at a depth of 5 to 7 feet. They were quite unmixed with other aquatics. In July and early August the long heavily fruited spikes floated in dense masses at the surface and gave to these areas of the water a characteristic brown look. Proliferations were not found on these plants during the summer; fruits, however, were more abundant than on any other form of *pectinatus*.

The gigantic form of *pectinatus* grows in deep water. Plants 8 feet long are common, although many average but 5 feet at the end of the growing season. This form grows in a substratum of sand and silt at depths varying from 6 to 12 feet in a region of the lake exposed to a more or less constant sweep of the wind. The plants, therefore, which grow practically to the surface of the water, are subjected at times to vigorous wave action. Altogether these environmental conditions favor a growth of remarkable luxuriance. The plants grow in banks, and so thickly as to preclude the possibility of encroachment by other forms of vegetation, though in shallow places, where the growth becomes sparser, a few scattered representatives of *P. crispus*, *P. perfoliatus*, and *Heteranthera dubia* occur.

This form of *pectinatus* begins growth early in the spring. In May, 1913, the plants already approached the surface of the water. On June 21, 1913, a plant bearing a single floral spike was found, although in several collections made thereafter neither flower nor fruit was obtained. This appears to be the first record of a floral spike on this form of *pectinatus*. From the collections made in November a few tubers were found on the tips of the foliage sprays of the plants that were uprooted from their natural moorings, although they were found more commonly on sprays that were floating in the drift. This latter observation is an agreement with Dudley (1886), who observed and described this form in Lake Cayuga. No rootstocks were secured, since attempts to uproot the plants at such depths with a grapple resulted always in breaking the stem just short of the subterranean system. This appeared to be embedded firmly and deeply in the substratum, at least more deeply than the length of the grapple teeth, which measured 4 inches. However, the bases of the erect stems, the parts which develop just above the rootstocks, possessed remarkable examples of proliferation. Thickened runners, more or less contorted, arose from leaf axils at the bases of the erect stems (fig. 50, A), terminated by large, elongate tubers. The bases of the stems were hard and woody, more especially so in the regions where they became detached from the underground system. This condition suggests a continuation of the woody structure in the subterranean parts. It may be inferred perhaps, from the general habit of the plant and the attendant conditions of growth, that the rootstocks are perennial, and that the basal runners, which bear in abundance large tubers and green shoots, are the chief propagative structures of this form of *pectinatus*.

POTAMOGETON ROBBINSII.

Although this Potamogeton is less well known than the other species, it is destined to be regarded as an important aquatic forage plant, first, because it is very prolific, and, second, because the foliage is very generally eaten. The habitat of this species, where it has been under observation, is not unlike that of *P. amplifolius*, with which it is often found in association. It has been observed in the quiet waters of lakes and ponds at depths of 3 to 5 feet in a substratum of rich, black mud. The stems ascend from a somewhat creeping base and branch profusely in a more or less two-

ranked arrangement, forming large, broad, flat sprays of foliage, which often cover the bottom in large patches. It is the rarest of all Potamogetons to fruit, at least in the situations where it was observed, but because of the tendency to branch profusely propagation is readily effected. The branches, especially those whose internodes remain short, become thickened and hardened through the storage of starch, and when detached function as propagative structures. This enlargement and induration may occur also at various points along the main axis that bears the propagative branches, so that the final dismemberment of the whole plant provides enormous possibilities in the multiplication of the species. Dismemberment may occur in the autumn, but the plant is hardy, and this natural separation of parts may be deferred till spring, then long rootlets develop at the nodes and establish the plant at once. The plant is tardy in beginning its growth in the spring, but this tardiness in growth is obviously advantageous to a plant that propagates mainly by vegetative means in the manner of this species. Moreover, a very material advantage accrues in that the full and complete foliage of this Potamogeton appears late in the season when many other aquatics, including Potamogetons, show signs of decay. Growth occurs during the winter. It is not great, however, and manifests itself only in a slight elongation of the branches, producing fresh, green tips of foliage, which are foraged upon by aquatic herbivores almost as fast as the leaves appear.

SUMMARY OF CULTURAL FEATURES.

The Potamogetons which yield important forage products fall into two groups: Those which produce abundant herbage in their leaves—*P. americanus*, *amplifolius*, *perfoliatus*, *crispus*, and *Robbinsii*—and those which develop a large supply of starchy food products in the tubers and tuberous rootstocks—*P. pectinatus*, *filiformis*, and *heterophyllus*.

The species which grow best in the currents of streams are *P. americanus* and *obtusifolius;* in deep water, *P. pectinatus*, especially the slender and gigantic forms of Dudley; in calcareous regions, *P. heterophyllus* and *filiformis;* in exsiccated places, *P. heterophyllus*.

The species appearing early in the spring are *P. americanus*, *zosterifolius*, *pectinatus*, *heterophyllus*, *crispus*, and *amplifolius;* those growing late in the autumn and continuing throughout the winter are *P. crispus*, *amplifolius*, and *Robbinsii*.

Abundant fruit is produced in *P. perfoliatus*, *obtusifolius*, and *filiformis*, and on *pectinatus* in most situations. Vegetative reproduction occurs freely in all species. The important vegetative structures are: Winter buds or hibernacula in *P. obtusifolius* and *zosterifolius;* modified branches in *P. crispus* and *Robbinsii;* tubers in *P. pectinatus* and *filiformis;* tuberous rootstocks in *P. heterophyllus*, and subterranean buds in *P. americanus*, *amplifolius*, and *perfoliatus*.

NATURAL AND ARTIFICIAL PROPAGATION.

The natural propagation of Potamogetons has been touched upon in a general survey of life conditions, and it has been seen that these plants propagate freely by means of various vegetative structures. At this point it is desirable to consider this method of propagation in greater detail, and to present data which will afford a means of comparison between the general seed habit and the tendency to produce vegetative propagative structures.

PROPAGATION BY TUBERS.

A conspicuous method of vegetative propagation is seen in the development of plants from tubers in *P. pectinatus* and *P. filiformis*. The tuber-forming habit of *pectinatus* has been described by Irmisch (1858), who carefully worked out the morphological details of the tubers in terms of the ordinary stem structure. His figures illustrate the development of tubers on detached parts of leafy stems, on the erect axis, and on the underground stems. It is not clear from which forms of *pectinatus* these drawings were made. In general, however, they bear a close resemblance to our most common representative of *pectinatus*, though no hint of the variability in this species is given beyond the fact that some plants were collected in deep water, and that the tubers were varied in shape, some being more cylindrical than others.

The work of Sauvageau (1894) confirms the observations of Irmisch as regards the tuber-forming habits of *pectinatus*, but this investigator also makes no allusion to the remarkable forms that exist in this species. His drawings, moreover, are, as he states, modifications of those by Irmisch. Both of these workers in this field recorded the time of tuber formation to be in the autumn. Jepson (1905) suggests an earlier development for those on the rootstock and the erect stem. He says: "The slender threads which develop one, two, and even three tubers at the end, are not only borne on the horizontal rootstocks and on the soil at the bottom of the ponds, but are also produced on the upright stems, and at the end of the season on the uppermost leafy portion."

Regarding the presence of tubers on rootstock, stem, and spray, the present investigation is confirmatory. Tubers have often been observed on all these parts of the plants. Additional figures and observations relate more especially to the season in which they occur and to their artificial propagation. Collections of plants made on the 15th of May, 1913, and thereafter throughout the growing season, show the presence of tubers in great numbers on the proliferating shoots of the rootstocks. Many of these tubers are well grown in May, though others subsequently arise on the extensions of the subterranean system which develop after this time.

Figure 41 represents the basal part of a small immature plant of *P. pectinatus* collected in shallow water June 20, 1913. Many plants at this time were more nearly mature and bore larger tubers, but it seemed desirable for illustration to select a small plant because in such all parts may be preserved intact during the collection of material, a task that is attended with considerable difficulty when the plant has attained a large size and great complexity of parts, especially in the subterranean region, where the underground stems are exceedingly brittle and tender. This figure (fig. 41) illustrates the general sequence of growth in what may be termed the typical vegetative life cycle of the plant. The order of development is as follows: The production of a leafy, erect shoot (C) from the tuber of the preceding season (A); the growth of the horizontal axis or rootstock (D); and the production of the stolon-like branch or runner which in turn bears a tuber or tubers at the end (B).

As the season progresses the tubers become solidly packed with starch in sufficient amount, apparently, to bring the plants developed from them to a very advanced stage of growth, at least to render them quite independent of the soil for a considerable length of time. Figure 45, B illustrates the typical condition in this respect when tubers suspended in aquaria without contact with the substratum produce the future propagative structure. Thus the continued dependence of the plant upon the stored starch in

the tuber would seem to be advantageous, especially if growth occurred under untoward conditions.

The tubers, hardened by the great quantity of starch that is packed into the tissues, normally pass through the winter in a dormant state. This, however, is quite easily disturbed, and by supplying continuously ordinary room temperatures the tubers may send forth shoots as early as October. Figure 45, noted above, illustrates such a response to growth conditions, the plant having been developed between the dates of October 22 and December 20.

The propagation of tubers in aquaria has shown that when tubers occur in twos, for example, figure 40, the larger one develops the shoot. The smaller one has never been seen to sprout unless by chance it became detached. In that case it developed an individual plant. It has been frequently observed that plants of this species when propagated in aquaria never attain their full size or vigor when deprived of a soil substratum, an observation that is in accord with the results of Pond's (1903) experiments on rooted aquatic plants.

The remarkable versatility of *P. pectinatus* as regards the origin of tuber-bearing runners has been clearly shown by Irmisch (1858). There is, moreover, in each of these situations, on rootstock, stem, and spray, a considerable variation in size and number of tubers. For example, an underground stem or rootstock may develop them at the ends of slender, stolon-like branches which arise from the axils of fertile nodes as shown in figure 43. These have been found singly or in pairs, large or small, depending upon the richness of the substratum and the size of the plant. Again, the rootstock itself may be terminated by tubers which occur singly, in pairs (fig. 42), or in threes (fig. 39). Plants bearing rootstocks of this character have been collected at various times during the growing season, and from each collection the specimens have shown comparatively short underground stems without other tuber-bearing structures. Some rootstocks have shown no tendency to produce tuber-bearing runners or tubers at the end of the horizontal axis, but send up a succession of leafy shoots from the fertile nodes. It is suspected, however, that had such plants been undisturbed tubers might have developed, especially since at the base of these upright shoots there was always a bud, either latent or showing a tardy development.

In autumn *pectinatus* develops tubers on the leafy spray. They are generally smaller than those which occur on the rootstock, but quite conspicuous because of their pale, yellow color. They are borne singly or in pairs at the ends of runners that are bright green and stouter than the stems from which they arise (fig. 44, B, C). These structures are readily distinguishable about the time the plant begins to show signs of decay. They may occur on attached or detached parts of the plant. The remarkable prolificity of these sprays is a characteristic of this species. Repeatedly detached parts of the leafy spray have been placed in aquaria and tubers have been developed in abundance until the spray became completely disorganized. It is interesting to note that when this species grows in the currents of the stream the tendency to form proliferations on the leafy spray is conspicuously lessened, although portions of these plants when caught in the drift and carried to quiet water readily produce them in the new environment.

For the most part tubers are more numerous on sprays devoid of fruiting spikes, although exceptions are frequent. In examples of this kind, figure 44, B, C, shows the

origin of tube-bearing structures, one arising near the base of the peduncle, the other solitary from the axil of a leaf. Figure 44, A, is a detail of such a spray showing the usual character of the runner. Runners arise also on the lower parts of the stem (Irmisch, 1858). These, like many on the tips of the spray, may develop so late in the autumn that tubers never mature. What their fate is during the winter can only be conjectured. It is a fact, however, that when placed in aquaria they continue to grow slowly and eventually produce small tubers, or remain for a time in a quiescent state and then send forth leaves and other runners.

In the gigantic form of *pectinatus* (Dudley, 1886) the tubers are elongate and large in size. They are born on runners at the bases of the stems just above the substratum of mud, and are therefore several feet beneath the surface of the water. Figure 61 shows the entire leafy axis with a tuberous runner attached at the base of the stem. This is the normal position for what appears to be the chief propagative structure of this form of *pectinatus*, and the usual condition at the approach of winter. The runner is seen in detail in figure 50. The tubers are yellowish in color, and when stripped of scales, which envelop them at this season, appear as in figure 51. The remainder of the runner is dark green in color, more or less contorted and tuberous, and hardened throughout by storage of starch (fig. 50, B). Secondary runners bearing tubers (fig. 50, 1, 2) are additional features in what withal is a remarkable propagative structure. Peculiar tuberous internodes, transition stages, perhaps, in the formation of tubers, appear frequently and characterize the more hardened and resistant portions exclusive of the terminal tubers (fig. 52). On germination a leafy shoot and runner are produced. Figure 55, an illustration of a similar feature repeated in a series, was developed in an aquarium from the terminal tuber of a small runner. It illustrates how resourceful in the propagation of this species so small a structure may become.

Young, green, leafy shoots arise from the fertile nodes of the runner (fig. 50, D) and doubtless function in perfecting the propagative structures of this persistent part of the plant, for at this time—that is, in the autumn—the leaves of the main axis begin to disorganize. The young shoots retain their greenness through the winter, remaining in a quiescent state meanwhile, and produce the main axis of the new plant the following spring. When these structures are transferred to aquaria, they pass through a winter-rest period, a period which is less easily disturbed, however, in this form of *pectinatus* than in others of the same species. Extreme plasticity is characteristic of various portions of the runner. Fertile nodes produce either tubers direct, or leafy tips, or runners, any one of which may in turn produce a runner. The tip of a secondary runner may produce a leafy shoot (fig. 53), and a tuber, instead of elongating its axis in the natural way, may develop precociously a reserve bud which produces the leafy stolon (fig. 54).

As in *pectinatus* generally, the detached sprays of the gigantic form show a greater tendency to produce tubers than the attached ones. Likewise the runner is the important structure which bears them. Such tubers may become very numerous. As many as 15 have been counted on a single plant (fig. 62). Detached portions of the plant bearing tubers float away in the drift, from whence they may or may not find a favorable place of growth in the spring. The tuber-bearing runners developed at the bases of the stems rarely become loosened from the tangle of vegetation at the bottom and must therefore repopulate the area year after year, encroaching but slowly on the surrounding region.

P. filiformis represents a tuber-forming species which produces these propagative structures apparently in the manner of *P. pectinatus*. Since material was collected but once during the summer, no definite data can be recorded regarding the details of tuber formation beyond the fact that the plants develop from tubers, as the collected materials show (fig. 36, 37), and that these tubers, whether they occur singly or in a series of two or more, have a likeness to those of *pectinatus*, in size resembling the common form and in shape approaching more nearly the deep-water form. In details of structure the tubers of *filiformis* are similar to those of *pectinatus*. Judging from the general habit of the plant it seems fair to assume that the tubers have arisen in the same way and that vegetative propagation would depend largely upon them.

PROPAGATION BY TUBEROUS ROOTSTOCKS.

The vegetative structures of *P. heterophyllus terréstris* are illustrated in figures 10 and 11. Morphologically they are a series of more or less shortened and hardened internodes richly provisioned with starch. They are borne at the terminal portions of the underground stems. Well-developed buds, the incipient, erect axes, occur at alternate nodes of the structures, while the intervening nodes remain sterile, as in the case of undifferentiated rootstocks. Figure 8 represents a typical plant collected early in May. At this season the plant is still submerged. The tuberous rootstock of the previous year sends up young, erect shoots from the fertile nodes, and extends the growth horizontally by an elongation of the terminal bud to form the new rootstock.

