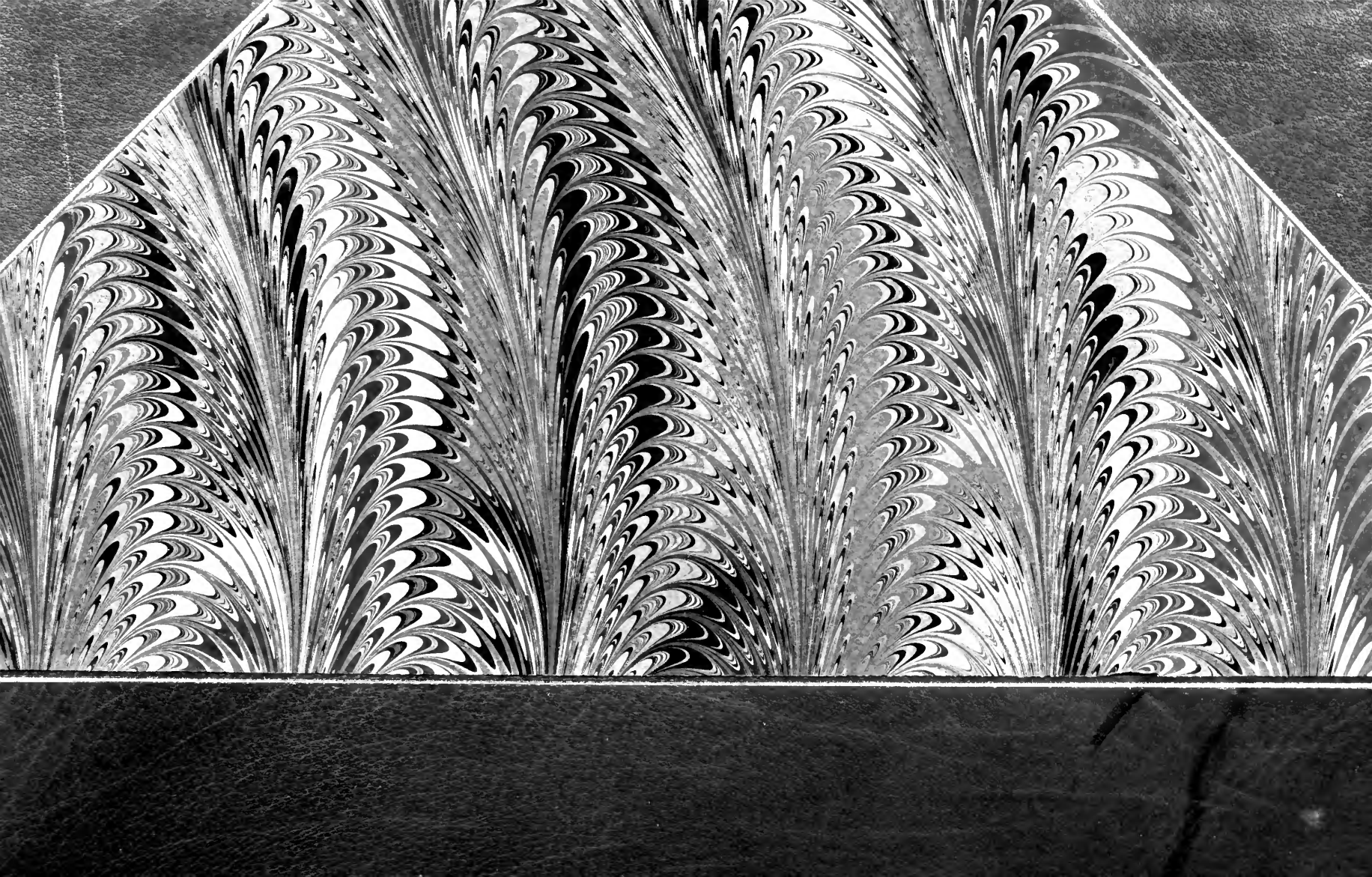


ZOÖLOGICAL BULLETIN

EDITED BY

C. O. WHITMAN AND W. M. WHEELER

THE UNIVERSITY OF CHICAGO

VOLUME I

BOSTON, U.S.A.
GINN & COMPANY, PUBLISHERS
The Athenæum Press
1898

CONTENTS OF VOL. I.

No. 1. — August, 1897.

No. 2. — September, 1897.

No. 3. — October, 1897.

No. 4. — December, 1897.

No. 5. — February, 1898.

No. 6. — May, 1898.

Volume I. *August, 1897.* *Number 1.*

ZOÖLOGICAL BULLETIN.

THE MORPHOLOGY OF THE PETROSAL BONE AND OF THE SPHENOIDAL REGION OF THE SKULL OF AMIA CALVA.

EDWARD PHELPS ALLIS, JR.

THE sphenoidal region of the skull of vertebrates is that part of the base and sides of the skull that corresponds to that part of the skull of man that is occupied by the sphenoid bone. This bone in man (No. 24, vol. ii, pt. i) lies between the ethmoid bone anteriorly and the occipital and temporal bones posteriorly, and has two portions, — an anterior, presphenoidal part, and a posterior, postsphenoidal part. The presphenoidal part of the bone contains a median element, the presphenoid, and two lateral elements, the orbitosphenoids, one on each side of the head. The postsphenoidal part of the bone contains a median element, the basisphenoid, and three lateral elements on each side, the alisphenoid, the sphenotic, and the internal pterygoid plate, the latter being developed from one of the appendages of the skull, the palatopterygoid bone, and hence not a part of the cranium proper.

The presphenoid first appears in man, according to Sutton (No. 29, p. 581), as a pair of nuclei on the inner sides of the optic foramina. These nuclei lie on the deep aspect of the perichondrium, and do not involve the subjacent cartilage

until they have attained some considerable size. They fuse first with the orbitosphenoids; then with each other, by sending a thin shell of bone across the dorsal aspect of the cartilage that separates them; and then with the basisphenoid. Much later they fuse with each other in the deeper parts of the separating cartilage.

According to Thane (No. 24, vol. ii, pt. i, p. 76) the presphenoid of man may develop from the two nuclei of Sutton, or may be "an independent growth." This latter possibility is perhaps of some considerable morphological importance, as will be later seen.

The basisphenoid first appears as two nuclei in the cartilage forming the floor of the pituitary fossa (No. 29, p. 580), that fossa being the sella turcica of the adult (No. 24, vol. ii, pt. i, p. 75).

In Amia the prefrontals of Sagemehl's descriptions and my own are the lateral ethmoids, or ectethmoids of English writers, the generally accepted homologues of the lateral masses of the ethmoid bone of man; and the petrosals are the proötics, the generally accepted homologues of the correspondingly named parts of the petrous part of the temporal bone of man. The sphenoidal region of the skull of Amia would accordingly be, if the bones alone were considered and the homologies implied in their names accepted, that part of the base and sides of the skull that lies between the prefrontals anteriorly and the basioccipital, squamosals, and petrosals posteriorly.

The region so defined in Amia contains, on each side of the head, four primary ossifications, three of which are called by Sagemehl (No. 25) the orbitosphenoid, alisphenoid, and postfrontal; while the fourth is considered by him as one half of a basisphenoid bone. The postfrontal of this nomenclature is the sphenotic of Bridge's descriptions of Amia (No. 7), and the postorbital ossification of my own (No. 1, p. 479).

The orbitosphenoid (No. 2, Figs. 8–11) lies between the orbito-nasal fenestration (No. 2) of the optic wall of the skull and the optic fenestra (No. 25, p. 202); the alisphenoid between the latter fenestra and the two trigeminal foramina. The orbito-nasal fenestra lies, in larvae of Amia, and hence

morphologically in the adult also, in front of the foramen by which the olfactory nerve leaves the cranial cavity. The optic fenestra is, according to Sagemehl, simply the greatly enlarged foramen of the nervus opticus, but it includes, in its posterior portion, as will later be shown, the tall orbital opening of my descriptions of the eye-muscle canal (No. 2), which opening transmits the oculomotorius, trochlearis, profundus, and abducens nerves, and is the fused foramina of those nerves and that of the vena ophthalmica (No. 2).

The orbitosphenoid and alisphenoid, both of which form parts of the side wall of the skull, thus lie respectively between the olfactory and profundus nerves, and the profundus and trigeminus. Posterior to the alisphenoid the petrosal occupies a similar position between the facialis and glossopharyngeus.

The profundus, trigeminus, facialis, and glossopharyngeus are all generally considered as segmental nerves; the olfactorius is sometimes so considered (Nos. 6, 18); and Marshall suggests (No. 18, p. 38) that the opticus also may possibly be of segmental value.

The orbitosphenoid, alisphenoid, and petrosal bones of Amia thus have, in general position, the same relation to segmental nerves that the several components of the occipitale laterale have (No. 2). This markedly interneural, and hence segmental or intersegmental, whichever it may be, position of the bones in Amia, thus gives no support whatever to Vrolik's conclusion (No. 32, pp. 240, 251) that the occipitale laterale and petrosal of fishes are ossifications of the side wall of the brain case, formed, respectively, around the vagus and facial foramina. The alisphenoid would naturally, under Vrolik's interpretation of the bones, be an ossification formed around the trigeminal foramina.

The postfrontal of Sagemehl, my postorbital ossification, forms part of the dorso-lateral edge of the skull in the region between the trigeminus and facialis.

The two so-called halves of the basisphenoid lie in the base of the skull in the region between the olfactorius and profundus, if not perhaps between the opticus and profundus.

In teleosts, in marked distinction to Amia, the basisphenoid is a single median bone. It is, moreover, a most inconstant element of the teleostean skull. According to Vrolik (No. 32, p. 276) it may be found in connection with an eye-muscle canal, or where that canal does not exist; there may be an eye-muscle canal and no basisphenoid, or neither eye-muscle canal nor basisphenoid; the bone may, in some fishes, be replaced by a ligament; and in still others it may be fused with the parasphenoid, and appear as a median, vertical process of that bone. It is said by Vrolik (No. 32, p. 254) to develop in Salmo as two lateral Γ-shaped nodules, which later fuse to form the single impair bone.

In all descriptions that I find of it, it lies between the extreme hind ends of the orbits and gives attachment and support to, or forms part of, the hind edge of a more or less developed membranous, ventral portion of the interorbital wall or septum. It may be preformed in cartilage (No. 32, p. 247), or it may develop wholly or in part in that membranous part of the interorbital septum to which it is destined later to give support (No. 32, pp. 254, 259, and No. 20, p. 139). In this latter case, in the single instance cited, the salmon, both Vrolik and Parker say that the interorbital septum itself is at first formed entirely of membrane, which later becomes invaded by cartilage.

The membranous portions of the interorbital region of the skull of Salmo, and hence of other fishes also, thus seem to represent simply an arrested condition in the development of the side walls of the cartilaginous cranium. Such being the case, the teleostean basisphenoid and orbitosphenoid, both of which develop either in the interorbital membrane or in the cartilage which may replace it, must be of primary, and not of secondary origin; and they would not necessarily cease to be strictly homologous with the corresponding bones in higher vertebrates, as Vrolik definitely says they do (No. 32, p. 259), simply because of their histologically somewhat different development in certain fishes.

In Amia the so-called basisphenoids are described by Bridge (No. 7, p. 613) as "two small osseous nodules," one on each side of the head, "resting upon and in part imbedded in the

cartilaginous, anterior clinoid wall." From the position of these nodules Bridge was led to conclude that they were probably ossifications that commenced in the strong fibrous membranes that close the optic fenestrae of the fish, and that they subsequently invaded the anterior clinoid wall. They were homologized by him, under some reserve, with the "prepituitary portion of the basisphenoid of other fishes"; but as there is, in fishes, no postpituitary portion of this bone, so far as I can find described, the portion of the bone so specifically defined by Bridge must have been intended to include the entire bone. With this conclusion, as applied to the entire bone of teleosts, Sagemehl entirely agrees, the principal reason given by him being that if the bones are not the homologues of the teleostean basisphenoid they would become ossifications peculiar to Amia alone, among all fishes (No. 25, p. 215). The disappearance of the wide cartilaginous bar that separates the two bones in Amia, and their subsequent fusion into the single impair bone of teleosts, is said by Sagemehl to be brought about by the compressive action of the additional recti muscles, which, in the latter fishes, find their way into the eye-muscle canal.

In my own earlier work on Amia I was led to strongly doubt this homology of the so-called basisphenoids of that fish with the teleostean basisphenoid. Later work, and a more special study of the region, convince me that it is wholly wrong, as is also the supposed homology of the teleostean basisphenoid with the similarly named bone of higher vertebrates.

In my earlier work (No. 2, pp. 406, 407) I found the so-called basisphenoids of Amia of variable size and form, not only in different specimens, but also on the two sides of the head in the same specimen; and I found them developing in connection with the origins of three of the recti muscles. I considered them, as Bridge did, largely of membranous origin, because they lay, in the adult, lateral to the internal carotid arteries, while in larvae, those arteries, after having pierced the basis cranii, ran upward and forward on each side of the head, along the lateral surface of the ventral portion of the cartilaginous, anterior clinoid bar. The further course

of each artery, and its relation to the clinoid bar, considered at the time as unimportant, was said to be upward along the anterior surface of the dorsal edge of the bar, internal to a projecting, anterior, dorso-lateral corner of the bar, the artery not again entering the cartilage at all.

In Scomber, work being done in my laboratory here by Dr. Dewitz shows the carotid artery on each side, running upward along the anterior edge of the lateral wing of the so-called basisphenoid of that fish. In Salmo it apparently has a similar course (No. 20, Pl. V, Fig. 7). The artery in Scomber and Salmo thus has exactly the same relation to the basisphenoid bone that it has in Amia to the cartilaginous, anterior clinoid bar.

Why, then, should the cartilaginous, anterior clinoid bar of Amia not be the unossified homologue of the basisphenoid bone of Scomber and other teleosts? It fulfills exactly all the conditions required; the otherwise perplexing difference in the relations of the internal carotids to the so-called basisphenoids in Amia and Scomber are naturally explained; and Amia would not differ from all other ganoids in possessing osseous rudiments of a basisphenoid, as Bridge was forced to assert (No. 7, p. 614). If such be the case the homologues of the two bones that cap the bar in Amia must be looked for elsewhere than in the teleostean basisphenoid. I consider each of them as a part of the orbitosphenoid of its side of the head, ossified from an independent center and not fused with the rest of the bone because of the great development of the optic fenestra. In support of this proposition it is to be noted that, in their general position, and especially in their relations to the internal carotid arteries, the two bones of Amia agree closely with the anterior clinoid processes of the orbitosphenoid part of the sphenoid bone of man; and that the orbitosphenoid in man must necessarily ossify from two different centers in those cases in which the presphenoid bone develops, as Thane says it sometimes does, independently of the pair of nuclei that first appear on the inner sides of the optic foramina (No. 24, vol. ii, pt. i, p. 76).