The underground stems acquire a distinctly tuberous appearance very early in the summer. At Myers Point, where the collections were made frequently, the tuberous character became apparent at the time when drought conditions began to prevail in the pools; that is, when the water level was reduced to such an extent that the submerged, leafy shoots gave place to the later-formed, erect shoots topped with tufts or rosettes of aerial leaves which rest upon the mud. Figure 12 represents a plant of this kind. By comparing the plants shown in figures 12 and 16 the origin of the tuberous rootstocks is clear. In figure 16 tuberous structures appear at the ends of the new underground stems, B and C. This tendency to produce the tuberous growth may appear early when the plant is still submerged, though it may be deferred till drought conditions prevail, when the new type of leaves forming the rosette above the ground function to produce the abundant storage of starch which is found in the mature tuberous rootstocks.

Some underground stems, throughout the growing season, continue to produce internodes nontuberous in structure (fig. 9), but they are exceptional rather than the rule. The tip of the rootstock that is destined to become tuberous generally shows this character very early. The internodes at the end do not elongate in the usual way, but appear serially in a more or less bead-like form (fig. 10 and 11). Figures 13 and 14 represent the tuberous rootstocks partially developed. Figure 10 shows a fully mature one. These structures, and many others in similar stages of development, were collected in July and it is interesting to note that while some are only approximately mature others are fully so thus early in the season. In November all evidences of other plant parts have disappeared and the tuberous rootstocks only are left isolated in the mud, where they remain in a quiescent state through the winter. A typical structure, as it

appears at the beginning of the winter, is seen in figure 11, although variations in the length and thickness of internodes are not uncommon.

Tuberous rootstocks have been transferred to aquaria, where the growth has corresponded exactly with that exhibited in the natural habitat except in one respect, the development of aerial tufts of leaves. But the explanation of this omission in the cycle of development is clear, since the plants remained submerged in the aquaria. The period of desiccation not having been interpolated, it is assumed that the tuber formation progressed in a natural manner for the species. Figures 16 and 17, drawn from aquarium specimens, show how in the purely aquatic phase of its existence the natural habit of growth and reproduction in this Potamogeton is reproduced under artificial cultivation.

PROPAGATION BY SUBTERRANEAN STEMS NOT TUBEROUS.

Among the species studied, *P. perfoliatus*, *P. amplifolius*, and *P. americanus* are propagated in this manner. The plants are carried over the winter by means of the terminal portions of underground stems, which are generally stouter than the ordinary ones and which bear conspicuous scaly buds. These buds are the incipient shoots from which the elaborate plant structures of the following season are developed. Sauvageau (1894) has figured this propagative structure for *P. perfoliatus* as he found it at the approach of winter. He states that the entire plant dies in autumn, except a few internodes which bear the buds for the continuation of growth in the spring. In figure 18 is represented a portion of an underground stem that survived the winter and produced the first few internodes of growth. The scales on the part that lasted through the year are distinctive in appearance. They are larger and looser than the ordinary ones, black in color, and leathery in texture (fig. 18, A.)

In *P. amplifolius* perennial parts are also found in the underground stem. Figure 7 represents the characteristic features of such a structure at the beginning of the winter. The young, erect shoots A, A, A, wit partially unfolded leaves at the tips, pass the winter unchanged and serve to promote rapid growth in the spring. The buds terminating the horizontal stems remain latent through the winter and on unfolding in the spring push out in all directions through the substratum. In these ramifications a subterranean system of interlocking stems and roots is developed that fixes the plant with exceeding firmness in the soil.

In *P. americanus* vegetative propagation is accomplished by subterranean scaly buds which generally grow in pairs at the end of the rootstock (fig. 4 and 5). The general structure of the bud resembles that of *P. perfoliatus*. It is an incipient shoot, possessing a succession of very short internodes and young leaves, with scales surrounding the whole axis. A small portion of the rootstock generally remains attached to the buds and persists through the winter.

PROPAGATION BY WINTER BUDS.

The winter buds afford the only means of vegetative propagation which have been observed for *P. zosterifolius* and *P. obtusifolius*. These structures develop at the ends of the shoots. The terminal internodes remain short and, becoming completely covered by closely overlapping leaves and stipules, form a hard, compact, cone-like bud.

Such buds become conspicuous during the month of August. Later when they are mature they easily fall away from the parent axis, which thereafter dies down completely. Being heavier than water, the buds sink to the bottom and by the middle of October they have either disappeared or have become entangled in the accumulations of Elodea, Myriophyllum, Ceratophyllum, etc., which still remain intact. In the disorganization of this mass of vegetation, a gradual settling of the entangled buds takes place and they eventually find lodgment with the others in the substratum of mud, where they remain in a quiescent state till early spring. Such buds may properly be called hibernacula, since they pass through the unfavorable winter season in a state of rest.

The general external aspect of the winter buds is seen in figures 33, 63, and 64. In size and form the two buds are quite similar but the leaves of *obtusifolius* are less stiff and harsh. In the internal structure of the bud (fig. 34) the typical branch-like character is apparent with the young leaves closely crowded toward the tip.

Plants of both species have been reared in aquaria by anchoring the buds in sand or mud. The latter operation is not necessary, however, since mature buds sink naturally to the bottom, but it was a precautionary measure against the disturbance of buds under observation in aquaria. The plants of *zosterifolius* thus propagated did not bloom, but produced winter buds; those of *obtusifolius* bore flowers and fruit early in August.

During the winter the loose leaves on the outside of the bud decay, but, on the whole, the entire bud is well preserved. This resistant character is more especially true of *zosterifolius*, in which many of the enveloping leaves of the bud persist long after the new plant has become established. In the spring the first sign of growth is manifested by a spreading of the inclosing leaves. Then follows the development of roots from successive nodes (fig. 35) and the elongation of the internodes at the tip of the bud. This elongation carries the young leaves forward and upward, and in a short time the general habit of the plant becomes apparent (fig. 65). The various stages in the growth of the bud in the spring are, in so far as they have been observed, similar in those two species of Potamogeton, except that *obtusifolius* lags behind *zosterifolius*.

PROPAGATION BY BURS.

P. crispus is the single example of such vegetative propagation. The first evidence of propagative structures by means of which the growth of this species is rapidly extended became noticeable early in May. At that time the so-called "burs" (fig. 22) made their appearance. They were enormously abundant, appearing in the axils of nearly all the leaves. Many of them became fully mature by the middle of the month, especially those which developed in pools of standing water where the daily temperature of the water was comparatively high. In the colder waters of spring pools and of the open lake these propagative structures, like the flowers and fruit, were retarded in development, maturing about two weeks later. As the summer advanced the development of the burs decreased until by the middle of July only scattered individuals were to be found.

As a rule, the burs occur in the axils of the leaves. They may, however, terminate the growth of the axis (fig. 30). In this latter position they may occur in pairs (Savaugeau 1894), often with a flowering spike. They may develop from the rootstock

directly (fig. 31), though this occurs but seldom. On the maturity of the bur detachment from the parent stem is an easy and natural process. The tissue just below the pointed base of the bur becomes softened and the burs fall away, either by their own weight or by accidental contact with other objects. On reaching the bottom, anchorage in the substratum is facilitated by the peculiar shape of the bur, a sharp-pointed, spindle-shaped structure that is heavier than water. A rest period occurs before germination takes place. This rest period is apparently a varied one, depending on the season when the bur is matured. Those which matured early in the season, in so far as it could be determined, germinated in the fall, and in October bore shoots from 6 to 10 inches long (fig. 59). Those maturing late passed the winter in the quiescent state and germinated early the following spring.

The slender, spicular burs (fig. 21) described by Irmisch (1858) and by Sauvageau (1894) were found more or less commonly in the axils at the base of the erect stem, and always few in number compared with the stouter form. It is interesting to note in this connection that these spicular burs appeared more abundantly on the so-called "state" of *P. crispus*, a plant with flat, not undulate leaves, said to be a young state of *crispus* (Fryer, 1900). In one of the spring pools from which collections were made the spicular buds predominated on what appeared to be matured plants of this flat-leaved form. The plants were never so vigorous looking as those in the other situations, and the appearance of the spicular burs upon them may be explained by differences in habitat. Generally they appear to be poorly conditioned plants, and from observations on their development it would seem that they are a starved state of *crispus* rather than a young state.

The development of the large bur (fig. 22), which Sauvageau (1894) described in part, has been observed in the field and in aquaria throughout the various stages, from its beginning as a small branch to its completion as a mature bur. Since the steps in the formation are essentially the same under natural or artificial conditions, observations will be presented on the material under control in the laboratory.

Vigorous looking plants were collected in the latter part of March and anchored in a soil substratum in aquaria with running water. Cuttings also were used, some of which were anchored in the soil and others allowed to float on the surface of the water. Three weeks later, short, stunted-looking branches appeared in the axils (fig. 26, A). They exhibited at once a noticeable thickness of the axis and later the peculiar denticulate appearance at the base of the leaves (fig. 22, *a*). When the diameter of the branch had become considerably augmented and the denticulate margin conspicuous, disorganization of the leaves commenced from the distal end and proceeded toward the base. Disorganization ceased at the tip of the denticulate base (fig. 22, *a*, 1). By this time the basal portion of the leaf was hardened, thickened, and horny like the axis, and the entire structure presented the characteristic burlike appearance. Figure 60 shows several small-sized denticulate burs in various stages of development.

Essentially the two kinds of burs are similar, differing only in certain minor details. In the bur shown in figure 22 the leaf bases are large and always denticulate, the buds in the axils are relatively small, and the internodes are short. In the spicular bur (fig. 21) the opposite is true. The leaf bases are small and spinous with a smooth margin, the buds are well developed, and the internodes are comparatively long. A difference

between them is also apparent in the time of occurrence and in position on the stem. Irmisch (1858) recognized a disparity between them and suggested a difference in origin, though he was not able to determine this for both forms. The spicular burs he found originating from the axillary buds of decaying, floating stems in autumn. The denticulate ones he found always mature and detached from the parent stem in muddy bottoms of pools. Sauvegeau (1894) describes and figures both forms of burs, giving their origin as well. My observations, however, are not in full agreement with their representation on the stem as expressed in Sauvageau's figures. According to his illustrations, both forms are abundant on the same branch and at the same season of the year. This has not been found to be the established order in vigorous and healthy-looking plants. Numerous collections of *P. crispus* indicate that when the denticulate burs are abundant—that is, in the early part of the growing season—the spicular burs are scarce, and if present on the same stem they are sparsely represented at the base of the axis. In every case the large denticulate bur seems to be the product of strong and vigorous-looking plants, and the spicular bur a result of poorly conditioned ones. That the spicular bur is a weakling would appear to be borne out by observations on their development. When grown in aquaria they have been found on sickly-looking plants and when germinating burs have been deprived of their vigorously growing shoots, small shoots bearing spicular burs have replaced them. In this instance a disturbance of the natural trend of growth would be the occasion of their formation. When the spicular burs germinate they produce shoots bearing leaves not crisped, but narrow and flat (fig. 25).

The internal structure of the bur is fundamentally like that of the ordinary stem. No new features appear in the tissues of any part of the bur, but starch grains are present in such great quantities that the cells become distended with them. In the fully developed bur (fig. 71) the cells become so greatly expanded that the air cavities are practically obliterated. It is to these distended cells so compactly stored with starch that the hardened, indurated character is due.

The accumulation of starch in the bur furnishes an abundant storage supply for rapid growth, after a rest period of greater or less prolongation, depending upon the time of formation. Burs formed early in the summer may germinate early in the fall, or, like those of later development, pass the winter in a quiescent state. Figure 23 shows a stage of germination which is usual in the early spring. It is obvious from the general appearance of the shoots that burs of this character passed the early part of the winter in the resting stage. At the same time burs much more advanced in stage of growth (fig. 32) are frequent, and it is assumed that these are comparable to burs that germinated in the fall (fig. 59) and grew but little during the winter. In aquaria a variable rest period is common. Under these conditions burs have been germinated after periods of six weeks and of three months.

In the germination of a bur there are as many possibilities for the production of stems as there are axillary buds on it, although usually not all of the buds germinate. The greater number of burs bear but one shoot eventually, but several may begin growth and produce short shoots (fig. 23). By experiment it has been found that when a bur is broken into bits with one bud per node, each bud will produce a shoot. In the development of a plant from the bur, progress in the growth of a shoot manifests

itself first by the establishment of an erect axis, from which very soon a subterranean system arises in the manner shown in figure 27. By further extensions of these axes the number of branches is greatly augmented and the capacity for multiplication greatly increased.

P. crispus, like most of the Potamogetons, propagates readily by detached stems. Many of these have been picked up in the drift along the lake shore where under favorable circumstances some, doubtless, find lodgment and establish new centers of growth. Besides, in the spring there have been found leafy axes which, while still remaining attached to the parent stem, lie prone upon the muddy or sandy substratum and, becoming rooted at the nodes, send up a long series of erect stems (fig. 20). In this manner *P. crispus* combines the rapid growth from stolons with the normal spread of the subterranean system and forms an effective means of possessing the soil.

The large number of burs which are developed indicate that they are the chief source of distribution in this species. Some plants doubtless develop from seed, though they can not represent any great number of the whole since comparatively few seeds mature. To obtain some data on this point a large number of young plants were pulled up and to the most of them a bur was attached, an observation which shows that, for the region at least, this structure was the chief agent of propagation. From the standpoint of prolificity, *P. crispus* represents a desirable species for cultivation. It remains to be shown that this abundant herbage is of importance in the economy of aquatic life. Data relative to this are recorded under the heading "Economic aspects of Potamogetons."

PROPAGATION BY FRAGMENTS OF STEMS.

In *P. Robbinsii* the propagation occurs exclusively by vegetative means, depending upon a more or less complete dismemberment of the plant. This breaking of the plant into propagative structures does not take place at random, but occurs at very definite points throughout the leaf-bearing part of the plant. At intervals along the axes of the stems, a few internodes develop which are very short, and in them starch is stored so abundantly that they become hardened and stiff and noticeably thickened in diameter. At the limits of these indurated regions where the stems appear constricted, the tissues soften when the structures are mature, and dismemberment becomes a natural operation. The process of separation is similar to that which is met with in *P. crispus* and which causes the detachment of the bud from its parent stem. Besides the main axes of the plant which break up into many potential units, there are also numerous short, axillary branches which possess the characteristic feature of the propagative structure. The internodes are likewise short and stiff and conspicuously augmented by the deposition of starch. Moreover, they are always provided with a growing terminal bud, a feature which facilitates rapid propagation. When an axillary shoot becomes 6 or more inches long it behaves like the main axis of the stem eventually breaking up into several propagative structures. In figure 67 is represented a single branch showing the constricted appearance which distinguishes a stem bearing more than one propagative structure.

In the spring, often before a general dismemberment of the plant occurs, very long, white rootlets are developed at the nodes (fig. 57). These rootlets serve to anchor the new growth, whether it be an attached part of the plant or a scattered fragment of the stem. The provision for the initial growth in these fragments of stems lies in the storage

of starch within the tissues. Starch is so abundant that the air cavities are considerably reduced by the distension of the cells (fig. 71). In portions of the stem where the tissues are not obscured by the deposition of starch, it is seen (figs. 69, 70) that mechanical tissue is scattered through the stem in greater abundance than is common in the other Potamogetons, serving to support the heavy sprays of foliage and to give the rigidity of stem which is characteristic of this species.

In *P. amplifolius* the tip ends of the branches function as propagative structures in a manner similar to *P. Robbinsii* (fig. 58). These structures appear in the autumn developing only at the tips of the branches. The internodes are short and thick and densely packed with starch. At the end there are a few partially unfolded leaves which continue to grow slowly or, at least, remain green all winter. These rapidly expand when the roots develop in the spring and the entire structure forms an effective and rapid means of propagation.

PROPAGATION BY SEEDS.

While the main purpose of this paper is a consideration of the vegetative means of propagation, yet it is important by way of comparison to present such data as are available on the propagation of these plants by seeds. In reviewing the literature on the seed germination of Potamogetons, it appears that Irmisch (1858) and Sauvageau (1894) have made the only contributions of importance.[a] Irmisch figures the germinating seeds and two small seedlings of *P. natans* but otherwise gives no data concerning them. Sauvageau found that *P. crispus*, *perfoliatus*, and *pectinatus* germinate in less than a year and that *P. natans* remains dormant three or more years. No figures accompany his account of their behavior.

In the course of the present investigation additional observations have been made on *P. pectinatus* and *P. americanus*. The seeds of both species were gathered in October and kept in cold storage through the winter. On January 24 seeds of each kind were placed in aquaria and kept at ordinary room temperatures. On February 14, the seeds of *pectinatus* began to germinate, but this process was very irregular, extending over a period of three or more weeks. These seedlings lacked vigor and nothing came of them. On March 15 other seeds of the same species were taken from cold storage and placed in aquaria as before. In this later planting germination was more uniform, the majority of seeds sprouting within a few days of each other. Subsequent growth was rapid and vigorous. It appears from the behavior of the seeds in the two experiments that the later planting is advantageous. Figures 46 and 47 represent seedlings of the second planting 3 and 5 days old, respectively. Figure 48 represents a seedling of the same species about 10 days old, and figure 49, one about 3 weeks old.

The seeds of *P. americanus* planted on January 24, showed no signs of life till May 5. Those of the second planting germinated between June 13 and 15. In this species also the later planting proved to be more successful. Figures 2 and 3 represent seedlings, respectively, 5 and 14 days old. When the seedlings were about 3 weeks old they were transplanted and kept in outdoor aquaria with running water till October. Figure 4 shows one of these seedlings which produced winter buds during the latter part of the growing season. These winter buds described in a preceding chapter are the vegetative

[a] In a recent publication by Esenbeck the seedlings of *P. coloratus* are described. (Esenbeck, Ernst: Beiträge zur biologie der gattungen Potamogeton und Scirpus. Flora, bd. 7, June, 1914, p. 151–212, fig. 59.)

propagative structures characteristic of the species. All of the seedlings produced them. Figure 5 represents the first shoot in a germinating winter bud. It may be assumed from the general behavior of the seedlings and the growth from the hibernacula that in this species vegetative structures only are matured the first year, and that seed formation is deferred at least until the second year.

At present definite knowledge regarding the young stages of Potamogeton, in general, is very meager and this is doubtless attributable to the fact that the plants are small and inconspicuous the first year and fail to develop fruit until one or more vegetative reproductions of the plant have taken place.

PRODUCTION OF SEEDS AND VEGETATIVE PROPAGATIVE STRUCTURES.

The abundance of *P. crispus* and *P. pectinatus* in the local flora have made it possible to observe the relative production of seeds and vegetative structures in a considerable number of these plants. Besides, the formation of the conspicuous vegetative structure in both species is practically synchronous with seed formation. The observations on mature plants selected at random form the basis of the following tables:

TABULATIONS OF PROPAGATIVE STRUCTURES IN POTAMOGETON CRISPUS, JUNE 16, 1913.

(A) BUR FORMATION.

Number of plants.	Denticulate burs.			Spicular burs, number.
	With burs only, number.	Plants with both burs and floral spikes.		
		Burs.	Floral spikes.	
4	1	..	..	..
1	1	..	..	1
2	..	..	1	..
4	2	..	..	..
1	..	2	2	..
9	4	..	..	..
2	..	4	..	1
3	..	4	3	..
1	..	..	2	..
1	5	..	..	2
1	..	5	2	1
11	6	..	..	..
6	..	6	1	..
6	7	..	..	1
3	..	8	1	..
2	..	8	2	..
1	..	8	3	..
9	8	..	..	..
2	9	..	..	1
1	9	..	..	4
2	..	9	2	1
12	..	10	..	..
1	..	10	1	..
1	..	10	2	..
5	11	..	..	..
1	..	11	2	2
1	..	12	1	1
3	12	..	..	2
1	..	12	3	..
1	15	..	..	..
1	..	15	2	1
1	21	..	2	2
100	111	135	32	20

TABULATIONS OF PROPAGATIVE STRUCTURES IN POTAMOGETON CRISPUS, JUNE 16, 1913—Continued.

(B) SEED FORMATION.

Plants bearing sterile spikes.			Plants bearing fertile spikes.			
Number of plants.	Number of spikes per plant.	Number of flowers on spike.	Number of plants.	Number of spikes per plant.	Number of fertile spikes per plant.	Number of seeds set.
12	1	6–7	4	1	1	2–4
10	2	4–7	3	2	1	1–2
16	3	5–8	6	3	1	1–5
6	4	5–9	2	4	1	2–8
2	5	5–8	1	6	1	2
3	6	5–7	1	2	2	6
1	7	5–7	1	9	1	1
50	139	...	18	53	19	...

Table A shows a preponderance of burs over floral spikes; table B, a preponderance of sterile spikes over fertile ones. A comparison of the tables A and B shows that bur formation exceeds seed production; that is, the important mode of increase is by vegetative means. It should be remembered in this connection, however, that the bur which is the most conspicuous is but one of several vegetative structures contributing to the rapid extension of this species, and that seed production is, therefore, even less important relatively than the tables suggest it to be.

TABULATION OF PROPAGATIVE STRUCTURES IN POTAMOGETON PECTINATUS, SEPTEMBER 30, 1913.

Number of plants.	Number of fruiting spikes and tubers on same plant.		Number of fruiting spikes only.	Number of tubers only.	Number of immature stolons.	Number of tubers on subterranean stems.
	Fertile spikes.	Tubers.				
1	..	..	4	..	2	(a)
1	..	..	..	10	1	1
1	2	17	..	..	3	(a)
1	..	..	..	18	1	(a)
1	..	..	..	25	2	(a)
1	..	..	..	5	..	(a)
1	1	6	..	..	..	(a)
2	2	7	..	..	4	(a)
b 1	..	12	..	..	..	(a)
1	5	..	..	..	..	1
2	..	..	..	2	..	2
2	..	..	..	..	..	1
3	1	3	..	..	2	(a)
1	3	11	..	..	1	(a)
1	6	2	..	..	1	1
1	..	..	..	..	..	6
7	..	..	..	..	..	2
1	..	..	..	..	3	(a)
1	..	..	..	..	..	4
1	..	..	..	15	..	(a)
1	..	..	..	8	..	(a)
2	..	..	..	..	..	3
1	..	..	..	..	..	5
35	24	71	..	86	31	43

a Imperfect record. b This plant bore one sterile spike.

The fertile spikes of *P. pectinatus* produce, in general, from 10 to 15 seeds. The tubers occur singly, in pairs, and in threes. Bearing these possibilities in mind, the tabulation of *P. pectinatus* indicates a close approximation to equality in the production of seeds and tubers. The small number of plants from which the data were collected is an objection which could be justly put forward, yet the results conform in general with field observations in restricted areas where the common form of *pectinatus*

predominates. Propagation by tubers is, as we have seen, the more rapid method and the one which produces a luxuriant foliage early in the growing season.

In view of the observations and experiments, it is clear that in any project in which the propagation of Potamogetons is an important feature, success will be measured by adherence to the general principle that vegetative reproduction is the dominant mode of increase in the genus Potamogeton.

ECONOMIC ASPECTS OF POTAMOGETONS.

In the study of the various phenomena attending the propagation of Potamogetons opportunity was afforded to observe, more or less closely, various aquatic animals which abounded on these plants. Their presence in such great numbers suggested the possibility that the Potamogetons might play an important rôle in the economy of life beyond that of mere shelter and support, or other mechanical and indirect relations which have been ascribed to the larger aquatic plants for many years.

It has been stated by Pond (1905) that—

> The larger aquatic plants, as such, are, while living, little used as food by aquatic animals, yet they greatly increase the surface available for the attachment of microscopic plant forms, which are eaten by smaller animals, and the latter in turn by the fishes.

In the very recent publication by Shelford (1913), bearing on the life relations of aquatic animals, but little importance is attached to the larger aquatic plants beyond the various mechanical and indirect relations that have so long been attributed to them. He says:

> The smaller aquatic animals are commonly either alga-eaters or predatory. The larger aquatic animals are commonly predatory or scavengers. The rooted vegetation is eaten only to a small extent. Small floating or swimming plants and animals are the basis of the food supply of larger animals. We could probably remove all the larger rooted plants and substitute something else of the same form and texture without greatly affecting the conditions of life in the water; that is, so far as the life habits of the animals are concerned. * * * Plants in water are of particular use to animals as clinging and nesting places.

Recent research bids fair to modify these generalizations by Shelford. Such a relation as Pond describes has frequently been observed in *P. pectinatus* in the autumn when myriads of midge (chironomid) cases have been found applied to the leaves (fig. 56). The leaves are not eaten but they are thickly covered with diatoms and other small algæ which, doubtless, afford foraging materials for the larvæ. A small caddis fly (hydroptilid) larva, with characteristic elliptical case, has also been observed in considerable numbers in the same relation with *pectinatus*, the larvæ apparently feeding on the epiphytic algal growth. The larvæ of both of these insects, after wintering on the algal-covered leaves, have emerged as adults in the spring. Other midges and caddis flies, flies (aquatic Diptera), moths (aquatic Lepidoptera), and beetles (Coleoptera) have been found in great numbers on the various species of Potamogeton. The other smaller invertebrate animals most frequently seen on these plants are Crustacea, snails, and worms.

Another interesting relation existing between the Potamogetons and aquatic insect forms is seen in the striking resemblance between the cases of a caddis fly (Leptoceridæ) and the stipules of the leaf of *P. americanus* (fig. 75). The cases in which both larvæ and pupæ dwell are attached along the stems and leaves in so characteristic a manner as to become almost, if not quite, indistinguishable from the plant parts.

Reighard (1894) has expressed in a table "a part of the imperfectly known relationships existing between the various groups of plants and the invertebrate animals on the one hand and the fishes on the other." One of the great gaps in the chain of relations therein expressed is a lack of definite knowledge concerning the rôle of the higher plants.

Some definite research in this direction has been begun. Recent investigations on the food habits of aquatic insects have shown that the larger aquatic plants do serve as forage materials. According to Hart (1895), the larvæ of *Nymphula* sp. (*Paraponyx*), an aquatic lepidopterous insect, feed voraciously on *Potamogeton natans*. Needham (1907) mentions the presence of *Nymphea advena* in the diet of *Chironomus albistria*, and Morgan (1912) found that the higher plant tissues formed an important part of the stomach content of May-fly larvæ. In view of these investigations the leaves and other edible parts of Potamogeton were closely scrutinized for evidences of their use as food. In my own investigations the first indication that the living tissues of Potamogeton was being eaten was seen in the young growing tips of *P. crispus*, which had been transferred from a pond to an aquarium in the laboratory. The leaves of several plants were mined by a small larval form which proved to be a chironomid (midge). The characteristic leaf mine is shown in figure 72. Miss Tilbury (1913), who was working in the Cornell laboratory on the feeding habits of the midge, taking advantage of this observation, reared her species, *Chironomus cayugæ* Johannsen, mainly on *P. crispus* and entirely on Potamogeton.

On examining the leaves of other Potamogetons it was found that practically all species were foraged upon to a greater or less extent. Larval depredations were most common on *P. Robbinsii*. In this plant the aquatic lepidopterous larva *Nymphula* sp. (*Paraponyx*) is the chief herbivore, and so voracious is its appetite that a large proportion of the growing tips are constantly being defoliated in the manner shown by figure 68. Portions of the leaf are cut out also by the larva, applied together by means of silk, and used as a protective case or retreat during the larval and pupal stages. *Nymphula* sp. is by far the most conspicuous larva feeding upon *P. Robbinsii*, yet other important smaller forms are numerous. The limy incrustation that accumulates very freely on *P. Robbinsii* offers apparently especial inducements to certain case-making insects, as midges and caddis flies. Such larvæ are exceedingly numerous on this species of plant, and the limy incrustation is the chief material used in the construction of the cases.

A few of the chironomid larvæ that were common on *P. Robbinsii* collected at North Fairhaven in October were segregated and fed exclusively on this Potamogeton. They passed successfully through the pupal and adult stages and proved to be the midge, *Chironomous aberrans*. The larval and pupal stages have been hitherto unrecognized in the life history of this species.[a]

The leaves of *P. amplifolius* were conspicuously mined by the dipterous larva *Hydrellia* sp. (Ephydridæ). The pupæ were collected on the leaves August 6. Several flies and their parasites were reared from them, emergence occurring between August 16 and 20. The larva makes a wide, irregular mine through the leaf, and in each case under observation pupates at the end of the mine toward the base of the leaf blade where the edges naturally roll together and form a protecting furrow (fig. 73). *Nymphula* sp. (*Paraponyx*) is also common on this Potamogeton and many of the young leaves are eaten by them. Oftentimes the larva cuts out a portion of the leaf for its case with the neat-

[a] Determinations of dipterous larvæ have been made by Prof. O. A. Johannsen; of caddis-fly larvæ, by Mr. J. T. Lloyd.

ness and precision of a leaf cutter bee (fig. 74), though usually there is less regularity of outline.

On the floating leaves of *P. americanus* collected early in August were found eggs of Paraponyx and of chironomid.[a] Those of Paraponyx covered broad areas of the under surfaces of the leaves and presented the appearance of minute six-sided cells of honeycomb, yellowish in color. In a few days the larvæ hatched and began at once to feed and to cut portions from the leaves for larval cases. Fryer (1888), in connection with his studies on *P. fluitans*, mentions that the larvæ of Nymphula (*Hydrocampa potamogata*) entirely destroy the floating leaves of this species, and thus indirectly induce the development of fascicles of leaves, structures which are analogous to the winter buds of *P. obtusifolius*. The eggs of the chironomid, which were found on the leaves of *P. americanus*, were inclosed in small elongate cases blackish in color, suspended from the edges and from the underside of the leaf, and from the petiole. These eggs hatched within a few days, but their entire life history was not observed.

The leaves of *P. obtusifolius* harbor a large number of chironomids, and apparently offer a valuable supply of food to many of them. A few of the larvæ were segregated in small dishes and supplied with fresh leaves of this Potamogeton. An undescribed species of *Chironomus* was reared. *Cricotopus trifasciatus* and *Tanypus flavellus* were the most abundant species on the leaves.