In fishes I can find no recorded instance of such a development of the orbitosphenoid from two centers, nor of a second-

ary separation of the bone into two parts by the optic fenestra. The bone is, however, a variable and inconstant element of the piscine skull. It may be wanting ; may be preformed in cartilage (Amia), or in membrane only (No. 32, p. 255) ; may be fused with its fellow of the opposite side of the head (No. 27, p. 68) ; and may be, as in Polypterus, fused also with both the alisphenoids and the teleostean basisphenoid to form a single impair bone, called by Traquair (No. 31, p. 170) the sphenoid bone, and by Pollard (No. 23), the orbitosphenoid. In Traquair's figures (Figs. 2, 3) the bone seems even to extend backward beyond and around the pituitary fossa. In Pollard's figures (Fig. 12), taken perhaps from a younger specimen, it does not extend so far.

Where the orbitosphenoid, in fishes, is found impair, and the cartilage of the interorbital part of the basis cranii has not disappeared, as in Polypterus and certain of the Characinidae, the median part of the bone lies on the dorsal surface of the cartilage, and is not seen from the outer ventral surface of the skull (No. 31, p. 170, and No. 27, Figures).

These facts all seem to indicate that the orbitosphenoids and so-called basisphenoids, once having appeared in any fish, as ossifications preformed either in cartilage or in membrane, can extend themselves in all directions, not only in the contiguous or adjacent cartilage but also, and by preference, in the membranes that line the optic and pituitary parts of the skull, and fill its orbital and interorbital openings. The same would seem to be true of other vertebrates also, for in man the orbitosphenoids of opposite sides of the head, in their later development, send, according to Sutton (No. 29, p. 582), "a thin lamella across that portion of the presphenoid which is anterior to the optic groove, thus excluding it from the cranial cavity." As the presphenoid first appears, according to Sutton, "on the deep aspect of the perichondrium," these thin lamellae of the orbitosphenoid must necessarily lie entirely superficial to the subjacent cartilage. Moreover, the sphenoid bone in man is subject to certain variations (No. 24, vol. ii, pt. i, p. 47) which are further important indications in this connection. A middle clinoid process is often found, sometimes connected by

a spiculum of bone to the anterior clinoid process, thus forming a foramen for the carotid artery, the carotico-clinoid foramen, which is strikingly similar to the carotid canal of my descriptions of Amia. The anterior and posterior clinoid processes may be similarly united by a spiculum of bone, which thus replaces a part of the thick glistening membrane of Amia ; and the petro-sphenoidal ligament may be ossified, thus forming a foramen through which the inferior petrosal sinus and the sixth nerve pass (No. 24, vol. ii, pt. i, p. 43), bone thus again replacing parts of the membrane of Amia. This membrane in Amia accordingly deserves more attention than has heretofore been given it.

The cranial cavity of fishes is said by Sagemehl (No. 26) to be filled with a mass of *Fettgewebe* or *Schleimgewebe*, usually voluminous, which occupies the entire space between the inner surface of the skull and a single vascular membrane which is closely applied to the outer surface of the brain. The outer and inner surfaces of this tissue are partially differentiated as limiting membranes, and between the inner of these membranes and the vascular membrane there is a slit-like pericerebral lymph space, which Sagemehl considers as the homologue of the subdural space of higher vertebrates. The single vascular membrane is accordingly considered by him as the pia mater and arachnoid together of higher vertebrates ; the voluminous mass of fatty or *Schleim* tissue, as the dura mater.

The outer, partially differentiated limiting membrane of the dura mater, so defined, is said by Sagemehl to be closely applied to the entire inner surface of the skull, excepting only the labyrinth recesses, and to be the periosteum or perichondrium, as the case may be, of the cranial cavity. It can be separated into two layers, the outer of which, alone, is the osteoblastic layer of the membrane, the inner layer being of a fibrous character.

In the spinal canal the outer limiting membrane of the cranial dura mater seems, from Sagemehl's descriptions, to be separated, as a separate membrane, from the rest of the dura mater ; for he says that the spinal dura mater does not lie, as the cranial dura mater does, against the inner surface of the

enclosing bone and cartilage, but is separated from that surface by a tough, fibrous membrane, which is the internal periosteum of the vertebral column. The dura mater lies inside this membrane, separated from it by an epidural space, and its external surface is simply a hardened superficial portion of the general tissue of the structure, and not at all the partially differentiated and partly osteoblastic limiting membrane of the cranial dura mater. The spinal and cranial durae are thus, from Sagemehl's own descriptions, not exactly similar structures, notwithstanding his definite statement to the contrary (No. 27, p. 470). It is perhaps not unimportant in this connection to note that fibrous bands, the interclinoid ligaments, are normally found beneath the dura mater in the pituitary region of the human skull (No. 24, vol. ii, pt. i, p. 47); and that the cranial dura mater, in many vertebrates, is subject to ossification, sometimes extensive (No. 24, vol. iii, pt. i, p. 183, and No. 27, p. 85), which does not occur, so far as I find recorded, in the spinal dura.

If now the skull of Amia be considered, we find a tough, glistening, fibrous membrane which forms the floor and sides of the interorbital and pituitary parts of the cranial cavity, closing at the same time the optic fenestrae, and forming the roof of the ventral portion of the eye-muscle canal, and the median walls of its upper, lateral chambers. The hind edge of this membrane is attached to the anterior bounding ridges of the labyrinth recesses, and, between those recesses, to the front edges of the median, horizontal processes of the petrosals. A separate and independent perichondrial or periosteal membrane, the histological character of which I have not attempted to investigate, lines the floor of the eye-muscle canal, and the lateral walls of its upper, lateral chambers. That this latter membrane is not simply a reflexed portion of the outer pericranial membrane seems to be sufficiently indicated by the fibrous tufts in which the free cartilaginous edges of the orbital opening of the eye-muscle canal are always seen to end in sections of larvae of Amia. Moreover, what is exceedingly important in this connection, a subpituitary or peripituitary canal is found in Lepidosteus entirely closed, toward

the orbits as well as elsewhere, by membrane (No. 27, p. 86). In this closed canal of Lepidosteus, which is considered by Sagemehl, as it unquestionably is, as the homologue of the apparently open eye-muscle canal of Amia, fatty tissue is found, similar, undoubtedly, to the fatty tissue found both in the apparently open canal and in the cranial cavity proper of Amia.

The eye-muscle canal in Amia and Lepidosteus, and hence probably in all fishes, is thus an intracranial space, opened secondarily toward the orbits, Lepidosteus presenting the primary condition of the canal in all fishes, and not a secondary one, as Sagemehl was led to conclude. It is also, according to Sagemehl's definitions of the cranial membranes, an intradural space, notwithstanding his indirect statement that it is extradural (No. 27, p. 87); and as it gives passage, in Amia, to certain nerves and arterial and venous vessels and lodges the Gasserian ganglion (No. 2), it is a space similar to, if not strictly homologous to, the cerebral sinuses and the cavum Meckelii of human anatomy. It is, however, in Amia, a space that certainly lies morphologically in, and not internal to, the membranous bounding walls of the primordial skull. It must accordingly lie in or external to, and not internal to, the cranial homologue of those extradural fibrous tissues of Sagemehl's descriptions that line the inner surface of the spinal canal of fishes. This is all sufficiently evident from Parker's statement (No. 20, p. 131) that in salmon larvae the membranous, interorbital cranial walls, later ossified as the orbitosphenoids, "pass down into the interorbital septum, which is continuous below the perichondrium of the tilted and coalesced trabeculae"; and Studnička's statement (No. 30, p. 618) that the membranous brain capsule of Ammocoetes "sich dorsal an die Trabeculae ansetzt."

The fatty tissue found in the eye-muscle canal, both in Amia and Lepidosteus, is then presumably similar, in general character, to that found in the membranous brain capsule of the Cyclostomata (No. 30), and naturally subject to chondrification, as in those fishes, and hence to subsequent or independent ossification. Such being the case the eye-muscle canal in different fishes, and the corresponding space in other verte-

brates as well, is naturally subject to much variation in form and size, according to the extent and manner in which its membranous walls adhere to each other and are chondrofied or ossified.

Further important evidence in support of these several conclusions is found in Jacoby's statement (No. 16, p. 81) that a certain dorsal process of the cartilaginous orbitosphenoid of human embryos indicates an earlier cartilaginous connection of the orbitosphenoids with the *Parietalplatten;* cartilage thus taking the place, in the early ancestors of man, of the thick, so-called dural membrane of Amia. In those early ancestors the alisphenoids must necessarily have been excluded from the bounding side walls of the cranial cavity proper, as they are in Amia.

The orbital and interorbital openings of the skull of fishes must now be considered.

The optic fenestra of Sagemehl's descriptions is considered by him as an enlargement of the foramen by which the optic nerve, on each side of the head, pierces the side wall of the skull (No. 25, p. 202). In the fresh skull this opening, in Amia, consists of two parts, one of which is closed by the anterior portion of the tough, fibrous dural membrane, which here forms a part of the side wall of the skull, while the other part of the opening is apparently open to the orbit and is the tall orbital opening of the eye-muscle canal of my descriptions. The posterior portion of the tough fibrous membrane lies inside the skull, internal to the alisphenoid, and is not exposed to the outer surface.

The optic nerve and the arteria ophthalmica pierce the anterior, exposed portion of the tough fibrous membrane, and enter the orbit at once. The other nerves that pierce the membrane pierce it in its posterior portion, or in the limiting region between its two portions, and enter the eye-muscle canal, from which they issue, by the orbital opening of the canal, into the orbit. The vena ophthalmica and the external rectus muscle, which enter the eye-muscle canal from the orbit by the orbital opening of the canal, do not pierce the tough, fibrous membrane at all.

There are thus in the recent state of Amia two morphologically distinct openings fused to form the single fenestra of Sagemehl's descriptions of the prepared skull. The same is true of all teleosts that I find described, but the conditions are there less obvious than in Amia. Moreover, in those teleosts in which the interorbital part of the skull is reduced to an impair interorbital septum, another opening is, or may be, added to the two optic fenestrae of Amia; the orbital region of the prepared skull of such fishes presenting three openings, two of which are lateral and one median. This is evident in Sagemehl's descriptions of Macrodon (No. 27, p. 67), and in the sectional view given by him of the orbital part of the skull of Erythrinus (No. 27, Pl. I, Fig. 8). The two lateral openings lead from orbit to orbit, and include, in their anterior portions, those perforations of the interorbital wall or septum that are said to be formed around the optic foramina. In their posterior portions they contain the orbital openings of the eye-muscle canal. The median opening lies between the postero-superior margins of the lateral openings and leads directly from the orbits into the cranial cavity. The flatter the hind wall of the orbit, and the larger the interorbital perforation, the more separate and distinct does the median opening of the brain case become, as Brooks' side view of the skull of the haddock plainly indicates (No. 8, Pl. V, Fig. 1). The lateral openings are the optic fenestrae as defined by Sagemehl; the median opening, wrongly called by him the optic fenestra in Macrodon, may be called the orbital opening of the brain case, or simply the orbital fontanelle. The optic fenestrae of Sagemehl are apparently the orbito-sphenoidal fenestrae of Parker's descriptions of Lepidosteus (No. 21, p. 480).

The optic fenestrae of teleosts, as above defined, are, in the fresh skull, entirely closed by membrane, excepting in their ventro-posterior portion, where the membrane on each side of the head is interrupted by the orbital opening of the eye-muscle canal. The anterior or antero-ventral part of the membrane of each side is, in those fishes in which a median, interorbital septum is found, fused with the corresponding part of the membrane of the opposite side to form that septum. The dorso-posterior

portion of the membrane of each side closes, in such cases, the corresponding half of the orbital fontanelle. Where there is an interorbital septum and no teleostean basisphenoid bone, as in the Characinidae and Cyprinidae, the membranes of opposite sides unite in their mid-ventral portion and are continued backward to form the membranous basisphenoid and the pituitary fossa, forming at the same time the floor of the cranial cavity and the roof of the eye-muscle canal. The orbital opening of the latter canal thus lies external to, and inferior to, this part of the membrane. This is all evident in itself from Brooks' and Sagemehl's several descriptions and statements, and is practically shown to be the case in one of Vrolik's figures of Esox lucius (No. 32, Fig. 9).

Through the orbital fontanelle, as above defined, the optic nerves are said by Sagemehl to issue, in all the teleosts described by him (No. 27, p. 70, and No. 28, p. 570). The nervus oculomotorius on each side is said by him to issue through the same opening, or through a special foramen in that part of the petrosal that lies lateral to, and in front of, the pituitary fossa. The trochlearis issues through the fontanelle in Cobitis and its related species, but in all the other fishes described it pierces the alisphenoid by a small foramen, the exact position of which is not given.

Each half of the orbital fontanelle in certain of these fishes thus corresponds exactly, in the structures it transmits, the optic nerve alone excepted, to the dorsal part of the tall orbital opening of the eye-muscle canal of Amia. In the other fishes described, it differs only in that the trochlearis and oculomotorius have become enclosed in the edges of the bones that bound the opening. In none of the fishes described is there an upper lateral chamber of the eye-muscle canal as in Amia.

The arrangement and disposition of the parts here under consideration thus indicate that that part of the internal fibrous layer of the external limiting membrane of the dura mater of Sagemehl's definitions that forms on each side of the head of Amia the median wall of the upper lateral chamber of the eye-muscle canal has, in all the teleosts described by Sagemehl, fused more or less completely with that subjacent, external,

perichondrial or periosteal layer of the same membrane, which in Amia forms a separate and independent lining of the inner surface of the lateral wall of the chamber. The dorsal portion of the tall orbital opening of the eye-muscle of Amia is thus, in these teleosts, closed toward the orbit; and the upper lateral chamber of the eye-muscle canal on each side of the head is reduced to certain intradural spaces, or to certain canals or chambers which traverse, or lie in, the side wall of the skull, and transmit the several nerves. The same is apparently true not only of all other teleosts, but also of all elasmobranchs, as the membranous interorbital walls of Chimaera and Callorhynchus plainly show (No. 13, Figs. 1, 2).

The two separate layers of the dural membrane of Amia having fused in other fishes, or never having separated to the extent found in Amia, whichever it may be, chondrification or ossification invades them from different sides and to different extents in different fishes.

In most teleosts the petrosal in particular undergoes a great development, often extending forward in the dural membrane, and also in the adjoining cartilage of the side wall of the skull, beyond the lateral edge of the pituitary fossa to the hind wall of the orbit, a part of which it forms. This marked characteristic of the teleostean skull, found already indicated in Amia, is accompanied by a relatively small development of the alisphenoid, that bone in teleosts seeming never to invade the dural membranes or adjacent cartilage to any great extent. The teleostean basisphenoid, where it is developed, also extends its horizontal wings on each side in the dural membrane, invading that part of the membrane that lies in front of the pituitary fossa, and even reaching and articulating with the anterior extension of the petrosal, or with the adjacent ventral edge of the alisphenoid. Esox represents an intermediate stage in this development (No. 32, Pl. XVIII, Fig. 9).