Other plant parts besides leaves were eaten. The tubers of *P. pectinatus* and the burs of *P. crispus* were devoured by the larvæ of Paraponyx and by the larvæ of the Chironomidæ.

The underground stems of *P. pectinatus*[b] are provided with large and numerous air spaces (fig. 66), and these were found to be an important air-supplying source for the Donacia larvæ. The larvæ attached to the subterranean stems of this Potamogeton were collected from the muddy substratum at North Fairhaven August 14, 1913. Stems on which the larvæ were not attached showed, quite generally, the characteristic punctures, or double scars, made by the caudal spines in tapping the air supply.

Jepson (1905) called attention to the value of the tubers of *P. pectinatus* in the diet of our wild game birds. He says, "The diving ducks, such as the canvasback and broadbill, eagerly seek these tubers, devoting most of their time to this pursuit until the duck-shooting season opens." McAtee (1911) and Thompson (1913) in their researches on the diet of wild game birds have shown that a large percentage of the food taken is Potamogeton.

The stomach content of 5 canvasbacks has come under my observation recently. One duck shot in October had been feeding in rich aquatic meadows where Potamogetons flourished with Myriophyllum, Elodea, etc. Its stomach was filled exclusively with tubers of *P. pectinatus*. Four ducks shot at the close of the season in January had apparently exercised a choice in the matter of food. Feeding in an abundant mixed vegetation, they had selected only Potamogeton—*P. Fresii* and *P. pusillus*. The parts of the plants available were the winter buds which at this season have settled in the mud at the bottom along with the hibernacula of Myriophyllum, Elodea, and other aquatic plants.

[a] During subsequent observations in June, 1914, masses of eggs almost infinite in variety and number have been found attached to the stems and leaves of the various Potamogetons. It would seem that these plants, diverse as they are in habit and form, offer especially suitable conditions for the attachment of the eggs of aquatic animals. The eggs of the water mite (Hydracarina) are exceedingly abundant. The eggs of insects that have been recognized are as follows: Stratiomyiidæ, Coriscidæ, Gyrinidæ, Donaciinæ, Hydrophylidæ, Pyralidæ, Cordulinæ (Tetragoneuria), Hydrobatidæ and Tricoptera.

[b] Since this observation was recorded Donacia larvæ have been found on the underground stems of *P. americanus*.

SPECIES OF POTAMOGETON AND THE ANIMALS FORAGING UPON THEM.

To facilitate reference, a list is given of the species of Potamogeton, together with the smaller animals which have appeared to be intimately associated with them. Other forms of animal life were often found upon these plants, but none seemed to be so characteristically on their own ground, so to speak, as the forms listed below. Those animals are starred (*) which have been observed feeding on the living plant tissue.

LIST OF POTAMOGETONS AND SMALL ANIMAL FORMS ASSOCIATED WITH THEM.[a]

Plant.	Animal.
P. americanus..... Insecta..	Diptera: Chironomidæ (undetermined). Lepidoptera: Pyralidæ— * Nymphula sp. (Paraponyx). Tricoptera: Leptoceridæ— Two species.
Mollusca..	Ancylus.
P. amplifolius..... Insecta..	Diptera: Ephydridæ— * Hydrellia sp. Chironomidæ— * Chironomus sp. * Tanytarsus sp. Tricoptera: Undetrmined. Lepidoptera: Pyralidæ— * Nymphula sp. (Paraponyx).
Mollusca..	Ancylus.
P. perfoliatus..... Insecta..	Diptera: Chironomidæ— * Tanytarsus sp.
P. crispus..... Insecta..	Diptera: * Chironomidæ (undetermined). Lepidoptera: Pyralidæ— * Nymphula sp. (Hydrocampa).
P. zosterifolius..... Insecta..	Diptera: Chironomidæ— * Tanytarsus sp. Lepidoptera: Pyralidæ— * Nymphula sp. (Paraponyx). Tricoptera: Undetermined.
P. obtusifolius..... Insecta..	Diptera: Chironomidæ— * Chironomous sp. Cricotopus trifasciatus. Tanytarsus flavellus.
P. pectinatus..... Insecta..	Diptera: Chironomidæ— Tanytarsus flavellus Cricopteris sp. Tricoptera: Hydroptilidæ (in autumn). Coleoptera: Donaciine— Donacia sp.
P. pectinatus..... Insecta.. (Gigantic form)	Diptera: Chironomidæ— Chironomous sp. Tanytarsus sp.
Crustacea...	Amphipoda (very abundant). Gammarus. Hyallela. Eucrangonyx.
Vermes..	Nais (very abundant in autumn).
P. Robbinsii..... Insecta..	Diptera: Chironomidæ— * Chironomus aberrans. Tanytarsus sp. Tricoptera— Undetermined. Lepidoptera: * Nymphula sp. (Paraponyx).

[a] A Potamogeton which came under casual observation only, *P. Epihydrus*, may be mentioned as an important addition to the plants actually foraged upon by insect larvæ. Several specimens of this species of Potamogeton, collected at Spencer Lake in August, were quite thickly dotted with caddis-fly larvæ (Leptoceridæ), which were feeding upon the fresh green leaves.

The Mollusca—Planorbis, Limnea, and Physa—were common on all of the Potamogetons.

These observations on the animal life associated with the Potamogetons afford an additional contribution to the biological relations of the Chironomidæ, Pyralidæ, Leptoceridæ, Hydroptilidæ, and Ephydridæ, groups in which one or more members have been observed in their feeding operations. Of these animals it has already been recorded by Reighard (1894) that the Chironomidæ are an important fish food. Scattered reference is made by others of the value of aquatic insect larvæ in the diet of fish. The fact that these insects eat the living plant tissue of the Potamogetons adds greatly to the importance of these plants from an economic standpoint.

CONCLUSION.

In all contributions bearing on the life conditions of the Potamogetons, the prominence of these plants in the shoal waters has been recognized, and where special effort has been directed toward the study of their life relations, an economic value has been ascribed to them. The present investigation affords further evidence of the economic value of these plants, and contributes the results of observation and experiment on the cultivation of several species. These results warrant the expenditure of additional thought and effort on what purports to be one of the most important resources of our lakes, ponds, and streams.

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EXPLANATION OF PLATES.

All of figures on Plates XXXIV–XXXIX, with the exception of the photo-micrographs, are photographs of plants floating in water, in aquaria, or in specimen jars.

PLATE XXII.

FIG. 1. *Potamogeton americanus*, seed, 1½ times natural size.

FIG. 2. *Potamogeton americanus*, seedling 5 days old, 1½ times natural size.

FIG. 3. *Potamogeton americanus*, seedling 14 days old, 1½ times natural size.

FIG. 4. *Potamogeton americanus*, seedling of four months, ½ natural size; A, winter buds.

FIG. 5. *Potamogeton americanus*, germinating winter bud, natural size; the last two winter buds belated in development. Aquarium specimen, January 24, 1914.

FIG. 6. *Potamogeton americanus*, rootstock with winter bud A, natural size. September.

PLATE XXIII.

FIG. 7. *Potamogeton amplifolius*, rootstock, ½ natural size; A, A, A, young shoots which continue to grow slowly through winter. November.

FIG. 8. *Potamogeton heterophyllus*, submerged plant, ½ natural size; A, tuberous rootstock; B, B, submerged shoots; *a*, *b*, and *c*, details of leaves. May 4.

FIG. 9. *Potamogeton heterophyllus*, rootstock not tuberous, natural size. July 7.

FIG. 10. *Potamogeton heterophyllus*, tuberous rootstock, natural size. July 7.

FIG. 11. *Potamogeton heterophyllus*, tuberous rootstock, natural size; A, A, incipient shoots November 17.

PLATE XXIV.

FIG. 12. *Potamogeton heterophyllus*, typical habit of land form, 1½ times natural size. Terminal portion of rootstock tuberous. July 7.

FIG. 13. *Potamogeton heterophyllus*, terminal portion of rootstock showing tendency to become tuberous, 1½ times natural size. July 7.

FIG. 14. Same, more advanced stage, 1½ times natural size. July 7.

FIG. 15. *Potamogeton heterophyllus*, aquarium specimen, natural size; A, tuberous internode placed in aquarium in November; B, B, new shoots and rootstock. January 26.

PLATE XXV.

FIG. 16. *Potamogeton heterophyllus*, aquarium specimen, natural size; A, B, C, D, new shoot and tuberous rootstocks in various stages of growth. March 14.

FIG. 17. *Potamogeton heterophyllus*, aquarium specimen, natural size.

FIG. 18. *Potamogeton perfoliatus*, winter shoot showing elongation of internodes, natural size; A, leathery scale; *a*, detail of scale. June.

FIG. 19. Growing tips of same.

PLATE XXVI.

FIG. 20. *Potamogeton crispus*, recumbent branch, showing development of new shoots on old stem, ½ natural size. March.

FIG. 21. *Potamogeton crispus*, spicular bur, 1½ times natural size. Aquarium. July.

FIG. 22. *Potamogeton crispus*, denticulate bur, ¾ natural size; *a*, detail of leaf with denticulate base, 2 times natural size; 1, line delimiting starch storage.

FIG. 23. *Potamogeton crispus*, sprouting bur, 1½ times natural size. March.

FIG. 24. *Potamogeton crispus*, denticulate bur with sprout, natural size. Aquarium. July.

FIG. 25. *Potamogeton crispus*, spicular bur with sprout, ½ natural size. Aquarium. July.

PLATE XXVII.

FIG. 26. *Potamogeton crispus*, cutting showing bur development, ½ natural size; A, immature burs. Aquarium. March 22–April 7.

FIG. 27. *Potamogeton crispus*, sprouting bur, showing development of rootstock and erect shoots, natural size.

FIG. 28. *Potamogeton crispus*, shoot with spicular bur at base, natural size.

FIG. 29. *Potamogeton crispus*, similar structure rooting above tip of bur, natural size.

FIG. 30. *Potamogeton crispus*, bur formation at tip of branch, 1¼ times natural size. Aquarium.

PLATE XXVIII.

FIG. 31. *Potamogeton crispus*, subterranean stem, showing bur in axil of scale, natural size. June.

FIG. 32. *Potamogeton crispus*, sprouting bur, ¾ natural size. March.

FIG. 33. *Potamogeton zosterifolius*, winter bud, ¾ natural size. October.

FIG. 34. *Potamogeton zosterifolius*, long section of winter bud, 1½ times natural size.

FIG. 35. *Potamogeton zosterifolius*, winter bud sprouting, ½ natural size. April.

PLATE XXIX.

FIG. 36. *Potamogeton filiformis*, habit sketch, ½ natural size. July.

FIG. 37. *Potamogeton filiformis*, detail of tuberous rootstock, 1¼ times natural size.

FIG. 38. *Potamogeton pectinatus*, young plant developing from tuber A, ½ natural size. May.

FIG. 39. *Potamogeton pectinatus*, series of tubers, slender form of Dudley, ¾ natural size.

FIG. 40. *Potamogeton pectinatus*, tubers, showing details of early growth, 1½ times natural size.

PLATE XXX.

FIG. 41. *Potamogeton pectinatus*, tubers of two successive seasons; A, old; B, young; C, erect stem; D, subterranean stem; 1½ times natural size.

FIG. 42. *Potamogeton pectinatus*, terminal portion of subterranean stem, 1½ times natural size. A condition which may be present from June to October.

FIG. 43. *Potamogeton pectinatus*, mature portion of rootstock bearing tuberous runners; A, tuber-bearing runner; natural size. September.

FIG. 44. *Potamogeton pectinatus*, spray showing fruiting spike and tuberous runners, A, B, C, ⅔ natural size; *a*, detail of runner; A, A, young green shoots.

PLATE XXXI.

FIG. 45. *Potamogeton pectinatus*, plant developed in aquarium, suspended in water, ½ natural size; A, old tuber which produced plant; B, young tuber.

FIG. 46. *Potamogeton pectinatus*, sprouting seed, 3 times natural size.

FIG. 47. Same, later stage, 3 times natural size.

FIG. 48. *Potamogeton pectinatus*, seedling about 10 days old, outer testa of seed removed, 1¼ times natural size; *a*, inner hard testa, showing characteristic lid-like portion thrust open, 1½ times natural size; *b*, seedling with hard testa of seed removed showing foot-like expansion.

FIG. 49. *Potamogeton pectinatus*, seedling three weeks old, natural size.

PLATE XXXII.

FIG. 50. *Potamogeton pectinatus*, gigantic form of Dudley, runner B, from base of erect shoot A, ½ natural size; C, tuber; D, D, D, young green shoots; 1, 2, secondary runners. November.

FIG. 51. *Potamogeton pectinatus*, gigantic form of Dudley, tuber devoid of scales, 1½ times natural size.

FIG. 52. *Potamogeton pectinatus*, gigantic form of Dudley, portion of a tuberous runner, natural size.

FIG. 53. *Potamogeton pectinatus*, gigantic form of Dudley, growing tip of secondary runner, natural size.

PLATE XXXIII.

FIG. 54. *Potamogeton pectinatus*, gigantic form of Dudley, sprouting tuber, 1½ times natural size. Aquarium, February.

FIG. 55. *Potamogeton pectinatus*, gigantic form of Dudley, tuberous runner, natural size.

FIG. 56. *Potamogeton pectinatus*, spray, showing cases of chironomids, natural size. September–November.

FIG. 57. *Potamogeton Robbinsii*, characteristic vegetative structure, rooting at nodes, 1½ times natural size. May.

PLATE XXXIV.

FIG. 58. *Potamogeton amplifolius*, rooted tip of branch, a propagative structure. April.

FIG. 59. *Potamogeton crispus*, germinating burs. October.

FIG. 60. *Potamogeton crispus*, burs in various stages of development. June.

PLATE XXXV.

FIG. 61. *Potamogeton pectinatus*, gigantic form of Dudley, erect axis of plant, 5 feet 2 inches tall, bearing runner at base of stem. November.

FIG. 62. *Potamogeton pectinatus*, gigantic form of Dudley, portion of leafy spray showing tubers. November.

PLATE XXXVI.

FIG. 63. *Potamogeton obtusifolius*, winter buds. October.

FIG. 64. *Potamogeton obtusifolius*, erect axes bearing winter buds. October.

FIG. 65. *Potamogeton zosterifolius*, sprouting winter bud. Aquarium specimen. February.

PLATE XXXVII.

FIG. 66. *Potamogeton pectinatus*, cross section through stem, showing numerous air spaces.

FIG. 67. *Potamogeton Robbinsii*, branch, showing points where dismemberment occurs, 1, 2, 3.

FIG. 68. *Potamogeton Robbinsii*, branch defoliated by larvæ of *Nymphula* sp. (*Paraponyx*). Larval cases, 1, 2, 3.

PLATE XXXVIII.

FIG. 69. *Potamogeton Robbinsii*, photo-micrograph of section through old stem, showing arrangement of mechanical tissue.

FIG. 70. Detail of fig. 69.

FIG. 71. *Potamogeton crispus*, photo-micrograph of section through stem of starch-filled vegetative structure; *a*, cell with starch grains. November.

PLATE XXXIX.

FIG. 72. *Potamogeton crispus*, leaves, showing characteristic leaf mining of chironomids.

FIG. 73. *Potamogeton amplifolius*, leaves, showing characteristic mines of *Hydrellia* sp.; *a*, *b*, pupa cases at end of mines. August.

FIG. 74. *Potamogeton amplifolius;* *a*, *b*, leaves, showing circular pieces cut away by larva of *Nymphula* sp. (*Paraponyx*); 1, larva in case; *c*, dead leaf, showing cases of *Chironomus* sp.

FIG. 75. *Potamogeton americanus*, spray showing attachment of cases of caddis fly (fam. Leptoceridæ); *a*, case of caddis fly; *b*, stipule of leaf. June 31, 1914.

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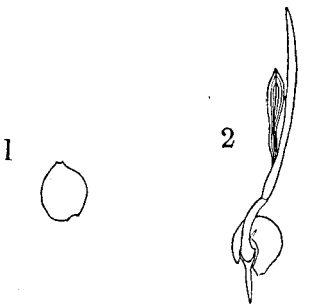
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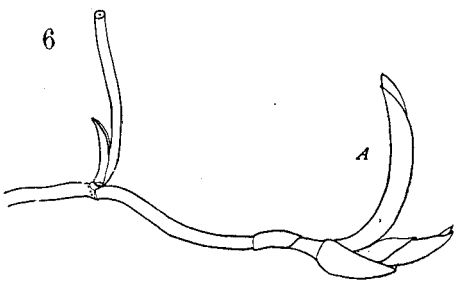
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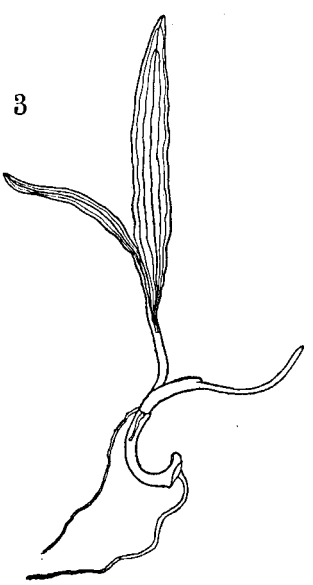
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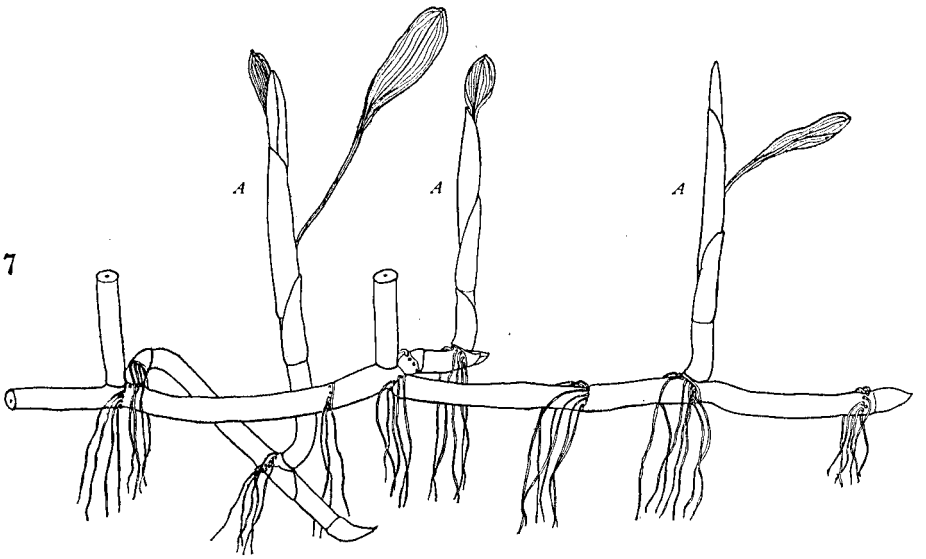

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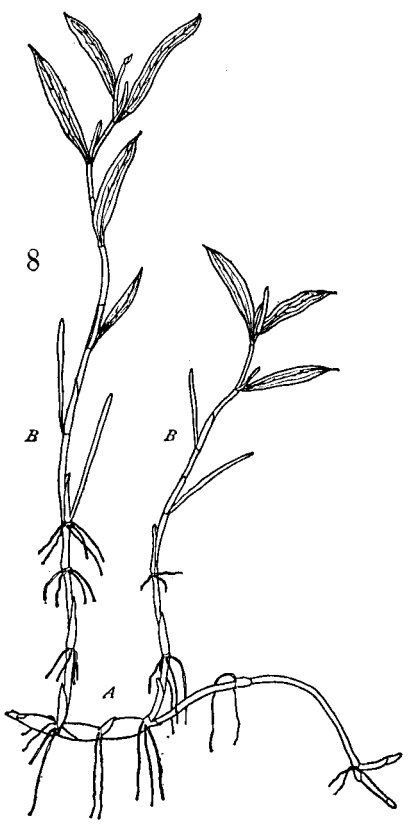

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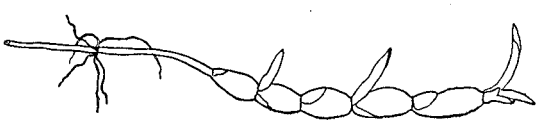

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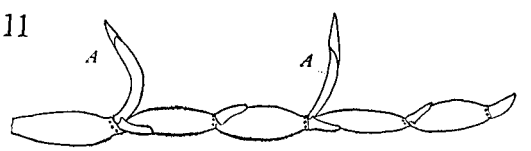

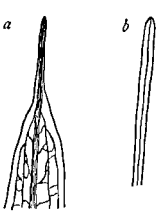

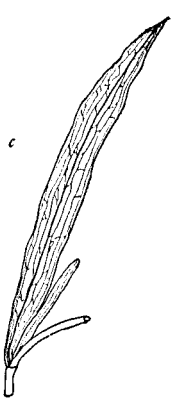

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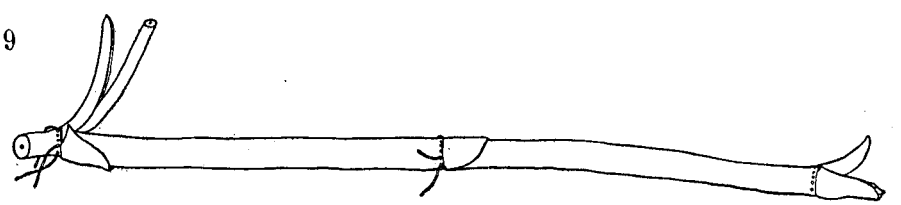

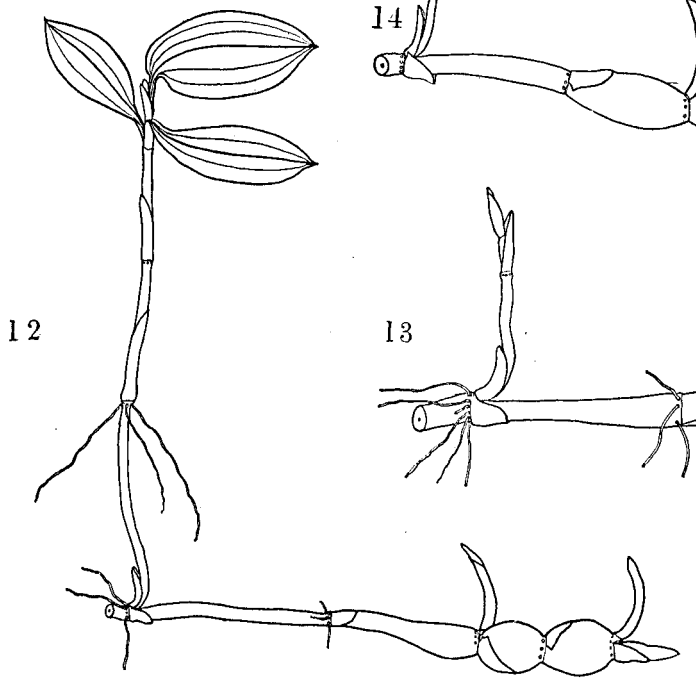
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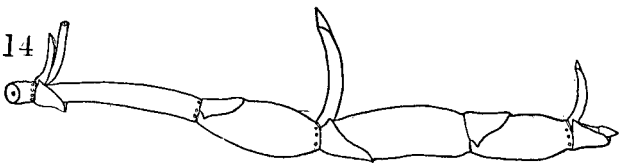
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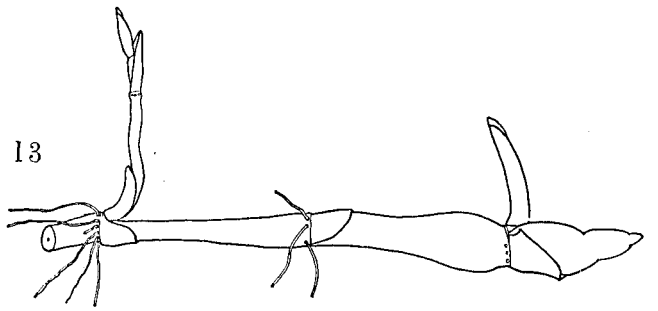
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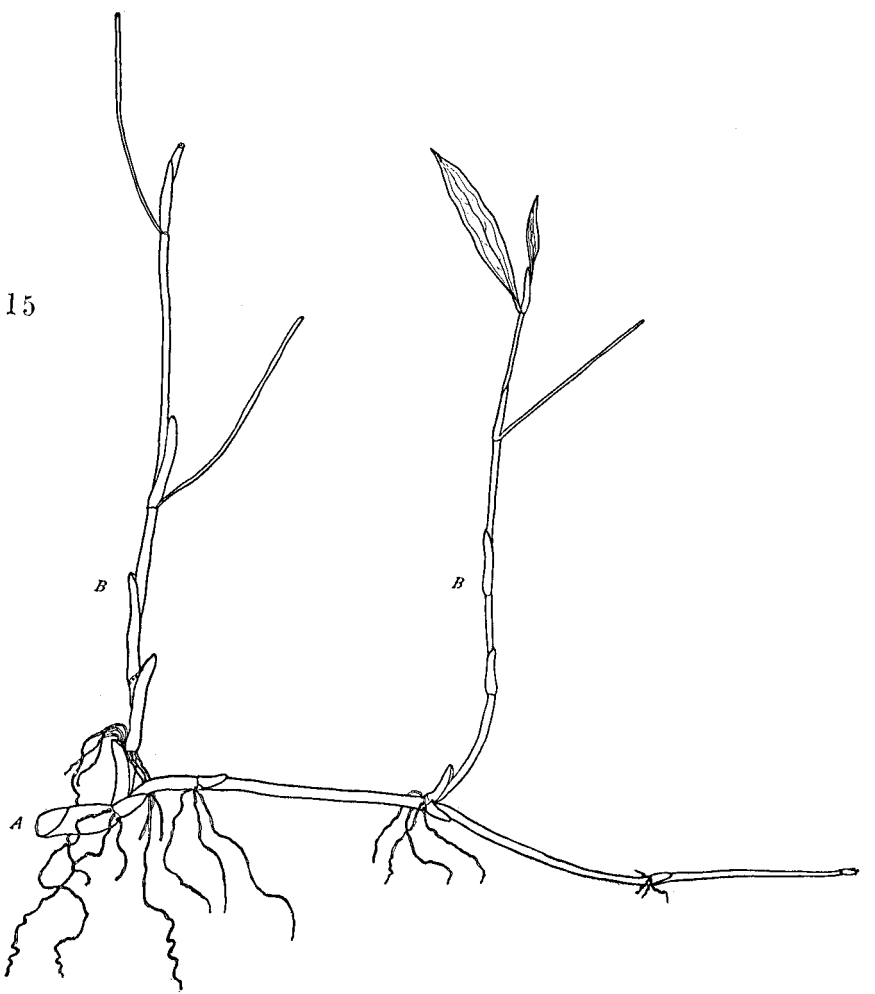
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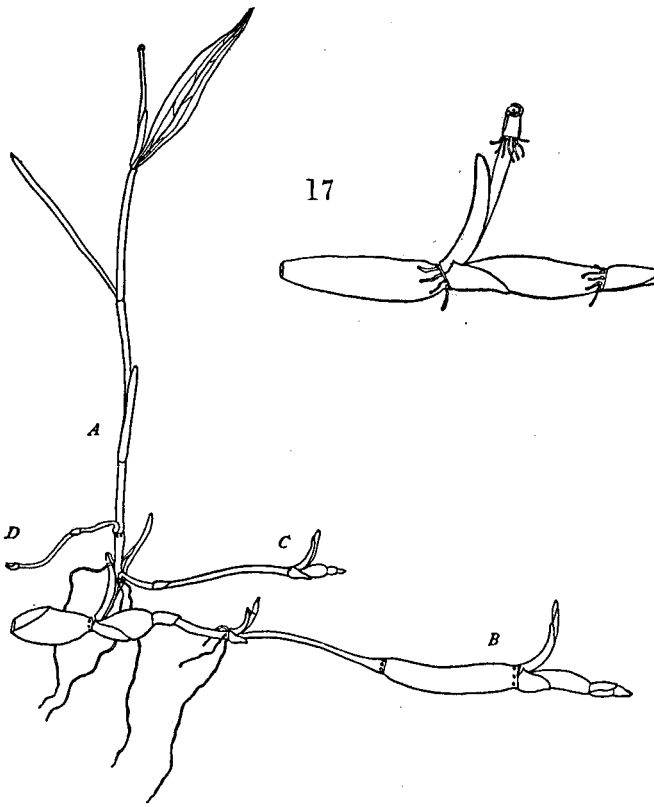

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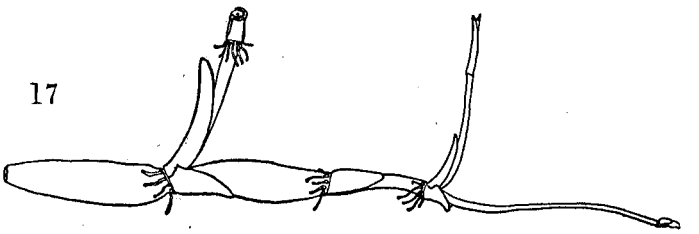

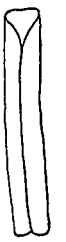

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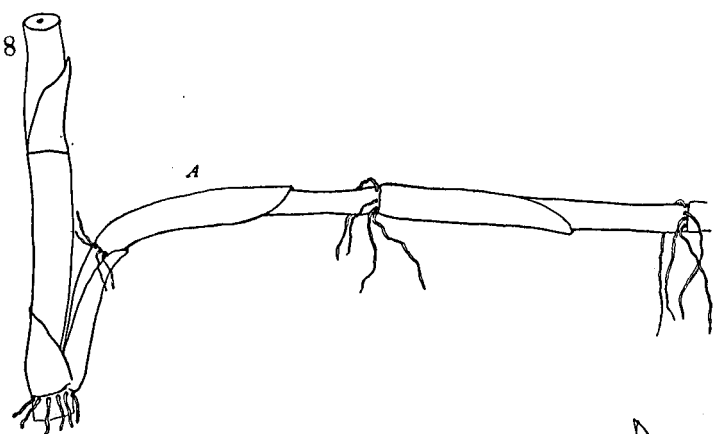