In Polypterus, in marked distinction to Amia and teleosts, it is the alisphenoid and not a petrosal bone that tends to occupy that antelabyrinthian region of the skull that lies between the trigeminal and facial foramina. In man, the complete invasion of this region by the former bone is plainly shown by a com-

parison of Jacoby's figures and descriptions of human embryos (No. 16) with the conditions found in the adult. Gegenbaur is even said by Jacoby (No. 16, p. 81) to have characterized the great development of an alisphenoid bone as a peculiarity of man.

In this invasion of the sphenoidal region of the skull by different ossifications, whether in fishes or in man, the relations of the nerves issuing to the several bones, as found in Amia, remain remarkably constant.

In teleosts, the opticus issues in front of or above the cartilaginous, membranous, or osseous basisphenoid, between it and the orbitosphenoid. The oculomotorius remains always in front of the alisphenoid, but may become enclosed in the anterior edge of the anterior extension of the petrosal. The trochlearis lies in front of the alisphenoid, or may become enclosed in the anterior edge of that bone. The profundus cannot be recognized in Sagemehl's descriptions of any of the fishes examined by him. In the haddock (No. 8, p. 171), what seems to be the nerve issues in front of the alisphenoid; and in Amiurus (No. 19, p. 275, and No. 34, p. 366), through the alisphenoid near that edge of the bone that articulates with the basisphenoid. The trigeminus in the Characinidae (No. 27, p. 70) issues through a single foramen in the anterior orbital part of the petrosal, its relation to the alisphenoid thus being uncertain. In the Cyprinidae, the ophthalmic branch of the trigeminus issues through a special foramen in the alisphenoid (No. 28, p. 568), the maxillary branches of the nerve issuing through a foramen which lies partly in the petrosal, and partly between that bone and the alisphenoid. In Leuciscina and Abramidina, the latter foramen is often separated into two parts by a bridge of bone; the upper part of the foramen, in such cases, transmitting the trigeminal nerves, and the lower part the vena ophthalmica. In the haddock and in Amiurus, the trigeminus issues with the facialis through a large foramen between the adjoining edges of the alisphenoid and petrosal, the foramen being often incompletely separated into two or more parts by bony spicules.

In man (No. 25, vol. ii, pt. i, p. 80), the opticus issues between the orbitosphenoid and presphenoid; the oculomotorius,

trochlearis, and abducens nerves, and the ophthalmic division of the trigeminus, the probable homologue of the profundus (No. 2, p. 000) issue through the sphenoidal fissure, between the orbitosphenoid and alisphenoid; the superior maxillary division of the trigeminus issues through the foramen rotundum, said by Thane to be cut off from the sphenoidal fissure, but from Jacoby's descriptions (No. 16, p. 67), lying apparently posterior to the alisphenoid; and the inferior maxillary division of the trigeminus issues through the foramen ovale, which is cut off from the foramen lacerum, which lies between the alisphenoid and periotic.

The sphenoidal fissure of man was shown in my earlier work (No. 2, p. 000) to agree strikingly in position and function with the tall orbital opening of the eye-muscle canal of Amia, and to be apparently the homologue of that opening. It is accordingly also, in whole or in part, the apparent homologue of one-half of the orbital fontanelle of teleosts.

In mammals, each half of the orbital fontanelle of fishes seems to be represented in a large opening, closed by membrane, which lies, according to Sternberg (No. 3, p. 147), between the two sphenoid bones and transmits the orbital nerves and vessels. The canalis craniopharyngeus lateralis of Sternberg, found in man and many mammals, is then the last remnant of the ventral part only of the tall orbital opening of Amia, and not a last remnant of the entire opening, as Bardeleben's statement of Sternberg's conclusions seems to indicate.

The petrosal bone, which lies in man posterior to the sphenoidal region of the skull, but which in fishes may, as shown above, invade that region to a considerable extent, and the facial nerve, which should lie morphologically in front of the petrosal, if that bone lies between successive segmental nerves, as its position in Amia seems to indicate, must now be considered.

The facial nerve in man leaves the primordial cranium, according to Vrolik, by the hiatus Fallopii and not by the stylomastoid foramen (No. 32, p. 308). This statement is based on the fact that in human embryos of from 12 cm. to 15 cm. in length, the nerve lies, after issuing from the hiatus, in a groove in the cartilage of the under surface of the skull (No. 32,

p. 307). It reaches that under surface, so far as can be judged from the figures and descriptions, through a large opening, which must be the foramen lacerum medium of the adult. Jacoby's figures and descriptions (No. 16) of a much younger embryo seem to confirm this. Vrolik's figures show little or no indication of a division of this opening into two parts by the lingula. Jacoby's figures, on the contrary, show the lower end of the opening cut off as a separate foramen by a strong cartilaginous bridge. The upper part of the opening in Jacoby's figures is simply a wide cleft in the cartilaginous side wall of the cranium, which transmits the third branch of the trigeminus, and apparently the second branch also. The ventral, completely separated, part of the cleft transmits the internal carotid artery, and is called by Jacoby in one place the canalis caroticus, and in another the foramen lacerum anterius (No. 16, pp. 66, 74). That it is not the foramen lacerum anterius of Thane's descriptions (No. 24, vol. ii, pt. i, p. 70) is sufficiently evident to need no comment.

In the adult man, the facial nerve does not traverse the foramen lacerum as it seems to in embryos. It is, however, still exposed to that foramen at the hiatus Fallopii, and, coming from the hiatus, the large superficial petrosal nerve crosses the foramen to reach the posterior orifice of the Vidian canal (No. 24, vol. ii, pt. i, p. 70), which it traverses with a branch of the external carotid artery. By the inner part of the foramen the internal carotid artery enters the cranial cavity, its groove being partially, and sometimes completely, separated from the rest of the foramen by the lingula (No. 24, vol. ii, pt. i, p. 70). The foramen spinosum and foramen ovale, both of which are cut off from the foramen lacerum, and may be, even in the adult, incompletely separated from it, transmit respectively the large and small meningeal arteries (No. 24, vol. ii, pt. i, pp. 47, 80). An irregular cleft, the petro-basilar fissure, extends from the foramen lacerum backward and outward to the jugular foramen (No. 24, vol. ii, pt. i, p. 68).

In Amia (No. 2, p. 000), the facial foramen perforates the petrosal and transmits both the facial nerve and the jugular vein. The facial nerve does not, however, leave the cranial

cavity proper by this foramen. After its exit from the brain it pierces a mesial membranous part of the anterior wall of the labyrinth recess, enters the upper lateral chamber of the eye-muscle canal, where it traverses the trigemino-facial ganglion, and then issues from the skull by the large facial foramen. Slightly below this foramen, and hence what would be in the human skull mesial to it, are the external carotid foramen and the external orifice of the internal carotid canal. Internal to the foramen, in the upper lateral chamber of the eye-muscle canal, the palatine branch of the facialis enters the posterior orifice of a canal, called in my descriptions the palatine canal, which runs forward between the parasphenoid and the ventral surface of the chondrocranium.

It is thus evident that but for the fact that the main external carotid artery of Amia enters the upper, lateral chamber of the eye-muscle canal, while in man its meningeal branches only enter the skull, there would be a striking functional resemblance between the facial and carotid foramina united, of Amia, and the foramen lacerum and jugular foramen together, of man. Moreover, that the difference in the course of the external carotid in the adult of Amia and of man is probably not of morphological importance, is evident from the fact that in embryos of the higher vertebrates (No. 27, p. 66) the external carotids have an intracranial course. Furthermore, what may possibly be related to that early condition, an external carotid may be, in rare cases, wanting even in the adult man (No. 24, vol. ii, pt. ii, p. 393).

In teleosts the same general relations of the parts here under consideration are found as in Amia, but there is great variation in the details of the disposition of the several parts, and the resemblances to the conditions found in man are not so striking as in Amia. The jugular foramen may become either partly or entirely separated from the facial foramen; the two carotid arteries may enter the eye-muscle canal by a single foramen, corresponding in position to the internal carotid foramen of Amia; and the facialis may have its exit by two foramina instead of by one (No. 27, p. 65, and No. 28, p. 559).

The trigemino-facial ganglion in Amia lies in the upper,

lateral chamber of the eye-muscle canal, the profundus ganglion in the orbital opening of that chamber. The ganglia are therefore both intradural in position, as the trigeminal ganglion is in man (No. 24, vol. iii, pt. ii, p. 234). The trigemino-facial ganglion lies in Amia immediately in front of the anterior bounding wall of the labyrinth recess and would lie on the dorsal surface of that wall if the hind end of the skull were flexed downward as it is in man. The anterior wall of the labyrinth recess is partly ossified as the petrosal. The upper, lateral chamber of the eye-muscle canal of Amia is thus both functionally and in position the equivalent, if not the homologue, of the cavum Meckelii of man.

In teleosts the *Wurzelganglien des Trigeminus* lie, according to Sagemehl (No. 26, p. 463), in the fatty portion of the dura mater, between the outer and inner limiting membranes of that structure. If the ganglia so defined form or belong to the Gasserian ganglion, that ganglion in teleosts occupies a position markedly different from what I find in Amia.

In other fishes parts of the trigemino-facial ganglionic complex may lie in canals in the side wall of the skull, and parts of it entirely outside the skull, as in Chimaera (No. 9), Laemargus (No. 10), and Acipenser (No. 12). In Necturus (No. 17) the several ganglia lie entirely outside the skull. Whether in these several cases, and in other similar ones, the ganglia are morphologically different in position from what they are in Amia, or not, depends entirely upon the positions of the two layers of the dura mater, and not upon the relations of the ganglia to the side walls of the skull. Unfortunately, the relations of the dura mater and its different layers to the ganglia are not given in any of the descriptions that I find.

The internal auditory meatus, the internal orifice of the canal by which the facial nerve in man leaves the cranial cavity, lies, according to Thane (No. 24, vol. ii, pt. i, p. 75), between the proötic and opisthotic portions of the petrous portion of the temporal bone. The proötic lies above the meatus, the opisthotic below it. The facialis in man, in that part of its course that traverses the side wall of the primordial cranium, cannot accordingly be considered as lying, in any

way, morphologically anterior to the proötic element of the temporal bone. If not simply ventral to that element it must lie morphologically posterior to it.

The petrosal, the supposed piscine homologue of the mammalian proötic, is, in Amia, a nearly circular bone, the front edge of which forms, according to Sagemehl (No. 25, p. 205), the anterior limit of the labyrinth region of the skull, notwithstanding the fact that the bone extends forward, as Sagemehl himself states, slightly beyond the anterior limiting ridge of the labyrinth recess. If Sagemehl's figures and my own (No. 2, Fig. 2) be examined it will be seen that the ridge here referred to runs from behind upward and forward across the inner surface of the petrosal, between the labyrinth recess and the upper, lateral chamber of the eye-muscle canal, and that the several foramina that perforate the bone all lie in front of the ridge. A similar but much less developed ridge is found in the Characinidae and Cyprinidae. That part of the petrosal that takes part in the formation of the labyrinth region of the skull in these fishes thus lies in marked distinction to the relation found in man, most decidedly posterior, instead of anterior, to the nervus facialis; and if the hind end of the skull in these fishes were to be flexed downward, as it is in man, so that the foramen magnum would come to lie on its ventral surface, the petrosal would necessarily lie ventral to the facialis, as the opisthotic does in man, and not dorsal to it as the proötic should.

Is then the petrosal of fishes the homologue of the opisthotic of man and not of the proötic? It certainly does not lie over the superior semicircular canal, the place assigned to the proötic by Thane in man (No. 24, vol. ii, pt. i, p. 75); nor does it form simply the fore edge of the periotic capsule, the place assigned to the bone by Parker in fishes (No. 20, p. 96); and it is the only bone in Amia that has any relation whatever to the ampulla of either the anterior or the posterior semicircular canals, the positions assigned respectively in fishes to the sphenotic and opisthotic. Of these two latter bones it could only be the opisthotic, the term sphenotic having been introduced by Parker for the postorbital ossification.

The intercalar of fishes, which is usually considered as the homologue of the opisthotic of higher animals, is certainly not that element of the skull, for it has in Amia (No. 2), contrary to Sagemehl's statement, and as Vrolik has already shown for teleosts (No. 32, p. 285), no primary relation whatever to the periotic capsule. The occipitale laterale, the only other bone in Amia that could be the opisthotic, is also not that element, since, in Amia, it is strictly confined, in its early development, to the postauditory region of the skull (No. 2).

Moreover, the bone that lies in Polypterus between the facial and vagus foramina, that is, in the position relative to those nerves that the petrosal has in Amia, is called by Traquair the opisthotic, and considered by him as that element of the skull fused with the epiotic (No. 31, p. 168). In Lepidosteus also the bone identified by Parker as the opisthotic has a similar position, and is similarly but less completely fused with the epiotic. In Polypterus there is no proötic bone. In Lepidosteus the proötic lies in front of the facial foramen (No. 21, Pl. XXXVIII, Figs. 1, 2), as it does also in the sturgeon (No. 22, p. 176). In Menobranchus the proötic occupies a similar position, the opisthotic lying, as in both Polypterus and Lepidosteus, posterior to the facialis, fused with the epiotic (No. 15, p. 188, and Pl. XXIX, Fig. 1). In Menobranchus no sphenotic bone is given. In Lepidosteus it is shown lying dorsolateral to the proötic, and is, in the oldest stage given, almost continuous with that bone. In Polypterus the postfrontal of Traquair is traversed by the lateral canals (No. 31, p. 181), and is therefore not the homologue of the postorbital ossification alone of Amia. The bone, whatever it may be, is firmly connected by suture with the parasphenoid, but is widely separated by cartilage from the opisthotic.

If, then, the petrosal of Amia is the homologue of the opisthotic, as its general relations to the facial nerve and the periotic capsule seem to indicate, the postorbital ossification, which is usually considered as the sphenotic, must in all probability be the homologue of the proötic. This ossification in Amia forms no part of the labyrinth recess. In many teleosts, on the contrary (No. 32, pp. 278–285), it lies above

the anterior end of the anterior semicircular canal, and forms the anterior boundary of the labyrinth recess, thus occupying exactly the place assigned by Thane to the proötic in man, and by Parker to the same bone in fishes. It adjoins anteriorly the hind edge of the alisphenoid, as it should; it lies inferior to the anterior end of the squamosal, as it also should; and it is the only primary bone of the skull of Amia that has any direct relation whatever to the spiracular canal, the homologue, according to Wright (No. 35, pp. 479, 488, 492), of the canalis tubo-tympanicus of higher vertebrates, from which the Eustachian tube develops.

The sphenotic nuclei of the sphenoid bone of man are said by Sutton (No. 29, p. 580) to arise, after the appearance of the alisphenoidal and basisphenoidal nuclei, as "earthy spots in the lingulae," and they alone of all the nuclei of the bone are so specifically characterized by him. According to Thane (No. 24, vol. ii, pt. i, p. 76) they form not only the lingulae, but also the adjoining parts of the carotid grooves. The corresponding regions in the skull of Amia and teleosts are occupied by the parasphenoid. My work accordingly leads me to accept Huxley's and Parker's conclusions that "the basitemporal rudiments of the parasphenoid" of Sauropsida are the homologues of the lingulae of man (quoted No. 29, p. 584), rather than Sutton's conclusion that the homologues of the latter bones are found in the sphenotics not only of Sauropsida, but also of fishes (No. 29, p. 585).

The postorbital ossification of Amia and other fishes, although universally called either the postfrontal or the sphenotic, must not be confounded with the postfrontal bone of Reptilia, which is, according to Parker (No. 20, p. 96) and Brooks (No. 8, p. 171), simply a membrane bone, the homologue doubtless of the postfrontal bone of my descriptions of Amia, or of that bone and one or more of the postorbital bones combined. The reptilian postfrontal is said by Bardeleben (No. 4) to be represented in man by the suprasquamosal or epipteric bone.

But one bone now remains to be considered, — the basisphenoid.

The basisphenoidal part of the sphenoid bone of man forms the floor and the anterior and posterior walls of the pituitary fossa. The corresponding parts of the skull of Amia are occupied by that part of the eye-muscle canal that lies immediately below the membranous sac that forms the pituitary fossa. The floor of this part of the canal is formed by a median portion of the dorsal surface of the parasphenoid, and by cartilaginous parts of the primordial cranium that are covered externally by the lateral portions of the parasphenoid. In teleosts the corresponding part of the skull is formed by the petrosals and parasphenoid, the petrosals invading and replacing almost entirely the cartilaginous parts of Amia. The teleostean basisphenoid lies in front of this region, in the place occupied by the presphenoid bone in man.

The parasphenoid of fishes and Amphibia is said by Huxley (No. 14, p. 27) to replace functionally the basisphenoid and presphenoid of higher vertebrates, and to become, in these latter animals, confounded with the basisphenoid. Parker states more definitely (No. 20, p. 138) that "in the bird, the basisphenoid borrows its ossifying center at first from the parasphenoid."

The mammalian basisphenoid bone is thus probably not developed in fishes because of the earlier development, in connection with the dentition of these latter animals, of a large parasphenoid bone which functionally replaces it. Whether the parasphenoid is developed directly from the plates that bear the teeth, by their fusion, as Sagemehl concludes (No. 27, pp. 186, 199), or independently of those plates, from the connective tissues underlying them, as Walther concludes (No. 33, pp. 67, 78), is evidently unimportant in this connection, excepting as the origin of the bone might determine or affect its ultimate more or less complete incorporation in the skull.

If then, in general summary, it be assumed that in some animal presenting in the sphenoidal region of its skull the features characteristic of Amia, the teeth that give origin, either directly or indirectly, to the parasphenoid bone no longer being needed, the bone itself begins to be replaced or absorbed by a primary ossification, the basisphenoid; that

the parasphenoid finally disappears entirely, as a separate ossification, excepting only that part of it that forms the anterior boundary of the internal carotid foramen; that the cartilaginous, anterior clinoid wall of Amia ossifies independently; that the alisphenoids instead of the petrosals invade, on each side of the head, the region between the foramina of the trigeminal and facial nerves; and that the postorbital ossification, as the hind end of the skull is flexed downward, descends onto the dorsal surface of the petrosal and fuses with it as a part of the periotic mass, — an arrangement would arise so markedly similar to that found in the human skull that the several homologies I have here sought to establish seem much more than simply probable. To definitely establish them, and to show whether the apparently interneural position of the several bones in Amia is really of vertebral significance or not, evidently still requires a wide range of careful anatomical and embryological work.

PALAIS CARNOLÈS, MENTON,
March 24, 1897.

BIBLIOGRAPHY.

1. ALLIS, EDWARD PHELPS, JR. The Anatomy and Development of the Lateral Line System in Amia Calva. *Journ. of Morph.* Vol. ii, No. 3. April, 1889.
2. ALLIS, EDWARD PHELPS, JR. The Cranial Muscles and Cranial and First Spinal Nerve in Amia Calva. *Journ. of Morph.* Vol. xii, No. 3. 1896.
3. BARDELEBEN, K. VON. Knochen, Bänder, Muskeln. *Erg. der Anat. und Entwicklungsgeschichte.* Bd. i. 1891, 1892.
4. BARDELEBEN, K. VON. Ueber das Praefrontale und Postfrontale des Menschen. *Anat. Anz. Ergänzungsheft zum xii. Band.* 1896.
5. BAUR, G., and CASE, E. C. On the Morphology of the Skull of the Pelycosauria and the Origin of the Mammals. *Anat. Anz.* Bd. xiii, Nos. 4, 5. Jan. 30, 1897.
6. BEARD, J. The System of Branchial Sense Organs and their Associated Ganglia in Ichthyopsida. A Contribution to the Ancestral History of Vertebrates. *Studies from the Biological Laboratories of The Owens College.* Vol. i. 1886.

7. BRIDGE, T. W. The Cranial Osteology of Amia Calva. *Journ. of Anat. and Phys.* Vol. xi, pt. iv. July, 1887.
8. BROOKS, H. ST. JOHN. The Osteology and Arthrology of the Haddock (Gadus aeglefinus). *Scient. Proc. of the Royal Dublin Soc.* Vol. iv (N. S.), pt. iv. January, 1884.
9. COLE, FRANK J. On the Cranial Nerves of Chimaera monstrosa (Linn.), etc. *Trans. of the Roy. Soc. of Edinburgh.* Vol. xxxviii, pt. iii (No. 19). 1896.
10. EWART, J. C. On the Cranial Nerves of Elasmobranch Fishes. Preliminary Communication. *Proc. of the Roy. Soc.* Vol. xlv, No. 278. March 7, 1889.
11. GAUPP, E. Beiträge zur Morphologie des Schädels. III. Zur vergleichenden Anatomie der Schläfengegend am knöchernen Wirbelthierschädel. *Morph. Arb.* Bd. iv, Heft 1. 1894.
12. GORONOWITSCH, N. Das Gehirn und die Cranialnerven von Acipenser ruthenus. Ein Beitrag zur Morphologie des Wirbelthierkopfes. *Morph. Jahrb.* Bd. xiii. 1888.
13. HUBRECHT, A. A. W. Beitrag zur Kenntniss des Kopfskeletes der Holocephalen. *Nied. Arch. f. Zool.* Bd. iii, Heft 3. May, 1877.
14. HUXLEY, T. H. A Manual of the Anatomy of Vertebrated Animals. New York, 1872.
15. HUXLEY, T. H. On the Structure of the Skull and of the Heart of Menobranchus lateralis. *Proc. Zoöl. Soc.* 1874.
16. JACOBY, MARTIN. Ein Beitrag zur Kenntniss des menschlichen Primordialcraniums. *Arch. f. mikr. Anat.* Bd. xliv, Heft 1. Sept. 15, 1894.
17. KINGSBURY, B. F. On the Brain of Necturus maculatus. *Journ. of Comp. Neur.* Vol. v. December, 1895.
18. MARSHALL, A. MILNES. The Morphology of the Vertebrate Olfactory Organ. *Journ. of Micro. Sci.* Vol. xix (N. S.). July, 1879.
19. MCMURRICH, J. PLAYFAIR. The Osteology of Amiurus catus (L.) Gill. *Proc. of the Canad. Inst.* Vol. ii, fasc. No. 3. Toronto, October, 1884.
20. PARKER, W. K. On the Structure and Development of the Skull in the Salmon (Salmo salar, L.). *The Bakerian Lecture*, May 30, 1872. *Phil. Trans.*, 1873.
21. PARKER, W. K. On the Development of the Skull in Lepidosteus osseus. *Phil. Trans.* 1882.
22. PARKER, W. K. On the Structure and Development of the Skull in Sturgeons (Acipenser ruthenus and A. sturio). *Phil. Trans.* 1882.
23. POLLARD, H. B. On the Anatomy and Phylogenetic Position of Polypterus. *Zool. Jahrb., Abt. f. Anat. und Ontog.* Bd. v, Hefte 3, 4. Oct. 24, 1892.
24. QUAIN'S Elements of Anatomy. Edited by E. A. Schäfer and G. D. Thane. London, 1892–1896.

25. SAGEMEHL, M. Beiträge zur vergleichenden Anatomie der Fische. I. Das Cranium von Amia calva (L.). *Morph. Jahrb.* Bd. ix, Heft 2. 1883.

26. SAGEMEHL, M. Beiträge zur vergleichenden Anatomie der Fische. II. Einige Bemerkungen über die Gehirnhäute der Knochenfische. *Morph. Jahrb.* Bd. ix, Heft 4. 1884.

27. SAGEMEHL, M. Beiträge zur vergleichenden Anatomie der Fische. III. Das Cranium der Characiniden nebst allgemeinen Bemerkungen über die mit einem Weber'schen Apparat versehenen Physostomenfamilien. *Morph. Jahrb.* Bd. x, Heft 1. 1884.

28. SAGEMEHL, M. Beiträge zur vergleichenden Anatomie der Fische. IV. Das Cranium der Cyprinoiden. *Morph. Jahrb.* Bd. xvii, Heft 4. 1891.

29. SUTTON, J. B. On the Development and Morphology of the Human Sphenoid Bone. *Proc. of the Zoöl. Soc.* London, 1885.

30. STUDNIČKA, F. K. Ueber die Histologie und Histogenese des Knorpels der Cyclostomen. *Arch. f. mikr. Anat.* Bd. xlviii, Heft 4. Jan. 28, 1897.

31. TRAQUAIR, RAMSAY H. On the Cranial Osteology of Polypterus. *Journ. of Anat. und Phys.* Second Series, No. 7. November, 1870.

32. VROLIK, A. J. Studien über die Verknöcherung und die Knochen des Schädels der Teleostei. *Nied. Arch. f. Zool.* Bd. i, Heft 3. June, 1873.

33. WALTHER, JOHANNES. Die Entwicklung der Deckknochen am Kopfskelett des Hechtes (Esox lucius). *Jen. Zeit. f. wiss. Zool.* Bd. xvi.

34. WRIGHT, R. RAMSAY. On the Nervous System and Sense Organs of Amiurus. *Proc. of the Canad. Inst.* Vol. ii, fasc. No. 3. Toronto, October, 1884.

35. WRIGHT, R. RAMSAY. On the Hyomandibular Clefts and Pseudobranchs of Lepidosteus and Amia. *Journ. of Anat. and Phys.* Vol. xix. July, 1885.

RECENT EXPERIMENTS ON REGENERATION.

CHARLES W. HARGITT.

DURING the summer of 1896 at the Marine Biological Laboratory the writer, while engaged upon the life history of some of the Hydrozoa, took occasion to repeat certain of the experiments of Loeb,[1] Bickford,[2] and others upon regeneration and heteromorphosis among hydroids, and to extend them to several other forms. In the following paper it is proposed to submit a synopsis of the several experiments made, with such results as have sufficient definiteness to justify record.

The work upon hydroids was restricted for the most part to the genera Eudendrium, Pennaria, and Clava ; though some of the experiments of the investigators named upon Tubularia, Margelis, etc., were repeated, and with results quite as they had reported. The work upon Eudendrium was quite as demonstrative as either of the former, and that notwithstanding the fact of its indisposition to take kindly to the artificial conditions of the aquarium and its rather complex character. Not only does Eudendrium readily regenerate excised parts, such as stalk, hydranth, root, etc., but it exhibits equally marked heteromorphism. Experiments upon the other genera mentioned were of the same general nature and results. Those upon Clava were somewhat limited, owing to the very limited supply obtainable during the present summer. No particular attention was devoted toward ascertaining immediate causes by which to account for the phenomena. That external conditions have much to do with certain aspects of it I have no doubt but that they are the chief, or primary, conditions seems at least an open question. Specimens placed under identically similar conditions do not respond with equal promptness nor with similar results. Many facts would seem to indicate the operation of intrinsic factors. But of this it is

[1] Biological Lectures, 1893, p. 37.

[2] *Journ. of Morph.*, vol. ix. p. 417.

not the purpose here to inquire in detail. Further investigations, some of which are under way, will be necessary to afford sufficient data for any adequate judgment.

The particular class of experiments to which I purpose to direct attention at this time pertains to another group of organisms, and one upon which, so far as I am aware, nothing directly has been done, though incidentally it has been referred to in connection with another subject, and will be duly noted in another part of the paper. The organisms referred to are the medusae. In connection with the previous work already noted, the thought of extending these experiments to the medusae occurred more than once, but owing to their peculiar delicacy and highly specialized character, was dismissed as of doubtful practicability. The presence, however, of considerable numbers of Gonionemus vertens, a preliminary report upon which was made during the previous year by Dr. Murbach, and the capacity of which to endure confinement in small aquaria was rather marked, revived the previous conception, and after reflection it was determined upon with some hesitation.

Accordingly a small number of these medusae were obtained and placed in small table aquaria, and upon them a series of extremely simple experiments made, such as the excision of a few tentacles, notching the margins, etc., more from a spirit of curiosity than of expectation. When, therefore, upon the following day I noted that the mutilations were healed in several cases and that there was an evident tendency toward a restoration of excised parts, interest was only exceeded by surprise. However, I found, upon a brief review of the work of Romanes[1] on the *Nervous System of Jellyfish*, etc., that he had called attention to the fact of the capacity of these organisms to regenerate mutilated tissues, but no work was done in connection with the observation. Later Eimer also made certain experiments upon jellyfishes of a similar sort, but without recording any tendency toward active physical regeneration, though demonstrating the recovery of nervous activity.[2] I may incidentally note the fact that in none of my

[1] Jellyfish, Starfish, and Sea Urchins, p. 103.

[2] Organic Evolution, pp. 345 *et seq.*

experiments did I observe that apparent paralysis resulting from the excision of the marginal nervous system which he found so striking. However, the fact that his work was done chiefly upon Scyphomedusae, while Gonionemus apparently belongs to the Hydromedusae, may sufficiently account for the difference. While in my experiments there seemed to be a sort of shock induced by extensive mutilations, it was, however, in no case of any considerable duration or effect upon the specimens.

With these simple results as an index of possibilities a considerable number of medusae were secured and a series of systematic excisions and mutilations was begun. The following synopsis will serve to indicate the scope and character of the experiments, though in most respects the record must be regarded as of the nature of a preliminary report, since the histological investigations to determine the deeper results upon the tissues are not yet sufficiently complete to be presented, and, moreover, some of the primary experiments need verification under other conditions than were then possible, or than the time at my disposal after they were undertaken was sufficient to complete. In view of the interest and significance of the work in its relations to current discussions, it has seemed advisable to give publicity to the facts thus far established, reserving to a later time further details upon the subject which may come from more extensive and critical experimentation.

Gonionemus vertens is a medusa of very interesting habits, and of a form and size well adapted to the work undertaken. It may not be amiss in this connection to record the fact that, so far as published records of its distribution are known, it had not, prior to the summer of 1895, been known on the Atlantic coast. I am informed by Prof. C. C. Nutting that during the summer of 1896 he found evidence at Newport of its occurrence there, but at what time I could not learn.

In Fig. 1 is shown in a somewhat diagrammatic form the more characteristic features of the medusa. In size it varies considerably, owing probably more to age than any other cause, averaging from one to two centimeters in marginal diameter.