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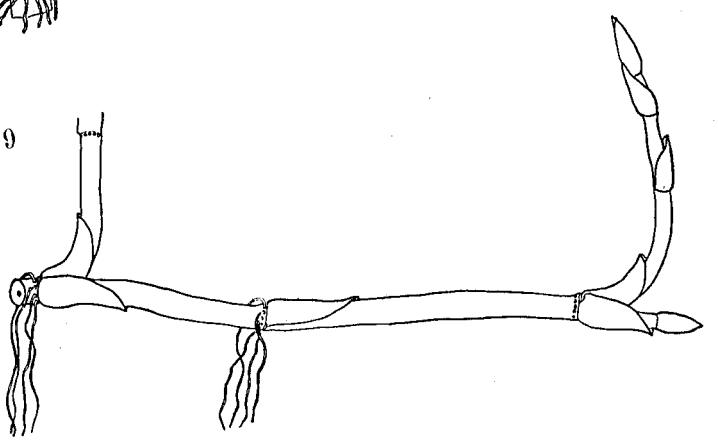

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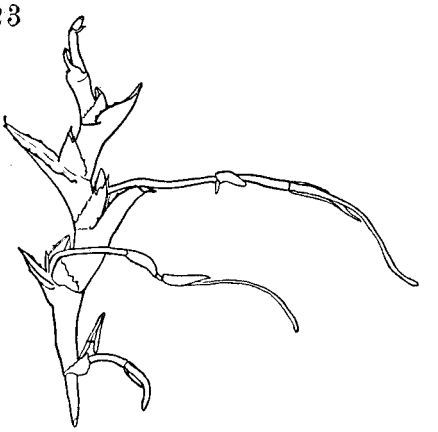
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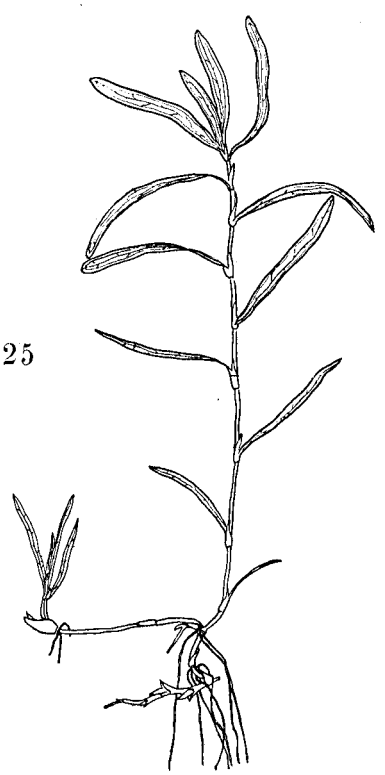
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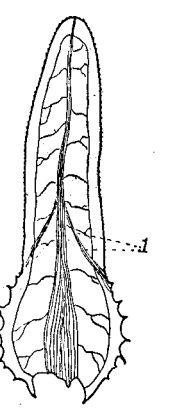
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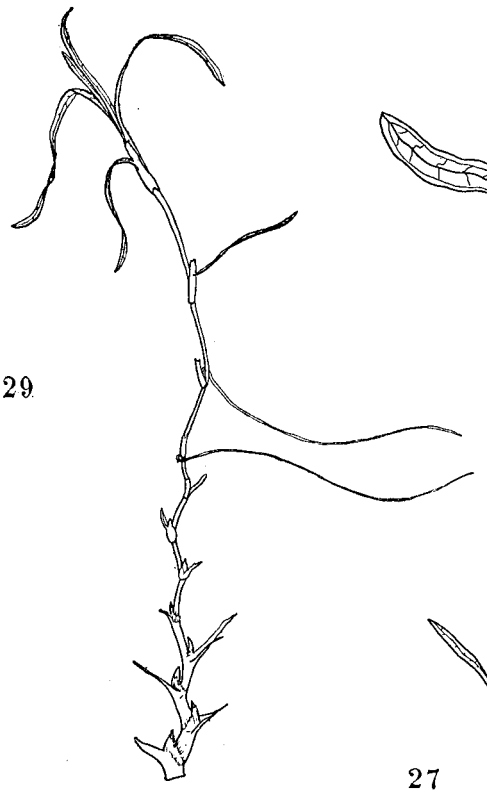

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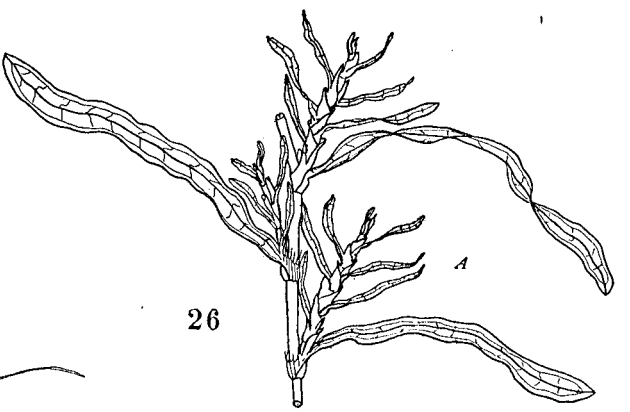

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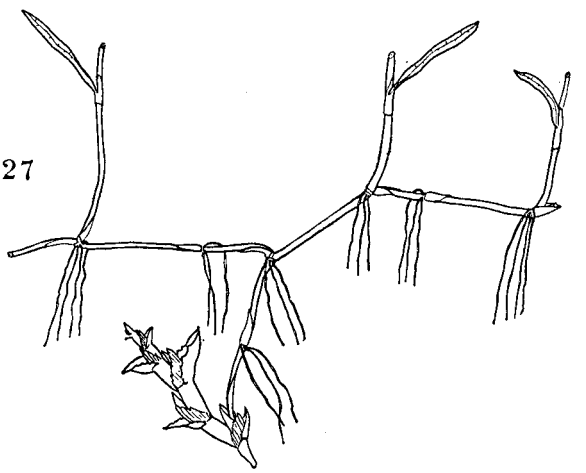

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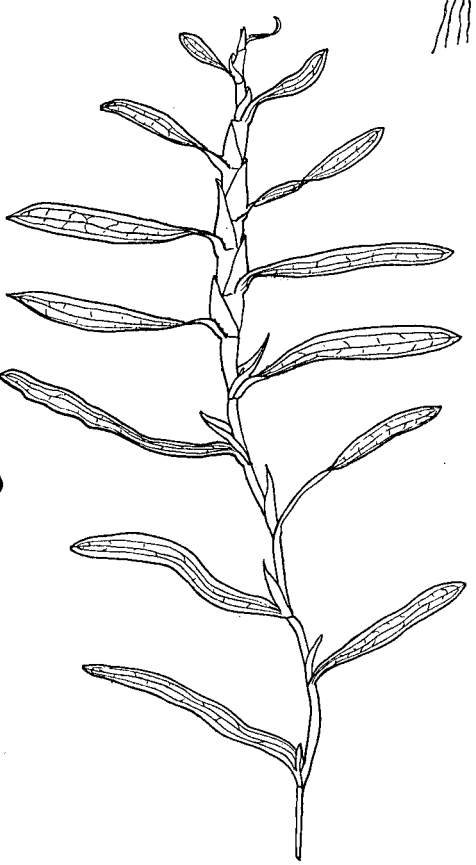

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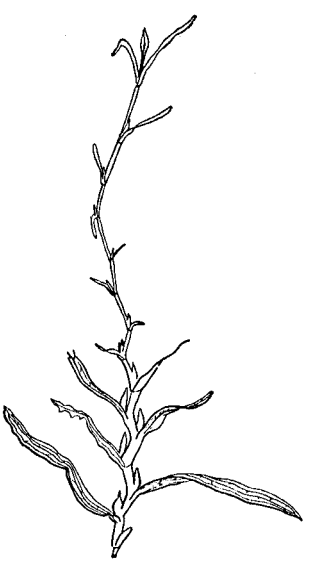

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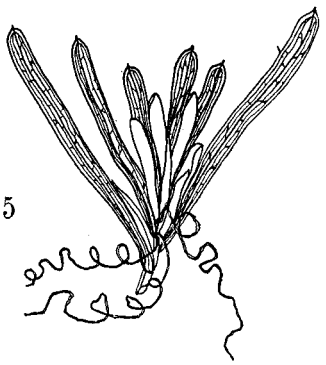

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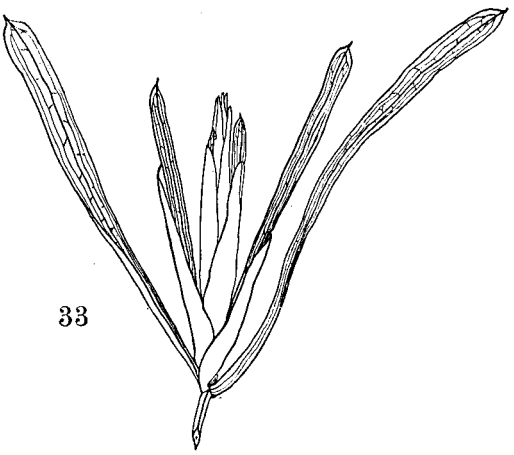

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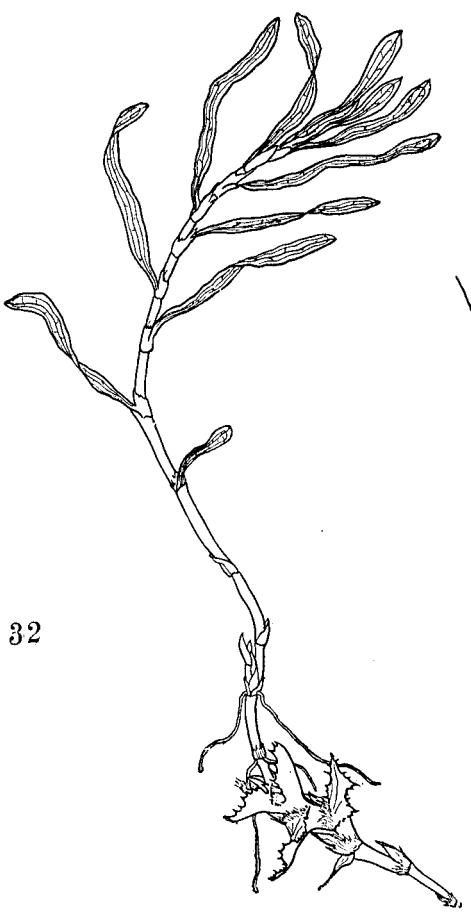

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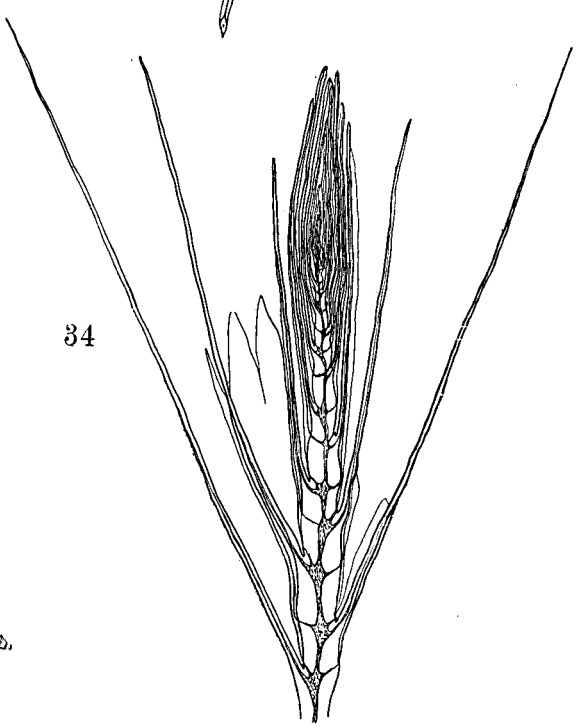

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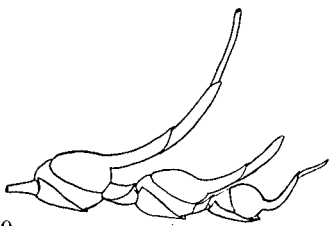

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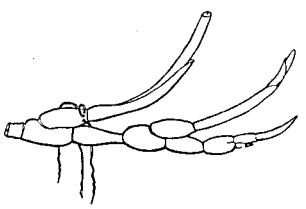

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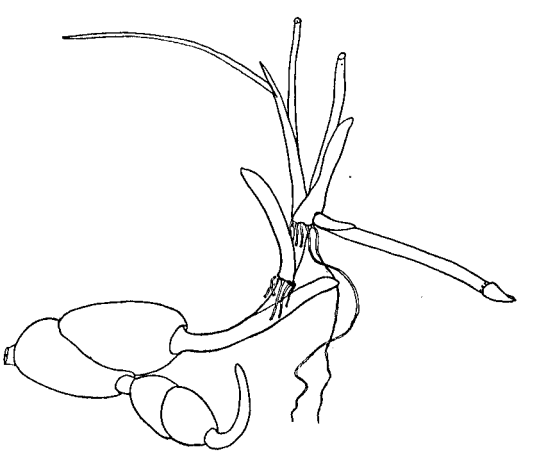

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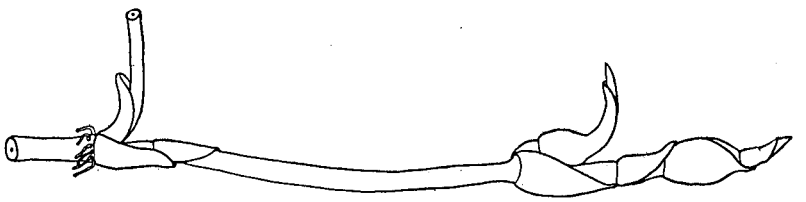

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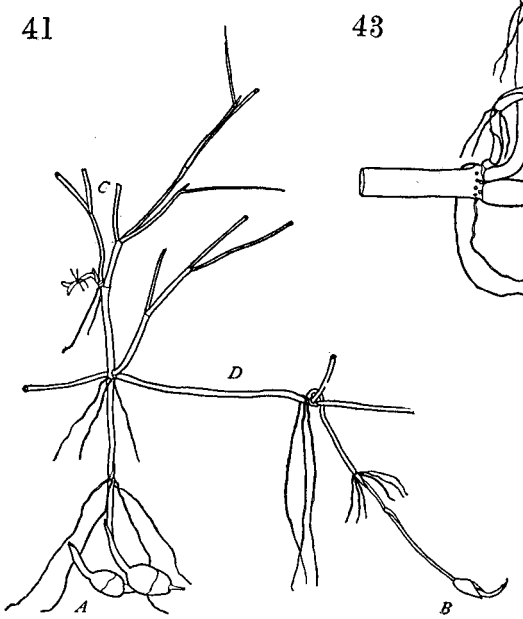

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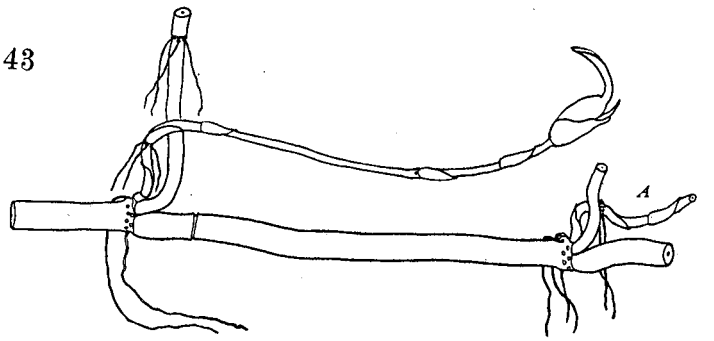