Not pausing to notice the simpler experiments already referred to, such as the slight incisions, or notching of the margins, excision of tentacles, etc., the first experiments consisted in excising portions of the margin of the umbrella, as indicated in Fig. 2. These were repeated upon about twenty specimens, removing portions of the body between the radial canals and portions including them. The regeneration of such portions was usually quite prompt, varying from two to four days, depending chiefly and naturally upon the size of the portion to be restored. In every case the regeneration seemed to be perfect, including radial canals, velum, and tentacles.

In a few specimens all the tentacles were removed by plucking them singly from their very roots with fine forceps. In

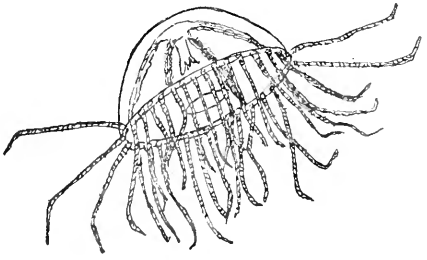


FIG. 1.

FIG. 2.

these cases regeneration followed in about the same time as in the others, but the tentacles were more slender and delicate than the ordinary ones.

In the next series the manubrium was excised close up to the stomach cavity, and indeed in some cases including the entire thickness of the aboral body wall. The results were as before, the manubrium being regenerated with promptness and completeness and in about the same time, depending as before upon the amount of matter to be restored. It may be noted in this connection that the excised manubria themselves continued to live, even for days, and to move about by a slow, creeping sort of motion, but did not show any appreciable tendency toward regeneration. The same was true of small particles from almost any part of the body, such as tentacles, bits of margins, etc. No special attention was given to determining from how small a portion of the animals regeneration

might be secured, but from the general tenor of the experiments I should doubt whether anything less than a full quarter of the body would reproduce a new individual. This, however, is merely inference based on the general facts observed. Specific experiments to be undertaken later will possibly show very different results.

In the third series the experiments consisted in vertical sections through the median portion of the body, dividing it as nearly as possible into halves, as indicated in Fig. 3, and with the result that each half became an independent and perfect medusa. In this case the restoration was somewhat peculiar. It would seem to be a sort of *recovery* of form and function rather than regeneration in the usual sense of that term. After the first brief shock of the operation, which in many cases was scarcely noticeable, was overcome, there was an evident effort of the half-medusa to assume the normal form by a contraction of the body so as to bring the cut edges of the bell together, the approximations of the edges taking place from above downward, or peripherally, and with the subsequent union or healing of the approximated margins. Complete restoration, resulting in the assumption of the original form, occurred in from three to five days. The new medusae were in most respects quite similar in form and action to the original, though of course of only about half the size. The time at my disposal was insufficient to observe whether there was subsequent growth of the specimens. In the recovery of the specimens I was not able, moreover, to observe any disposition to regenerate the additional radial canals necessary to complete the symmetry of the original. This, however, does not seem to be an important matter, since there does not seem to be a special necessity for a definite number. And in this connection it may be worth while to note a very considerable variation in normal forms as to the number of radial canals.

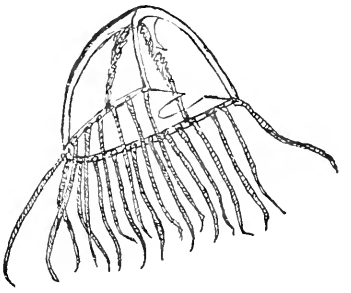


FIG. 3.

In Gonionemus, as in most Hydromedusae, the number is four. But in the specimens studied in this connection it was not rare to find three or five or six. This is the more remarkable in that Bateson[1] has been able to record but rare instances of such variation among Hydromedusae, instancing those noted as among the most striking illustrations of the "discontinuity of meristic variation."

A fourth series consisted in sections of the medusa in a horizontal plane, as indicated in Fig. 4. As will be perceived, this is clearly the most crucial of the entire series, since the animal is divided into such parts that if regeneration occur at

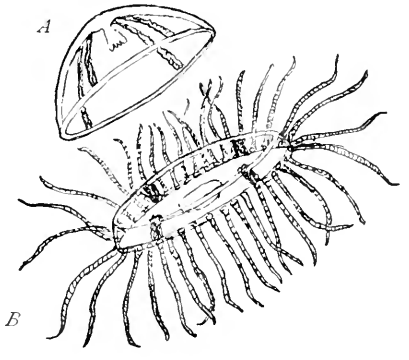


FIG. 4

FIG. 5.

all it must be *de novo*, from tissues devoid of any direct identity of form or function. But crucial as is the test, it was none the less successful. In Fig. 4, *A* and *B* represent the central and marginal portions of the divided medusa. In Fig. 5, *A* and *B* represent the corresponding portions after regeneration had occurred. *A* in this figure needs no special explanation. That which is first regenerated in this portion is the marginal canal. Next following this is the regeneration of the tentacles. As will be noticed from the figure, they are but rudimentary or bud-like. The unprecedented hot weather which occurred just at this time made extremely difficult the preservation of specimens in a healthy condition under the artificial conditions of

[1] Bateson: Material for Study of Variation, pp. 424 *et seq.*

the aquarium, and many perished before regeneration of these organs had gone forward to any considerable extent. Of its reality, however, there is not the least doubt. The rate of regeneration in these cases was much slower than in the former, as would be naturally expected, in most cases requiring a fortnight to afford conclusive indications of the new organs.

Most marked in many respects is the condition indicated in *B* of Fig. 5, the resultant of *B*, Fig. 4. That is, the excised margin had become a new medusa. But in this case, as in the vertical sections, the process appeared as more a restoration of form through a marked and continued contraction of the marginal walls, and a final union of their upper margins to form the dome of the new medusa. Again there seems to have been no evidence of growth in substance, which would in this case have been impossible, since the absence of mouth or gastric cavity would render the taking of food entirely out of question, and there would also be a draft upon any reserve energy in the tissues in the mere maintenance of life and the usual waste incident to existence. And this fact of itself makes the results more interesting ; namely, that in a minute fragment of the nature of the one under consideration there seems to be an intrinsic potency to recast itself into the morphological equivalent of the original ; and that this disposition was manifest in even the minutest portion from any part of the body. So marked, indeed, was this tendency that it almost seemed as if there were present some occult organic crystallization prepotency, if such a phrase or comparison be tolerable.

Such in brief is an outline of the facts concerned in the experiments. Their significance in relation to others of like character and to problems of current biological importance will be more or less apparent without special emphasis. It may not be amiss, however, to refer briefly to some theoretical considerations upon which they would seem to have special bearing.

Concerning the problem of the more primitive character of hydroid or medusa no additional light is afforded. If the more highly developed and specialized nature of the medusa is

appealed to as evidence of its derivation from the hydroid type, the capacity of the medusa for regeneration and the tenacity with which it maintains the medusan features would seem to point with equal force in the opposite direction. While this alone is not sufficient to settle a problem involving so many factors, it none the less suggests the propriety of caution and the necessity for further evidence than has yet been adduced before any dogmatic or final statement is made.

Concerning heteromorphosis the experiments were entirely negative. A definite polarity seemed evident in every part of the organism. It mattered not how small the fragment, or from what source, or however varied the conditions under which placed, the results uniformly indicated a definite intrinsic orientation.

If this might seem to imply a more fixed and constant heredity and therefore to point toward a more primitive condition than is usual in the hydroid, it is sufficient to call attention to the no less fixed and inflexible polarity of Hydra. It would seem, therefore, that on these points the facts are to be taken simply for what they are, and not as affording any definite basis upon which to speculate.

SYRACUSE UNIVERSITY, SYRACUSE, N. Y.
October, 1896.

THE METAMERISM OF NEPHELIS.

A Contribution to the Morphology of the Nervous System, together with a Description of Nephelis Lateralis.

CHARLES LAWRENCE BRISTOL.

Nephelis differs from most of the leeches in the absence of well-marked sense organs on the first ring of each metamere, and hence the means usually efficient for determining metamerism in other leeches is wanting. Professor Whitman had already worked out the distribution of the nerves in Clepsine and found a complete agreement between this and the sensillae (sense organs). It was proposed to apply the same method to Nephelis, therefore, in which form the sensillae were not so apparent.

A large number of individuals were collected from widely different localities, from Wood's Holl, Mass., to Wolf Lake, near Chicago. Color markings proved useless as criteria for determining species, and the method of counting the rings and noting the variations of size was used. A careful study of all the individuals resulted in placing all the forms in one species, — Nephelis lateralis. This name was chosen because of its priority. The generic name Nephelis was retained also for its priority, though Dr. Blanchard, in France, has brought the name Erpobdella into his descriptions.

Description. The size of the adult varies from 4 cm. to 10 cm. at rest. Anterior to the sexual organs the body tapers slightly to the mouth ; posterior to them it continues about the same size until just in front of the anus, whence it tapers to the sucker. A transection of the body is lenticular, though in the clitellar region it approximates a circle. The body flattens in swimming, as in Hirudo and Macrobdella. The color varies from a light chocolate brown free from pigment to almost a coal black free from any light areas. The total number of annuli is 106 to the acetabulum. The oral

sucker is not prominent, and the anal sucker is small. The male orifice lies normally between annuli 36 and 37. The female orifice lies normally between annuli 38 and 39.

The first pair of nephridiopores lies between annuli 16 and 17, at the posterior edge of the seventh (VII) metamere. There are four pairs of nephridia anterior to the male orifice, while posterior to them are 13 pairs of pores, the last pair lying between annuli 96 and 97. The nephridiopores are separated by five annuli. The anus is dorsal and lies behind the 104th annulus. The clitellum consists of 15 annuli — from 28 to 42 inclusive. It includes the last four rings of metamere X and the first of metamere XII.

The whole number of metameres is 34. In the body region they consist of five annuli each, as is shown externally by the nephridiopores. In the terminal regions these are more or less reduced and the limits were determined by the innervation as follows :

I consists of the prostomium ; II of annuli 2 and 3 ; III of a single ring, 4; IV of a single ring, 5; V of 6, 7, 8; VI of 9, 10, 11. Metameres VII to XXIV, inclusive, are normal, composed of five rings each. XXV consists of annuli 102, 103, and the anterior half of 104 ; XXVI, of the posterior half of 104 and 105. XXVII consists of 106, the last annulus and the acetabulum, the top part of the sucker. XXVIII to XXXIV consist of the sucker disc.

The number of eyes is six ; and they are arranged in pairs: the first pair lies in the second annulus and looks forward ; the second pair lies wholly in the fourth and looks outward and backward; the third pair lies partly in the fourth and partly in the fifth and looks outward and backward.

Habitat. Nephelis is found in fresh-water brooks and ponds wherever food is plentiful. They are not parasitic, but feed on the débris of animals as does Aulastoma. They are usually found partly buried in mud or sand or on the under side of submerged objects.

Nervous System. The nervous system consists of three well-marked divisions: the central, the sympathetic, and the intermuscular systems.

The *central system* lies in the ventral blood sinus underneath the alimentary canal. It consists of a "brain," or supraoesophageal part, together with a fused mass of ganglia, the suboesophageal ganglia, a chain of 18 ganglia (neuromeres), and a fused mass of ganglia, the anal ganglia. The neuromeres are joined together by paired connectives, and between the connectives lies a small bundle of nerve fibers known as the median nerve, or Faivre's nerve. Within each pair of connectives lie two "colossal" cells.

The general shape of a body neuromere is a flattened ellipsoid with a slight axial groove. The bodies of the neurons lie in six capsules, arranged about the body of the ganglion in pairs as follows: one pair on the ventral side and one pair on each lateral face. Within the ganglion are two "colossal" cells similar to those in the connectives. Two pairs of nerves arise from the ganglion and divide so as to innervate the somite, as will be described. The anterior pair arises from two roots which quickly fuse, and hence this pair is the homologue of the first two pairs of the three pairs of nerves in Clepsine. Just outside of the ganglion, between the two pairs of nerves, lie two "colossal" cells, one on each side, the prolongations of which fuse into the nerve trunks. These have been called "Leydig's cells."

Every factor that enters into a body neuromere is found in every neuromere in the central system.

The anterior nerve innervates the fourth and fifth annuli of the preceding metamere and part of the first annulus of its own metamere. The posterior nerve innervates the few dorsal sensillae of the preceding fourth and fifth annuli, the principal dorsal sensillae of the first annulus, and the lesser dorsal sensillae in the second and third annuli. Comparison with Whitman's description of Clepsine shows this distribution to be identical with that found by him.

Applying the above plan to the terminal fused parts of the nerve chain, — the "brain" and suboesophageal ganglia in the head region and the anal ganglia in the posterior end of the body, — we have been able to analyse them completely.

The anal ganglia comprise ten neuromeres; the three anterior neuromeres differing but slightly from normal body neuromeres, while the succeeding seven are modified by condensation. These latter represent the seven neuromeres in the anal ganglia of Clepsine, while the former mark the extent to which fusion has gone on in Nephelis beyond that in Clepsine. In the former the nerves emerge in two pairs with the "Leydig's cells" between them; in the latter they emerge fused into a single trunk with the "Leydig's cell" lying alongside. The distribution shows the limits of the metameres as given above.

In the anterior end of the nerve chain, the "brain" and suboesophageal ganglia, though much more modified, yield to the plan of the body neuromere, and again the distribution readily defines the limits of the metameres. The suboesophageal ganglia consist of five neuromeres. II lies on the side of the collar, while III to VI are closely fused together, yet each neuromere of this region contains every factor that goes to make up a body neuromere.

The nerves of the last neuromere, VI, emerge as in a body neuromere, the others as single trunks. In neuromere VI the "Leydig's cell" lies between the nerves; in V it lies in the angle of the first branching; in the others it lies alongside the trunks.

The "brain" or neuromere I lies above the oesophagus. In it, as in the other neuromeres, is found every unit that is found in a body neuromere. The "brain," therefore, does not differ morphologically from any other neuromere. The distribution in this fused region shows on analysis the same morphological arrangement as in the body metamere, and the division of this region into metameres as given above was derived from this source.

The Intermuscular Nerve Ring. In the second and fifth annuli of each body metamere, between the layers of the longitudinal muscles and the circular muscles, are found two rings of nerve cells and fibers. Each ring receives fibers from the central system and from sensillae on the surface, and gives rise to fibers that go to the central system and to the muscles. At definite and constant points about the ring are ten groups of

bipolar cells, and these cells resemble the "Leydig's cells" above mentioned and the "colossal" cells of the connectives. These intermuscular nerve rings are joined longitudinally by ten lines of bipolar cells, a single cell in each line joining two consecutive groups of bipolar cells. The cell body of these cells lies about midway between any two rings. These connective bipolar cells are also strictly comparable to the "Leydig's cells." This system is complete in that a ring may receive stimuli, send forth motor stimuli, and send impulses to other parts of the same system. In its application to the origin of a metamere composed of five annuli it confirms Whitman's proposition that the five-ringed metamere arose from the three-ringed metamere by the doubling of the second and third Clepsine rings. The third annulus in Nephelis is the posterior half of (Clepsine) two, and four in Nephelis is the anterior half of (Clepsine) three.