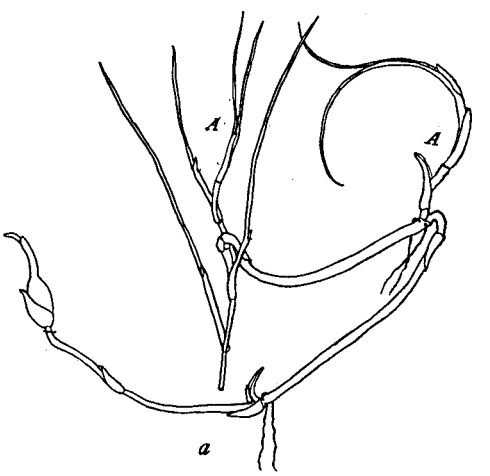

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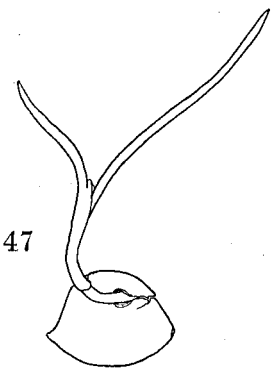

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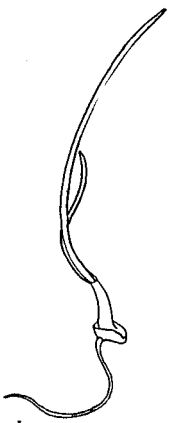

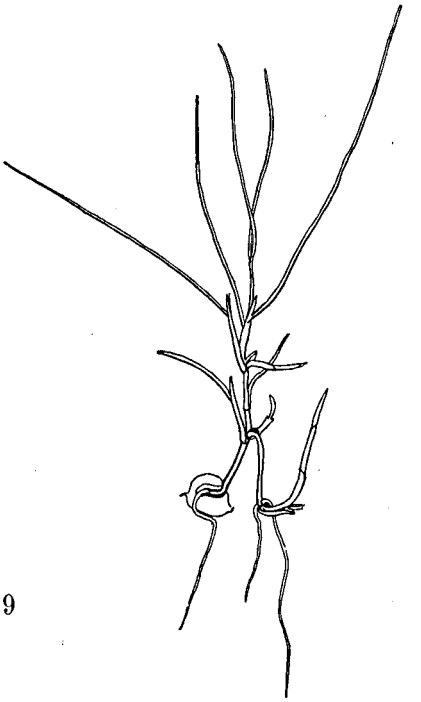

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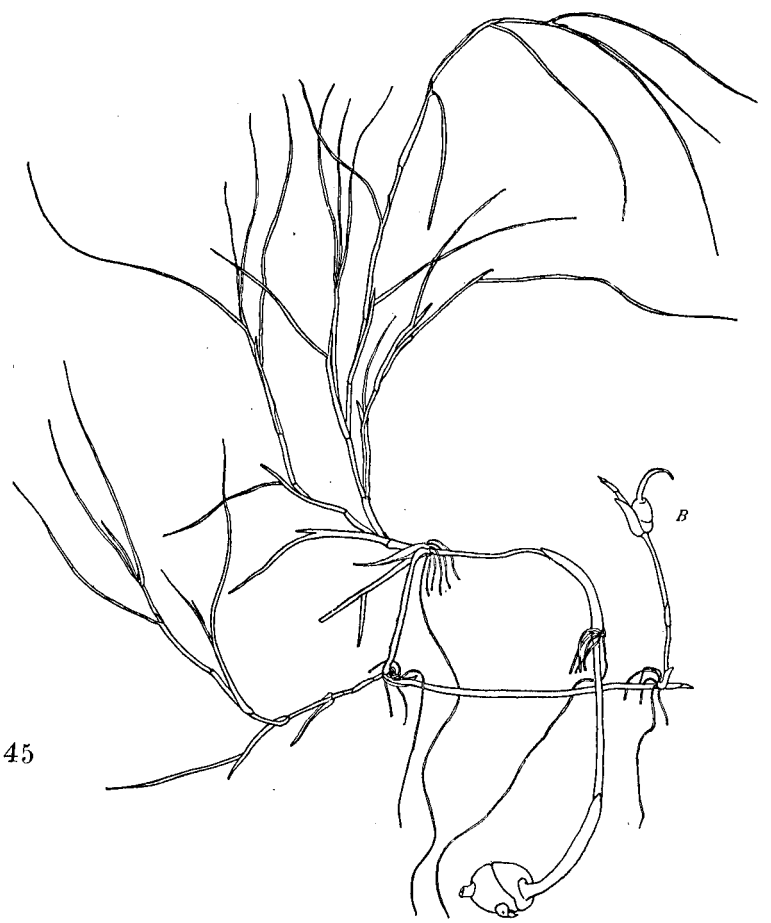

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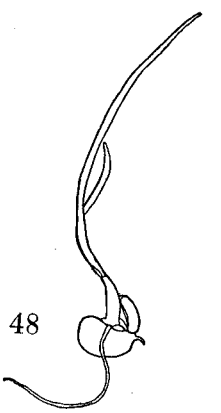

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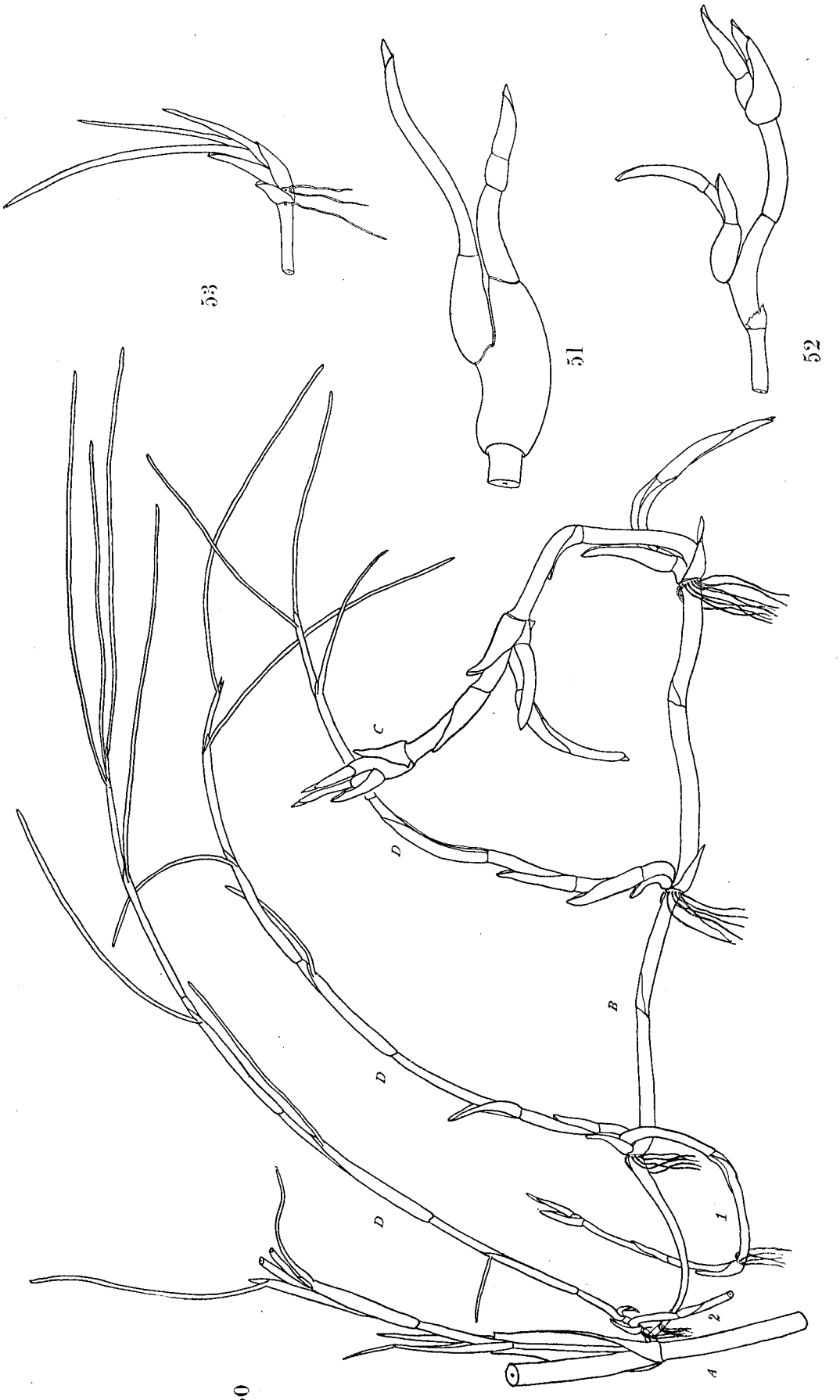
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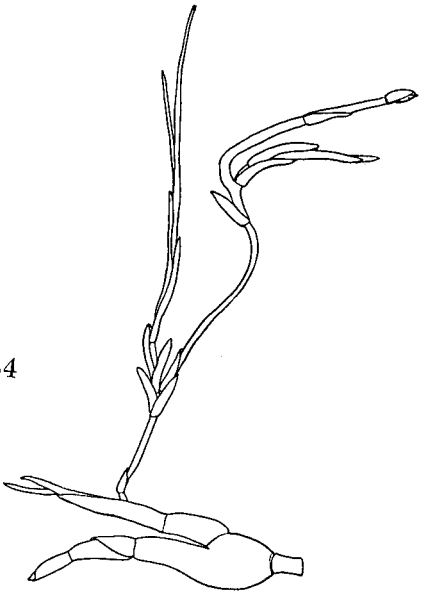

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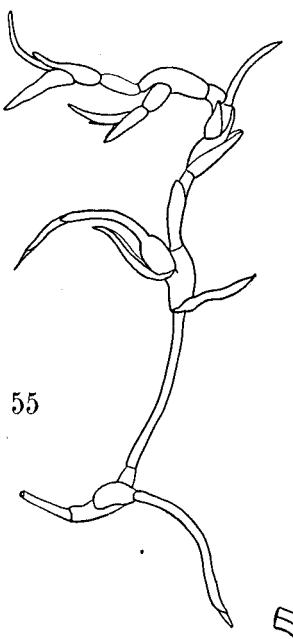

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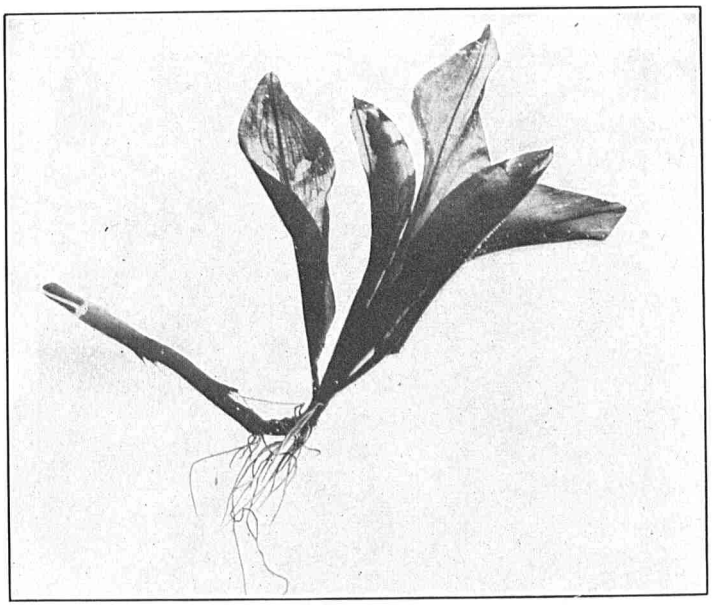

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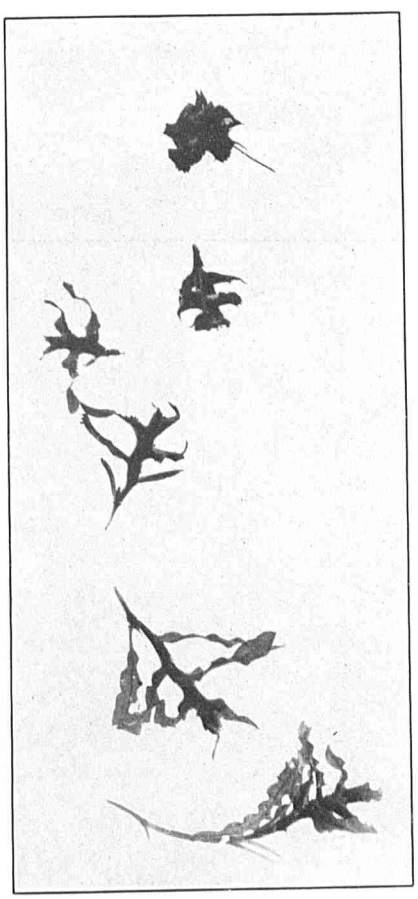

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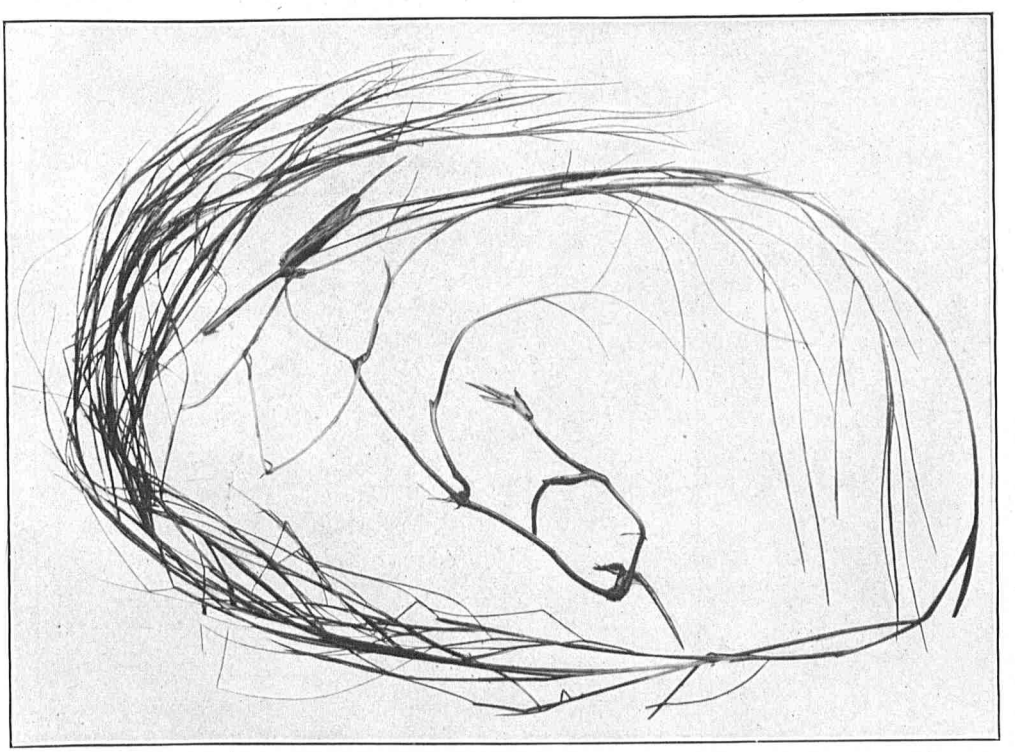

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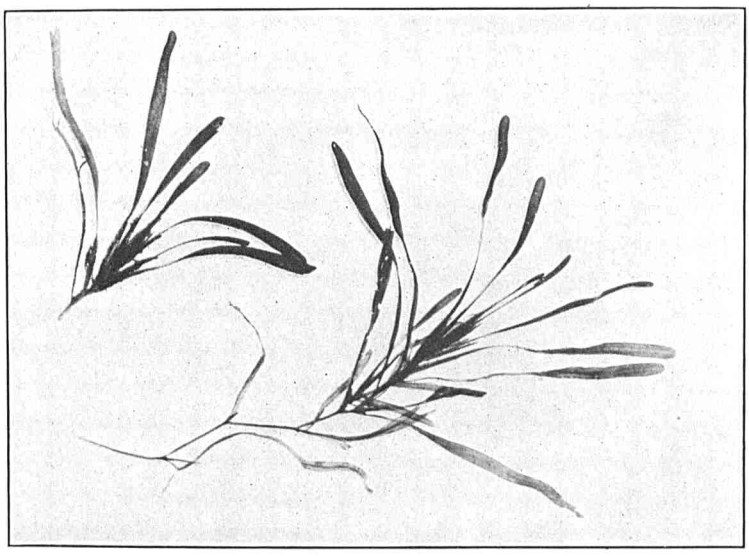

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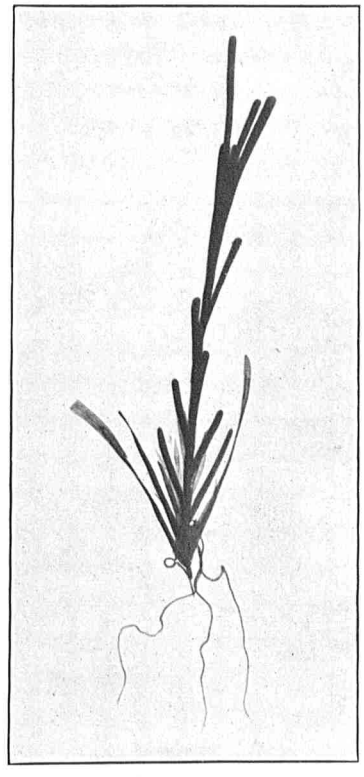

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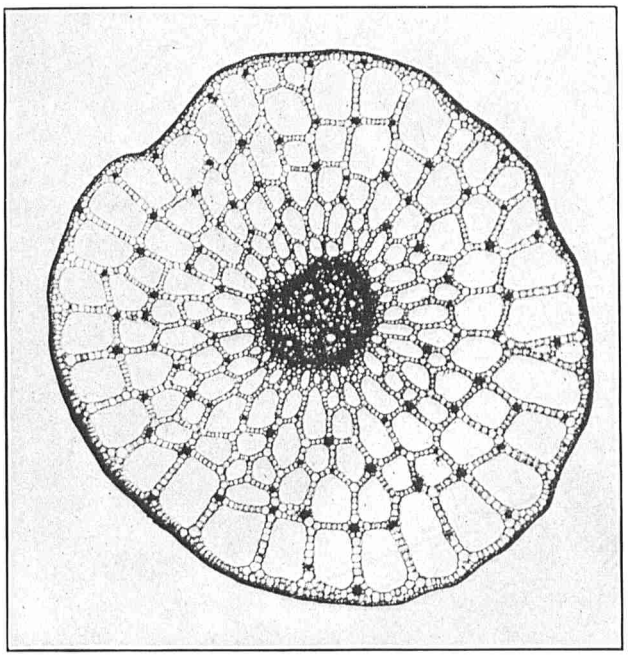

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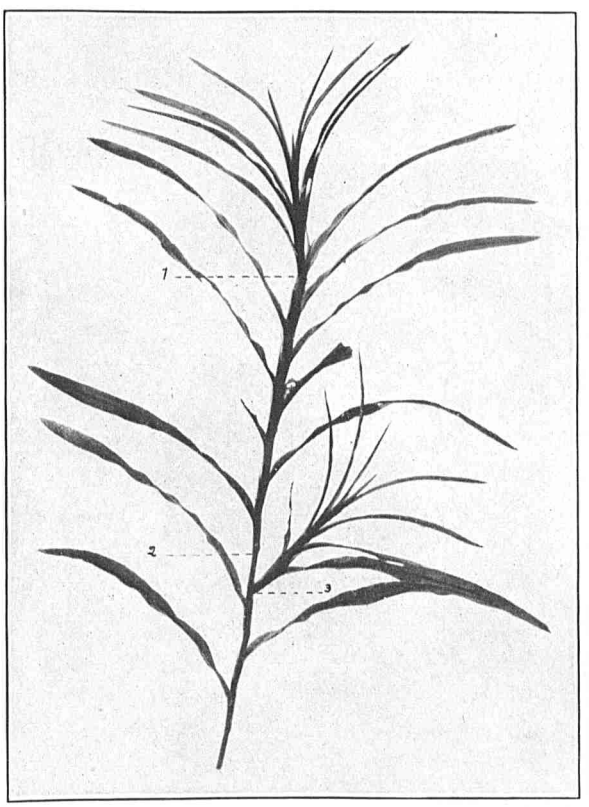

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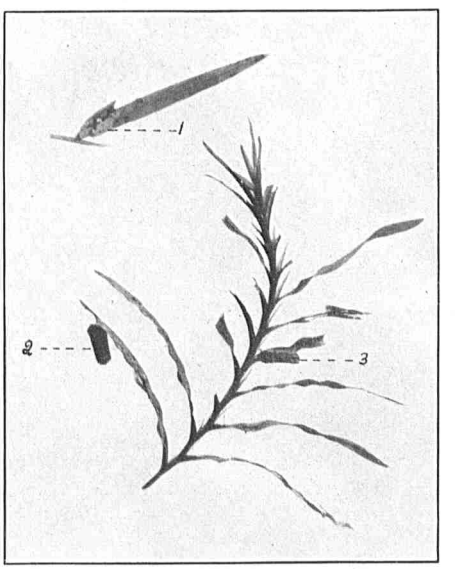

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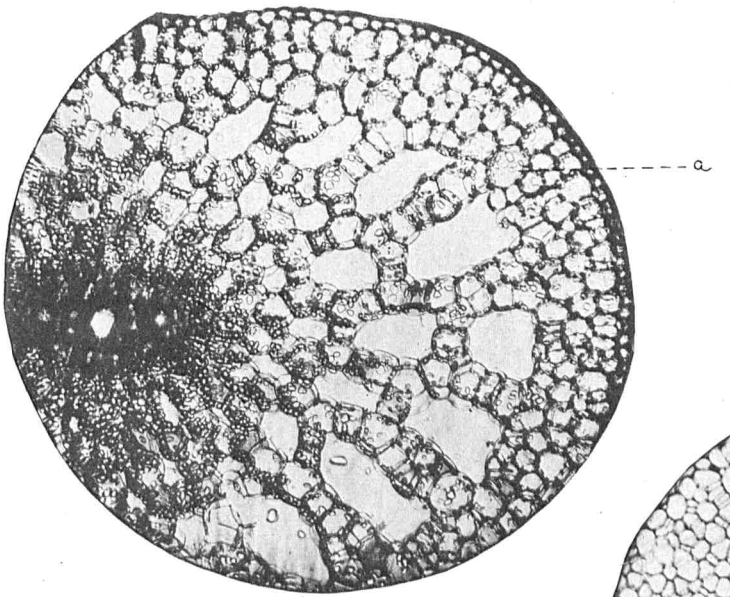

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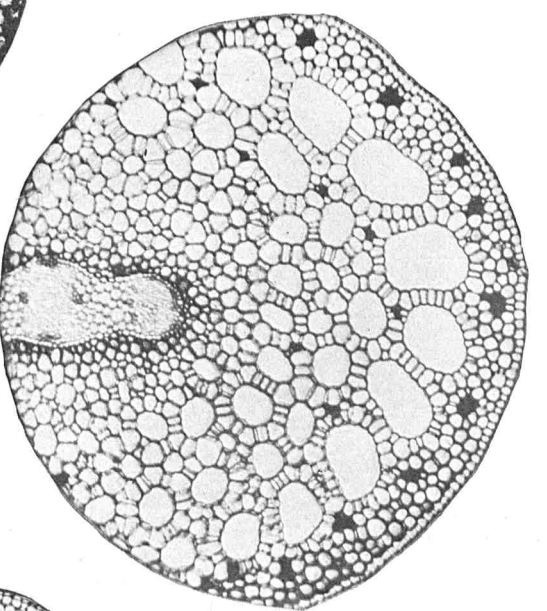

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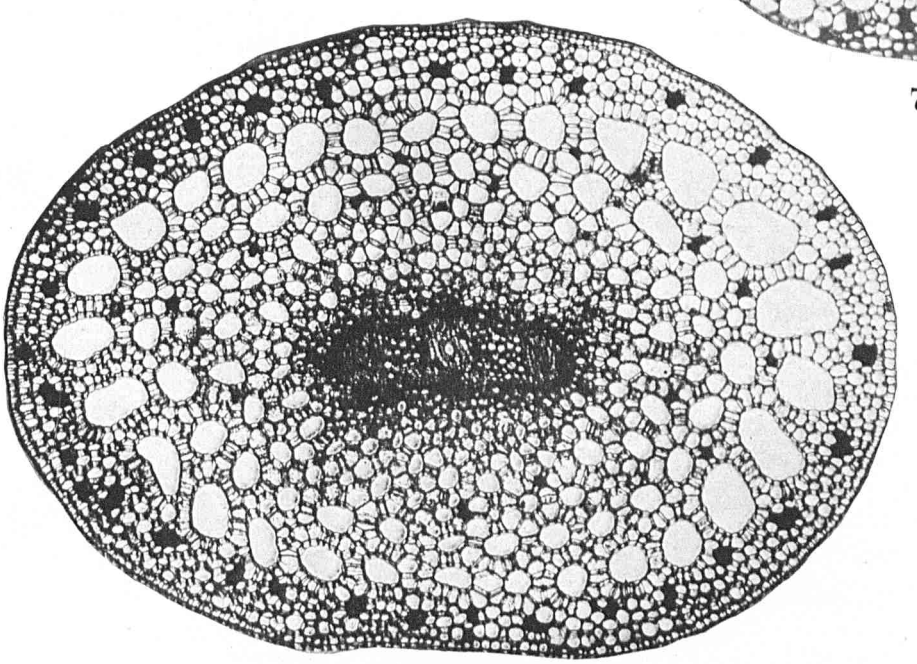

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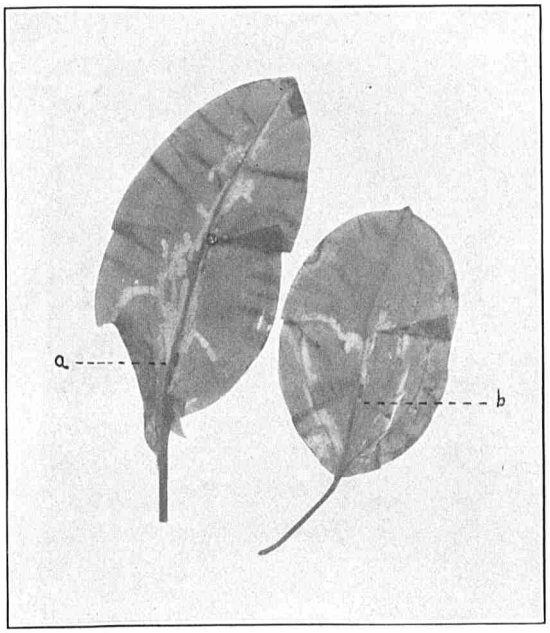

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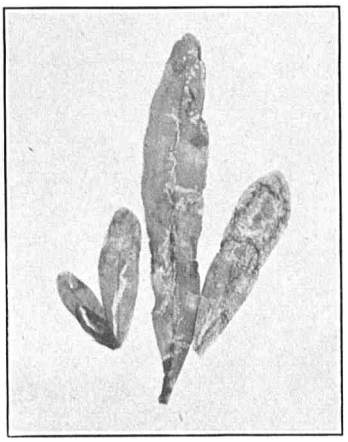

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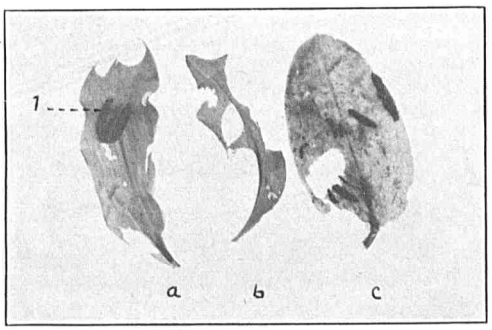

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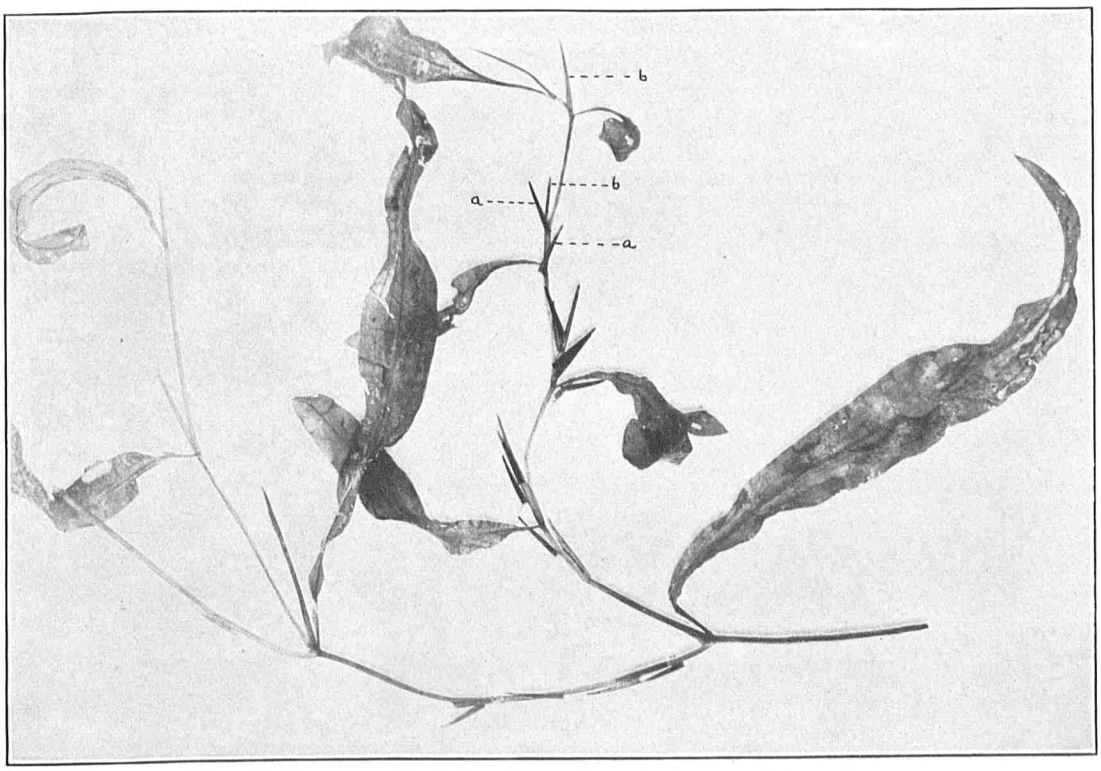

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