The Sympathetic System. This is well developed over the wall of the entire alimentary tract. It is connected with the central system at the collar. It passes off from the collar in three pairs of branches, a dorsal, a lateral, and a ventral, which soon fuse and form an intricate meshwork with the fibers from the many multipolar cells on the muscular wall. There is some evidence that the sympathetic system persists in the post-anal region as a remnant of the system that functioned when the anus was terminal.

NEW YORK UNIVERSITY,
February 1, 1897.

REMARKS ON THE QUESTION OF INTERCALATION OF VERTEBRAE.

G. BAUR.

PROFESSOR H. C. BUMPUS ('97) has published in the last number of the *Journal of Morphology*, vol. ii, No. 2, February, 1897, a paper on the skeletal variations of *Necturus maculosus* Raf.[1] Radiographs of one hundred alcoholic specimens were prepared, and the variations of the number of vertebrae with reference to the position of the sacrum were determined. G. H. Parker ('96, pp. 711–717) had already examined 27 specimens and found the following variations :

The pelvis is attached to the 19th vertebra in 19, to the 20th vertebra in 6 specimens. In one case the 19th vertebra had a well-developed sacral rib on the left side, the right sacral rib being on the 18th vertebra. In another specimen the sacral ribs were on the 20th on the left and on the 21st vertebra on the right side. Among these 27 specimens were found, therefore, two with asymmetrical sacral vertebrae, to which the pelvis was attached obliquely.

Bumpus' results are the following:

Number of Specimens.	Number of Presacral Vertebrae.	Sacral Vertebra. Ribs attached at the Left.	Right.
64	18	19	
28	19	20	
7	18	19	20
1	18	20	19

1 "*Maculosus*" is the correct name given by Rafinesque in the *American Monthly Magazine* and *Critical Review*, vol. iv, No. 1, New York, November, 1818, p. 41 (*Sirena maculosa*). In 1819 he called it *Necturus maculatus*, *Journ. de Physique*, vol. 88, 1819, p. 418, and in 1820 *Necturus maculosus*, Annals of Nature, or Annual Synopsis of New Genera and Species of Animals, Plants . . . Discovered in North America by C. S. Rafinesque. First annual number, for 1820. Transylvania University, March 1, 1820, Lexington, Ky.

In 127 specimens the ilium was therefore attached either to the 19th vertebra 83 times, or to the 20th 34 times, or to the 19th and the 20th obliquely 8 times, or to the 20th and 21st obliquely once.

The reason of this variation is this: The ilium is attached very loosely to the sacral ribs by ligament, as in *Proteus* and *Amphiuma*. There are no distinctly modified sacral vertebrae, and hence it is not surprising at all that the ilium is not attached always to the same vertebra. The sacral region has not the power of developing sacral ribs at several points on both right and left sides, but the pelvis may attach itself — for it becomes secondarily united with the vertebral column — to any of the vertebrae of the pelvic region. In *Necturus* we have all the variations possible for the attachment of two consecutive vertebrae, the 19th and 20th; only in one case the ilium reaches the 21st vertebra, and in this case the pelvis is oblique.

The Amphibia are descended from fishes in which the pelvis is never in connection with the vertebral column. In *Necturus* and *Proteus* the ilium is very loosely connected with the distal processes of the sacral ribs. *Necturus* and *Proteus* belong to the *Proteida*, the only group of living Amphibia with a free paroccipital [opisthotic]. The attachment of the ilium to a definite vertebra has not yet become constant. If one hundred specimens of a lizard, of a crocodile, or of a turtle should be examined, exceedingly few variations would be found. The great variability in the attachment of the pelvis to the vertebral column in the tailed Amphibia is a result of the loose connection between both. Very many more cases of asymmetrical sacra have been described in the tailed Amphibia, than stated by Parker, who knows only that of Lucas ('86, p. 561) in *Cryptobranchus* (*Menopoma*). They were described as early as 1818 in *Triturus* (*Molge*) *cristatus* Laur. by C. A. S. Schultze ('18, p. 379), and in 1825 by Cuvier ('25, p. 414); in *Triturus vulgaris* L. and *Salamandra salamandra* L. (*maculosa* Laur.) by Claus in 1876 ('76, p. 23); in *Megalobatrachus maximus* Schlegel by Schmidt, Goddart, and Van der Höven in 1862 ('62), by Claus in 1876 ('76, p. 29); in *Cryptobranchus* (*Menopoma*)

alleghaniensis Daudin, by Mayer in 1835 ('**35**, p. 78), Hyrtl in 1864 ('**64**, pp. 264–272), and Claus in 1876 ('**76**, p. 29).

Nearly all these cases have been recorded by Claus in 1876 in an excellent paper: "Verschiebungen des Darmbeins und der Sacralregion der Wirbelsäule von Amphibien." This very important paper has been overlooked by both Parker and Bumpus. Claus says: "Unter Ausschluss des ersten als Halswirbel zu bezeichnenden Wirbels und des letzten oder Sacralwirbels repräsentirt die Wirbelsäule des Rumpfes [bei den geschwänzten Amphibien] eine gleichmässig gestaltete Dorsolumbalregion von mächtiger Ausdehnung, *deren hintere Grenze aber bei der Verschiebung und Lageveränderung des zum Os ileum bezogenen Sacralwirbels nach der Caudalregion hin keineswegs unveränderlich, vielmehr mannigfachen Schwankungen ausgesetzt ist.* Für diese thatsächlich stattfindende Bewegung des Darmbeines in der hinteren Grenzgegend des Rumpfes glaube ich eine Reihe unzweideutiger Beweise vorlegen zu können. Anstoss zu den mitzutheilenden Beobachtungen gab mir der Vergleich von zwei in der hiesigen [Wiener] Sammlung aufgestellten Menopomaskeleten, von denen das eine die von Hyrtl beschriebene asymmetrische Gestaltung des vorderen Caudalwirbels zeigt. Diese und ähnliche für mehrere Eidechsen nachgewiesenen Asymmetrien, diez war unter dem Begriff der 'Assimilation' subsummirt, damit aber in ihrer Bedeutung noch keineswegs verstanden waren, legten mir den Gedanken nahe, dass es sich bei diesen Bildungen nicht etwa um *abnorme Missgestaltungen*, sondern um *allmälige Verschiebungen des Os ileum handelt*, welche ein Vorwärtsrücken des Kreuzbeins vorbereiten; und mit einer regelrechten Lageveränderung desselben als Uebergangsstufen in Verbindung zu bringen sein möchten."

We see that Claus had fully recognized the true nature of these conditions. The following is a table compiled from different authors; many of these cases have been recorded by Claus.

All the variations in the position of the sacral vertebra given in this list are produced by the shifting of the pelvis.[1]

[1] The same conclusion was reached by Jhering in his study of the spinal nerves, Ueber das peripherische Nervensystem der Wirbelthiere, Leipzig, 1878.

Variations in the Presacral and Sacral Vertebrae in the Caudata.[1]

Genera and Species.	Number of Specimens.	No. of Presacral Vertebrae.	Sacral Vertebra. No. Sacral Ribs attached at the Right.	Left	Author.	Date.
Triturus cristatus Laur.	1	15	16	17	Schultze	1818
Triturus Rafinesque, 1819, for *Triton* Laurenti, 1768, preoccupied by	3	16	17		Cuvier	1825
Triton Linné.	3	16	17		Wiedersheim ('75, p. 116)	1875
Molge Merrem, 1820.	3	16	17		Claus	1876
	1	16	17	18	Cuvier	1825
	1	17	18		Cuvier	1825
	1	18	19		Claus	1876
Triturus vulgaris Linn. (taeniatus Schneid.)	1	13	15	14	Claus	1876
	4	15	16		Wiedersheim	1875
	4	15	16		Claus	1876
Triturus palmatus Schneid.	4	13	14		Wiedersheim	1875
(*helveticus* Leydig)	4	13	14		Claus	1876
Salamandra salamandra Linn.	2	14	15		Claus	1876
(*S. maculosa* Laurenti, 1768)	9	15	16		Cope ('89, pl. XXXVIII)	1889
	9	15	16		Claus	1876
	2	15	16	16 17	Claus	1876
	1	15	16	17	Claus	1876
	1	16	17		Claus	1876
Salamandra atra Laur.	4	15	16		Claus	1876
	4	15	16		Cuvier	1825
	?	16	17		Cuvier	1825

Diemyctylus torosus Eschsch. (Taricha torosa)	3	13	14		Claus	1876
Pleurodeles waltlii Michah.	5	15	16		Claus	1876
Amblystoma punctatum Linn. (Salamandroides venenosus, Daud. Amblystoma argus, Dum. et Bibr.)	4 (3)	15	16		Claus	1876
	4 (1)	15	16		Cope ('89, pl. XIV and XV)	1889
Chondrotus tenebrosus Baird and Gir.	1	15	16		Cope ('89, pl. XXII and XXIII)	1889
Amblystoma tigrinum Green (Siredon)	3	16	17		Claus	1876
Spelerpes ruber Daud. (*Bolitoglossa rubra*, Dum. et Bibr.)	1	18	19		Claus	1876
Spelerpes porphyriticus Green. (*Spelerpes salmoneus*, Holbr.)	1	19	20		Claus	1876
Desmognathus niger Green. (*Amblystoma nigrum*, Dum. et Bibr.)	1	16	17		Claus	1876
Geotriton fuscus Bonap.	1	15	16		Wiedersheim	1875
Salamandrina perspicillata Savi.	1	14	15		Wiedersheim	1875
Nezturus maculosus Raf.	2	18	19		Claus	1876
	1	18	19		Hoffmann ('73, p. 52)	1873
	1	19	20		Cope ('89, pl. I)	1889
	1	19	21	20	Mayer ('35, p. 78)	1835
Cryptobranchus alleghaniensis Daud.	4 (3)	19	20		Claus	1876
	4 (1)	19	20		Cope ('89, pl. V and VI)	1889
	2 (1)	19	20	21	Hyrtl	1864
	2 (1)	19	20	21	Claus	1876
	1	19	20 21	20 21	Huxley ('78, p. 752)	1878
	1	19 1	20 21	21	Lucas ('86, p. 561)	1886
	1	20	21		Claus	1876

[1] *Caudati* (Urodèles), Duméril, 1806; *Ecaudati* (Anoures), Duméril, 1806. (Duméril, A.M., Constant. Zoologie Analytique, ou Méthode Naturelle de Classification des Animaux. Paris, MDCCCVI, pp. 90, 91, 94, 95.)

VARIATIONS IN THE PRESACRAL AND SACRAL VERTEBRAE IN THE CAUDATA. — *Continued.*

GENERA AND SPECIES.	NUMBER OF SPECIMENS.	NO. OF PRESACRAL VERTEBRAE.	SACRAL VERTEBRA. NO. SACRAL RIBS ATTACHED AT THE		AUTHOR.	DATE.
			RIGHT.	LEFT.		
Andrias scheuchzeri Holl	1	20	21		von Meyer ('45, pl. X)	1845
Andrias tschudii v. Meyer	1	21	22		von Meyer ('60, pl. IX, fig. 1)	1860
Megalobatrachus maximus Schlegel	1	19	20		Claus	1876
	1	20	21		Claus	1876
	1	20	21		Baur	
	1	20	21		Claus	1876
	1	20	united with first caudal		Schmidt, Goddart, und van der Höven ('62)	1862
	2	1 20	22	21		
		1 20	22	21	Claus	1876
	1	21	22		Schlegel ('38)	1838
	1	21	22		Hyrtl ('65, p. 43)	1865
Proteus anguineus Laur.	1	29	30		Hyrtl ('65, p. 43)	1865
	1	29	30		Hoffmann	1873
	1	30	31		Cuvier ('45, p. 429)	1825
Amphiuma tridactyla Cuv.	1	62	63		Hyrtl	1865
Amphiuma means Garden	1	62	63		Cope ('89, pl. X)	1889

The most striking case of increase of presacral vertebrae during the ontogeny of one species is shown by *Branchiosaurus amblystomus* Cred., a Stegocephalian from the Permian of Germany. The number of the presacral vertebrae is smaller in the younger specimens, and gradually increases until the adult stage is reached. Credner ('86, p. 620) gives the following table:

Length of Vertebral Column from Posterior Border of Skull to the Anterior End of the Sacral Vertebra.	Number of Presacral Vertebrae.
19	20
23	20
25	20
26	20
27	20
28	21
30	21
32	21
33	21
37	22
39	23 or 24
43	25 or 26
48	25 or 26
50	26
52	26
54	26 or 27
56	26 or 27

We have here the highest number of sacral variations that has been observed.

Having considered the Amphibia, we may now pass over to the Reptilia. Hyrtl ('64, pp. 264–272) has described in 1864 asymmetrical sacra in the following lizards: *Lophura amboinensis* Schlosser, *Amphibolurus* (*Grammatophora*) *barbatus* Cuv., and *Tupinambis* (*Ctenodon*) *nigropunctatus* Spix. In the living Crocodilia the number of the presacral vertebrae is 24, which are all procoelous or concave-convex. There are two sacrals: the first one is concave-plane, the second plane-convex, and the first caudal is biconvex; all the following caudals are like the cervicals, procoelous.

Among eleven skeletons Reinhardt ('73, pp. 221–228) found three with three sacral vertebrae.

1. *Caiman sclerops* Schneid. The last lumbar is transformed into a sacral. There are only 23 presacral vertebrae.

2. *Crocodilus americanus* Laur. (*acutus* Cuv.). There are three sacrals; the first two are the normal; the first is concave-plane, the second plane-plane, and the third plane-convex; all the following are procoelous, and the first bears a chevron. The first caudal has been taken up by the sacrum. There are, however, only 23 presacral vertebrae.

3. *Crocodilus americanus* Laur. Three sacrals. The first caudal is transformed into a sacral vertebra, and there are 24 presacral vertebrae.

4. Another case with three sacral vertebrae has been described by Case ('96, pp. 231–233). There are 24 presacral vertebrae. The 25th has the sacral ribs inclined backwards and becoming slender. The 26th has strong thick ribs; and the 27th has also well-developed ribs articulating with the ilium. This vertebra is seemingly biconvex. The first chevron is attached between the 28th and 29th. The first caudal has been taken up into the sacrum. Both sides are symmetrical.

5. I have described a case in *Alligator mississippiensis* Daud. The last lumbar possesses small sacral ribs, which, however, do not reach the ilium. In front of this there are 23 presacral vertebrae ('86, p. 690).

6. *Crocodilus americanus* Laur (*acutus* Cuv.), No. 129, B. Museum, Cambridge, England. The 25th vertebra has a strong presacral rib on the right side; it is much weaker on the left, and united with the center. The 26th shows typical sacral ribs; the 27th bears on the left side a strong free sacral rib, on the right side a weaker rib but free. The ilium is supported on the left principally by the 25th and 26th, at the right by the 26th and 27th vertebrae. The 28th is biconvex. Here we have an asymmetrical sacrum. This case was published by me in 1889 ('89, p. 240).

7 and 8. In two specimens of *Crocodilus porosus* Schneid. (*biporcatus* Cuv.) in the Reichsmuseum of Leyden there are only 23 presacral vertebrae ('89, pp. 240, 241).

In all these cases we have shifting of the pelvis.

Professor Bumpus does not believe in intercalation, but there are true cases of intercalation, as I shall show. The Gavial case has been accepted by Parker, but not by Bumpus. The original description was published in 1886 ('86, p. 689, also '91, p. 334). "Bei einem Exemplar von *Gavialis gangeticus* finde ich 25 praesacrale Wirbel; zwischen dem 9. und 10. Wirbel ist ein solcher eingeschaltet, wie aus der Configuration der Dia- und Parapophysen genau bestimmt werden kann." It is a well-known fact that in all living Crocodilia with normal vertebral column there are 24 presacral vertebrae, two sacrals, and many caudals. All the presacral vertebrae are concave-convex; the first sacral is concave in front, plane behind; the second plane in front, concave behind; the first caudal is biconvex. In the Gavial with 24 presacrals and the Gavial with 25 presacrals, the two sacrals and the first caudal are absolutely identical in structure. In the first case the first caudal is the 27th, in the second the 28th. Therefore one vertebra must have been intercalated between two of the 24 presacral vertebrae. I determined these vertebrae as the 9th and 10th. Here are my reasons. It is very well known that the vertebrae of the Crocodilia are very different in form, passing from the atlas to the sacrum, and these differences have been very well described by Huxley in his *Anatomy of Vertebrates* (1871). Omitting a consideration of the first two vertebrae, the atlas and axis, concerning the identity of which there can be no doubt in the two specimens, we may examine the following vertebrae. The other cervicals all possess ribs with distinct and long capitula and tubercula — the latter attached above the neurocentral suture to the neural arch, the former to the centrum below the neurocentral suture. The body of each cervical rib, after the second and as far as the seventh and eighth, is short and prolonged in front of, as well as behind, the junction of the capitulum with the tuberculum; and the several ribs lie nearly parallel with the vertebral column and overlap one another. The ribs of the eighth vertebra are a little longer than those of the seventh; the ninth rib is very much longer than the eighth, and has a terminal cartilage.

The points to which the capitula and tubercula of the ribs are attached are raised into tubercles, and by degrees these become elongated into distinct capitular and tubercular processes, — diapophysis and epapophysis, — between which in the third to the tenth vertebrae, the neurocentral suture passes. In the ninth and the tenth the diapophysis ascends to the level of the neurocentral suture, and in the eleventh it ascends with its upper half to the neural arch, being traversed by the neurocentral suture. At the same time the epapophyses become more and more elongated. In the twelfth vertebra a sudden change in the character of the transverse processes takes place. There is no true diapophysis any longer ; the capitulum is also connected with the long epapophysis at the anterior middle portion. In all the following vertebrae up to the lumbar region the ribs are connected with the strong epapophyses. *In all the Crocodilia the first vertebra which carries the ribs completely on the transverse process, is always the twelfth.* In *Gavialis*, with 25 presacral vertebrae, it is the thirteenth. There are 12 vertebrae behind this one in all the Crocodilia and the abnormal Gavial; therefore in front of this one a vertebra must have been added. By careful comparison it was found that this additional vertebra is intercalated between the ninth and tenth. The vertebra resembles the ninth and tenth in having the capitulum completely placed on the center just on the level of the neurocentral suture. In all the Crocodilia there are two such vertebrae; in the abnormal Gavial, three. In this case we have no shifting of the pelvis, but true intercalation.

In my paper on intercalation I made the following remark : "If intercalation takes place at all, we ought to expect traces of it in such forms which show a great increase in the number of vertebrae, for instance, snakes, different families of lizards, and plesiosaurs" ('91, p. 333). The enormous number of 435 of the vertebrae in *Python molurus* Linn. has certainly not been reached by adding vertebrae at the distal end, but by the intervertebral increase of the smaller numbers. It is evident that intercalation can only be proved by the presence of half-divided vertebrae, and it is very interesting, and certainly not accidental, that in snakes such half-divisions have been observed quite frequently.

Python molurus Linn. (*P. tigris* Daud). In a skeleton of this snake in the Museum of the Royal College of Surgeons of England in London, No. 602, the following peculiarities of structure are to be seen. Up to the 147th inclusive the vertebrae are normal, each having a pair of transverse processes and a pair of ribs. The next vertebra is normal anteriorly, and as far as the level of the posterior surface of the transverse processes, save that its neural spine is rather small from before backwards. The transverse processes bear a pair of normal ribs. But behind this pair of transverse processes the parts begin again, rising again into a neural spine, and growing outwards into a second pair of transverse processes, with a second pair of normal ribs. Posteriorly again the parts are normal. These two segments are not the result of ankylosis, but of imperfect, division.

In the same specimen the 166th vertebra is normal on the left side, bearing one transverse process and one rib, while on the right side there are *two* complete transverse processes and *two* ribs. The 185th shows the same condition, being double on the right side and single on the left. (Owen, 1853, p. 123; Baur, 1889, p. 333; Bateson, 1894, pp. 103–105.)

Python sebae (Gmelin), Museum Brussels. The 195th vertebra is single on the right side, and double with two ribs on the left. Besides 195 is ankylosed with 196. (Albrecht, 1883, pp. 21–34, Pl. II, Figs. 1–4; Fürbringer, 1888, vol. ii, pp. 975, 976; Baur, 1889, p. 332; Bateson, 1894, p. 105.)

Python sp., Cambridge University Museum. 168th vertebra double on the left side. (Bateson, 1894, p. 105.)

Hydrus platurus Linn. (*Pelamis bicolor* Daud), No. 763, Yale University Museum. The 212th vertebra simple on the left side, with one rib; double on the right side, with two ribs. (Baur, 1889, p. 333.)

Cimoliasaurus plicatus Phillips (Plesiosauria), No. 48.001, British Museum, London. Cervical vertebra divided on one side with two ribs. (Lydekker, 1889, p. 238; Baur, 1889, p. 333; Bateson, 1894, p. 105.)

In *Python* there may be as many as 435 vertebra, and in *Cimoliasaurus plicatus* we have 44 cervicals.

The increase of the number of cervical vertebrae in the Plesiosauria is certainly not produced by a shifting backwards of the shoulder girdle, but by addition of new vertebrae by intercalations, as will be seen from the following table:

TABLE SHOWING THE NUMBER OF VERTEBRAE IN PLESIOSAURIA.

	CERVICALS.	DORSALS.	SACRALS.	CAUDALS.
PLIOSAURIDAE				
Pliosaurus evansi Seeley	19–20			
Peloneustes philarchus Seeley	20–21			
PLESIOSAURIDAE				
Plesiosaurus rostratus Owen	28	20	2	34
Plesiosaurus macrocephalus Owen	30	20	2	18+*
Thaumatosaurus megacephalus Stutchbury	30	26	2	34
Plesiosaurus hawkinsi Owen	31	23	2	—
Cryptoclidus oxoniensis Phillips	31	23	2	23+
Plesiosaurus guilelmi-imperatoris Dames	35	20	2	37
Plesiosaurus conybeari Sollas	38	21	2	5+
Plesiosaurus homalospondylus Owen	38	22	2	25+
Plesiosaurus dolichodeirus Conybeare	41	21	2	30+
Muraenosaurus plicatus Phillips	44			
ELASMOSAURIDAE				
Elasmosaurus platyurus Cope	72	20	2	23+

Everybody who will examine this table must admit that the increase of the number of cervical vertebrae can only be explained by intercalation. We see, therefore, that intercalation really occurs.

All the cases enumerated in the table of the Amphibia caudata and the cases of the Crocodilia demonstrate the shifting of the pelvis, a process which I have always admitted.

In the Ecaudata (Batrachia) we find the same processes, as has been shown in an excellent paper just published (after I had written my paper) by W. G. Ridewood, "On the Development of the Vertebral Column in Pipa and Xenopus," *Anat. Anz.*, xiii, Bd. 10, April 1897, No. 13, pp. 359–376, with 4 figures.

* + means that the tail is not completely preserved.

Ridewood has discussed fully all the known cases of variation in the number of presacral vertebrae in the Ecaudata. He makes the following remarks (p. 366): "Throughout the whole group of the Anura (Ecaudata) the number of presacral vertebrae, and consequently the morphological position of the sacrum, is remarkably constant; and the wonder is that variations and abnormalities are not more common. When variation in the number of presacral vertebrae does occur, the explanation is to be sought, not in the intercalation or excalation of vertebrae, which, as Parker ('96) has already pointed out, are to be looked upon as very rare occurrences, but rather in the shifting of the ilium forwards or backwards on to the vertebra in front of or behind the normal. The vertebrae are from their mode of development intimately connected with the myotomes of the body, but the pelvis is less directly influenced by the primitive segmentation. It is a matter of little import whether it develops a little in front of or a little behind its normal position, and, in whatever position it develops, it seeks to gain attachment to that part of the axial skeleton which happens to be nearest. The vertebrae respond, and their lateral parts become modified accordingly in size and shape.

"In Anura (Ecaudata) those diapophyses which, during development, happen to come nearest to the upper extremities of the ilia enlarge in anticipation long before they come into actual contact with the pelvis. The diapophyses so affected are usually those of the ninth vertebra, and so this has come to be regarded as the normal sacral vertebra; but it may be those of the eighth or the tenth, or even a combination of these, forming a compound sacrum. The tenth vertebra is, like those succeeding, only 'potential,' and as a rule does not differentiate; but when from proximity of the ilium an additional strain is thrown upon the resources of the somite, its latent capacity for development is awakened, and a well-formed vertebra with strong diapophyses results.

"*As soon as we admit that, in Anura at least, any vertebra can become sacral, and that it only requires the stimulating presence of the iliac cartilages to induce an exaggerated development of the diapophyses, all the mystery of abnormal sacra is dispelled,*

whether the abnormality is due to the asymmetry, or to the compound nature of the sacrum, or to a combination of these."

With these very pertinent remarks I close this paper.

University of Chicago,
Laboratory of Palaeontology, May 6, 1897.

BIBLIOGRAPHY.

1883. Albrecht, Paul. Note sur une hémivertèbre gauche surnuméraire. de *Python Sebae* Duméril. *Bull. Mus. Roy. Hist. Nat. d. Belgique* Tome ii. 1883. pp. 21–34, Pl. II, Figs. 1–4. Bruxelles, 1883.

1894. Bateson, William. Materials for the Study of Variation. London, 1894. pp. 103–105, Fig. 10, I, II, pp. 123, 124.

1886. Baur, G. Osteologische Notizen über Reptilien. *Zool. Anz.* ix. Jahrgang, 1886. No. 238, pp. 689, 690.

1889. ——— Revision meiner Mittheilungen im Zoologischen Anzeiger, mit Nachträgen. *Zool. Anz.* xii. Jahrgang, 1889. No. 306, pp. 240, 241.

1891. ——— On Intercalation of Vertebrae. *Journ. of Morph.* Vol. iv, No. 3. Boston, 1891. pp. 331–336.

1897. Bumpus, Hermon C. A Contribution to the Study of Variation (Skeletal Variations of Necturus maculatus Raf). *Journ. of Morph.* Vol. xii, No. 2. February, 1897. pp. 455–478, Pls. *A*, *B*, and *C*.

1896. Case, E. C. Abnormal Sacrum in an Alligator. *Amer. Nat.* March, 1896. pp. 232–234. 2 figures.

1876. Claus, C. Beiträge zur vergleichenden Osteologie der Vertebraten. 1. Rippen und unteres Bogensystem. 2. Verschiebungen des Darmbeins und der Sacralregion der Wirbelsäule von Amphibien. *Sitzungsb. d. k. Akad. d. Wiss., math.-nat. Classe.* Bd. lxxiv, Abt. i. 1875. pp. 785–818, Pls. I–III. Wien, 1876.

1889. Cope, E. D. The Batrachia of North America. Washington, 1889. Pls. 1, 5, 6, 10, 14, 15, 22, 23, 37, Fig 1.

1886. Credner, Hermann. Die Stegocephalen aus dem Rothliegenden des Planenschen Grundes bei Dresden. vi. Theil: Die Entwicklungsgeschichte von *Branchiosaurus amblystomus* Cred. *Zeitschr. deutsch. Geol. Ges.* Jahrgang 1886. pp. 576–633, Taf. XVI–XIX. und 13 Textfiguren.

1825. Cuvier, G. Recherches sur les Ossemens Fossiles. Tome v. 2 me Partie. Paris, 1825. pp. 413, 414, 429.

1888. Fürbringer, Max. Untersuchungen zur Morphologie und Systematik der Vögel. Amsterdam, 1888. Vol. ii, pp. 972–991.

1873. HOFFMANN, C. K. Amphibien in Bronn's Klassen und Ordnungen. 1873. pp. 51, 52.

1875. HUXLEY, T. H. Article "Amphibia" in *Encyclopaedia Britannica*. Ninth ed. Vol. i, p. 752, Fig. 2, *A* and *B*.

1864. HYRTL, JOSEPH. Ueber Wirbelassimilation bei Amphibien. *Sitzungsb. d. k. Akad. d. Wiss., math.-nat. Classe*. Bd. xlix. Wien, 1864. pp. 264–272.

1865. ——— Cryptobranchus japonicus. Vindobonae, 1865. 4°. p. 43.

1878. JHERING, H. v. Ueber das peripherische System der Wirbelthiere. Leipzig, 1878.

1886. LUCAS, F. A. The Sacrum of Menopoma. *Amer. Nat.* Vol. xx, p. 561. 1886.

1889. LYDEKKER, RICHARD. Catalogue of the Fossil Reptilia and Amphibia in the British Museum. Part ii. London, 1889. p. 238.

1835. MAYER, A. F. J. C. Analecten für vergleichende Anatomie. Bonn, 1835. p. 78.

1845. MEYER, HERMANN VON. Zur Fauna der Vorwelt. Fossile, Säugethiere, Vögel und Reptilien aus dem Motasse-Mergel von Oeningen. 1845. Taf. 10.

1860. ——— Salamandrinen aus der Braunkohle am Rhein und in Böhmen. *Palaeontographica*. Bd. vii, pp. 47–73. 1860.

1853. OWEN, A. Descriptive Catalogue of the Osteological Series Contained in the Museum of the Royal College of Surgeons of England. Vol. i, p. 123. London, 1853.

1896. PARKER, G. H. Variations in the Vertebral Column of Necturus. *Anat. Anz.* Bd. xi. 1896. pp. 711–717. 2 figures.

1873. REINHARDT, J. Anomalier i krydshvirvlerne hos krokodilerne. *Videnskab. Meddel. fra d. naturhist. Foren.* Kjöbenhavn (Aaret, 1873), 1873–74. pp. 221–228. Also abstract in Gervais: *Journal de Zoologie*. iii. 1874. pp. 308–312.

1833. SCHLEGEL, H. Les Batraciens in Phil. Frz. de Siebold, Fauna Japonica. Fol. maj. Lugduni Batav. 1833. p. 132. Pl. VIII.

1862. SCHMIDT, F. J. J., AND J. GODDART, en J. van der Hoeven, jun. Aanteekeningen over de Anatomie van den *Cryptobranchus japonicus*. *Naturk. Verhand. Holl. Maatsch. d. Wetensch.* Harlem. xix deel 1 stuck. 1862.

1818. SCHULTZE, C. A. S. Ueber die ersten Spuren des Knochensystems und die Entwicklung der Wirbelsäule in den Thieren. J. F. Meckel, *Deutsch. Arch. f. d. Physiol.* Bd. iv, Heft 3. 1818. p. 379, Anmerkung.

1875. WIEDERSHEIM, ROBERT. Salamandrina perspicillata und Geotriton fuscus. Versuch einer vergleichenden Anatomie der Salamandrinen. Genua, 1875. p. 116.

Volume I. *September, 1897.* *Number 2.*

ZOÖLOGICAL BULLETIN.

PRELIMINARY NOTES ON DISTOMUM PATELLARE, n. sp.

MARY M. STURGES.

For the material used in making this description I am indebted to Dr. Whitman, by whom this Distomum was found in the bladder of Triturus (Molge) pyrrhogaster Boie of Japan. The specific name was suggested to Dr. Whitman by Professor Leuckart.

The total length averages in the living specimen 4.5 mm., and of this the length of the neck forms about two-fifths. The body, which is nearly circular, averages 3 mm. at its widest part and is .4 mm. thick. The ventral sucker is a trifle larger than the oral sucker and averages .65 mm. in width.

Anatomical. — The cuticle has no spines, but is covered with small wartlike thickenings among which papillae more conspicuous in size and shape, the sensory papillae, are irregularly scattered. The sensory papillae average 50 μ apart on the ventral surface and somewhat more on the dorsal, and are largest around and within the borders of the suckers, the body papillae projecting 5 μ, those of the suckers 10 μ, above the level of the cuticle. The cuticle lines the external openings of the various ducts, ceasing where their epithelium begins. Laurer's duct and the excretory vesicle, which have no epithelium, are lined throughout by a thin membrane which is con-

tinuous with the cuticle, but lacks its wartlike thickenings. Underlying the cuticle is the subcuticula, which consists of

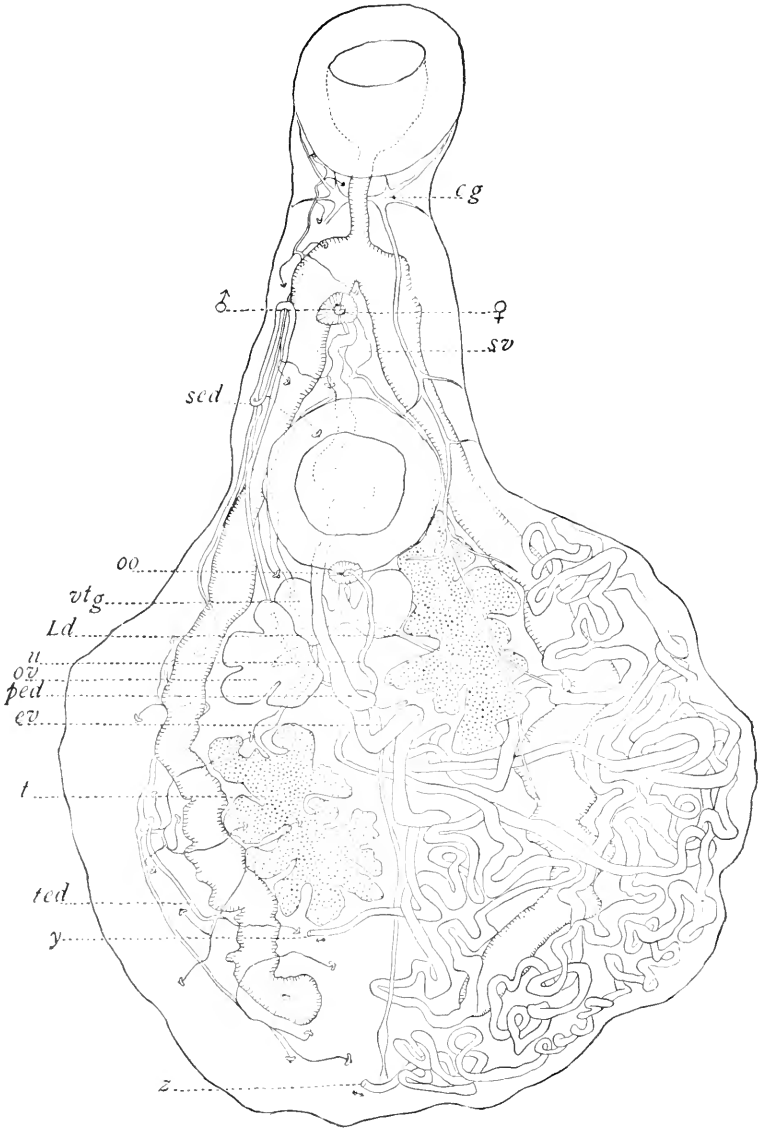


FIG. 1. — Ventral view. *cg*, cerebral ganglion; *ev*, excretory vesicle; *Ld*, Laurer's duct; *oo*, ootype; *ov*, ovary; *ped*, primary excretory duct; *sed*, secondary excretory duct; *sv*, seminal vesicle; *t*, testis; *ted*, tertiary excretory duct; *u*, uterus; *vtg*, vitelline gland; *y–z*, uterus upon right side, omitted in figure.

an external limiting membrane into which muscle fibers are inserted, and an internal non-cellular reticulum in which the dermal muscles are embedded. A continuation of the subcuticula sheaths the inner surfaces of the suckers. The dermal musculature shows the usual arrangement, — outer circular, middle longitudinal, and inner diagonal fibers. The oral sucker shows its adaptation to food-taking by the especial development of sphincter fibers, while its extrinsic muscles are weak ; the ventral sucker has few sphincters, but its base is fairly sheathed in a powerful extrinsic musculature of protractors and retractors, which pass in various directions from its sides to the body wall, so that the sucker could readily be shifted in position. The cellular structure of the parenchyma is distinct. The peripheral or subcuticular cells are smaller and more deeply staining than the rest, which look in comparison pale and vacuolar. The subcuticular cells are most conspicuous in the neck, but are coextensive with the cuticle. In the suckers they are also scattered among the vacuolar parenchyma cells.

D. patellare resembles in internal anatomy (Fig. 1) *D. folium*, which is described by Looss [1 (a)] as occurring in the bladder of certain fishes. The digestive tract has no salivary glands and no pharynx, although the thickened cuticle and musculature of the oesophagus may present traces of a pharynx. The intestinal epithelium has long abundant cilia. Each cerebral ganglion gives rise to two anterior and two posterior nerves, all of which give off branches which pass toward the body wall. No commissures were seen between the main trunks or their branches. The excretory system resembles more closely than that of *D. folium* that of *D. cygnoides* which occurs in the bladder of the frog and which Looss [1 (a)] considers closely related to *D. folium*. The excretory vesicle, which is much elongated and has a slight fusiform expansion in front of the excretory opening, has no epithelium, but the walls of the excretory ducts from the point where they leave the vesicle

[1] Looss, A.: (a) Die Distomen unserer Fische und Frösche, *Bibliotheca Zoologica*, Leuckart u. Chun, 1894.

(b) Zur Frage nach der Natur des Körperparenchyms bei den Trematoden, *Abhand. sächs. Gesell. d. Wiss.*, 1893.

consist of a distinct epithelium. The capillaries are not usually given off from short secondary or tertiary ducts as in *D. cygnoides*, but leave the primary or secondary trunks directly, so they are often of considerable length. The number of flame-cells varies and is seldom the same on both sides; there are usually from twenty-five to thirty on a side. The excretory ducts and capillaries are convoluted, owing probably to body contraction. The ovary lies, in more than half the specimens studied, upon the right side, in the rest of the specimens upon the left; a similar right or left-handed position of the ovary is described by Sommer [2] in *D. hepaticum*. The vitelline glands are two small compact lobes, lying close together, one on either side of the median line, just behind the ventral sucker. The uterus fills the circular body with its long, intricate coil; the contained eggs are thin-shelled and ellipsoidal, their axes measuring 25 μ and 17.5 μ. There is no cirrus, and neither male nor female genital opening is especially muscular. The male copulatory apparatus lies above the female apparatus and opens slightly in front of it into the common genital sinus.

The points most noteworthy in *D. patellare* are, externally, its pan shape, internally, its lack of pharynx and cirrus, the collection of its vitelline glands into two small compact lobes, the elongation of its excretory vesicle, and the great length of its uterus.

Histological.— The following histological points were studied:

1. Cuticle, subcuticula, and subcuticular cells.
2. The relation of the giant cells to nerves and muscles.
3. Flame-cells and capillaries of the excretory system.

1. *Cuticle, subcuticula, and subcuticular cells.* — The cuticle is a homogeneous, densely staining, single layer averaging 3 μ thick. In the genital openings and oesophagus it becomes much thicker (5 μ – 6 μ) and shows a sort of stratification, staining alternately light and dark in layers parallel to the body surface. It encloses the nervous cores of the sensory papillae which are bulb-shaped and highly refractive, but I could find no "pore-canals," ducts, or traces of nuclei. Between cuticle and subcuticula, which are usually closely applied, a narrow space is

[2] SOMMER, F.: Zur Anatomie des Leberegels, *Zeit. f. wiss. Zool.*, Bd. xxxiv, 1880.

sometimes seen which is traversed by delicate fibrils connecting the two layers; these fibrils are noted by Leuckart,[3] Brandes,[4] and Looss.[1(a)] When the cuticle is torn away, the fibrils break at the subcuticula. Their distribution and appearance preclude the idea that they are inserting muscle fibrils or, as Brandes suggests, the ducts of glands; it seems probable that they arise by the formation of vacuoles in the base of the cuticle and indicate a close connection between cuticle and subcuticula. The space traversed by these fibrils looks like a vacuolar layer and may correspond to the "vacuolar middle layer of the cuticle" described by some authors (Monticelli,[5] E. Walter,[6] Poirier[7]), their "basal layer of the cuticle" being what is here called the limiting membrane of the subcuticula. When cuticle meets epithelium it does not pass over it, under it, nor by transition into it, but ceases abruptly. There is in *D. patellare* no evidence that the cuticle is shed; the existence of well-differentiated nerve-endings in the cuticle seems, indeed, evidence to the contrary.

The tissue between the cuticle and the subcuticular cells, the subcuticula, has usually been described as undifferentiated. In *D. patellare* the outer part or limiting membrane is much more distinct from the inner reticular part in some specimens, where it is a thin, deeply staining sheet $\frac{1}{2}\ \mu$ thick, than in others, where it averages 1 μ thick, stains slightly, and passes almost imperceptibly into the subjacent reticulum. The limiting membrane forms sheaths for the inner surfaces of the suckers, where it contains fibrils which are probably elastic; it receives the insertion of muscle fibrils, and passes by transition into the basement membrane of ducts into whose external openings it accompanies the cuticle. The fine meshwork of the intermuscular reticulum, which shows no cellular structure

[3] Leuckart, R.: Die Parasiten des Menschen, Aufl. ii, 1886.

[4] Brandes, A.: Zum feineren Bau der Trematoden, *Zeit. f. wiss. Zool.*, Bd. liii, 1892.

[5] Monticelli, Fr. Sav.: Studii sui Trematodi endoparassiti, *Zool. Jahrbücher*, Suppl. III, 1893.

[6] Walter, E.: Untersuchungen über den Bau der Trematoden, *Zeit. f. wiss. Zool.*, Bd. lxv, Heft 2, 1893.

[7] Poirier, J.: Contribution à l'histoire des Trématodes. *Arch. d. Zool. expér. et gén.*, 2 sér., tome III, 1885.

and is often obscured by granules, is bounded internally with more or less distinctness by the subcuticular cells. The term subcuticula has been used interchangeably for the structures here called limiting membrane and intermuscular reticulum; thus Leuckart,[3] Braun,[8(a)] Monticelli,[5] E. Walter,[6] and Stafford[9] describe the limiting membrane as subcuticula, while Brandes,[4] Looss,[1(a)] and Braun[8(b)] describe as subcuticula the intermuscular reticulum. This fact together with the appearance of the subcuticula in *D. patellare* suggest that the limiting membrane is differentiated from the intermuscular reticulum, and that in some forms it may be noticeably developed, and not at all or only slightly in others. From the thickness and general appearance of the subcuticula it seems probable that it was once composed of cells, though no trace of these exists in the adult *D. patellare*.

There is hardly a point in the histology of Trematodes which needs study more than that of the subcuticular cells. The existence of a subcuticular cell layer, non-glandular, yet differing in appearance from the rest of the parenchyma, is maintained by Leuckart,[3] Ziegler,[10] Macé,[11] Looss,[1(a)(b)] E. Walter,[6] Schuberg,[12] and Goto[13] in Monogenea; the subcuticular cells are described as glandular by Brandes,[4] Monticelli,[5] Blumberg,[14] Poirier,[7] and Katheriner,[15] Brandes asserting and Monticelli denying that they form a continuous layer; while Saint-Rémy[16] denies the existence of any differentiated subcuticular cells in some forms.

[8] BRAUN, M.: Bronn's Klassen und Ordnungen des Thierreichs, Bd. Vermes. Trematoda. (a) Digenea. (b) Monogenea.

[9] STAFFORD, J.: Anatomical Structure of Aspidogaster conchicola, *Zool. Jahrbücher*, Bd. ix, Heft 3, 1896.

[10] ZIEGLER, H. E.: Bucephalus und Gasterostomum, *Zeit. f. wiss. Zool.*, Bd. xxxix. 1883.

[11] MACÉ, E.: Recherches anatomique sur la grande Douve du foie, Paris, 1881.

[12] SCHUBERG, A.: Zur Histologie der Trematoden, *Arbeit. a. d. zool.-zoot Inst. Würzburgs*, Bd. x, 1895.

[13] GOTO, S.: Ectoparasitic Trematodes of Japan, *Journ. of Coll. of Science, Imp. Univ. of Japan*, 1894.

[14] BLUMBERG, C.: Ueber den Bau des Amphistoma conicum, *Inaugural-dissertation*, Dorpat, 1871.

[15] KATHERINER: Die Gattung Gyrodactylus, v. Nrdm, *Arbeit. a. d. zool.-zoot. Inst. Würzburgs*, Bd. x, 1895.

[16] SAINT-RÉMY, G.: Matériaux pour l'anatomie des Monocotylides, *Revue biologique du Nord d. l. France*, Ann. v, 1892.

In some specimens of *D. patellare*, the typical subcuticular cells form a continuous layer, most conspicuous in the neck ; in others they are modified here and there into glandlike cells; and in others modification has progressed so far, the glandlike cells degenerating, others losing their typical character, that the typical cells are confined to certain regions. In all the specimens studied these cells were found in their typical condition at the margins of the body, especially in the posterior end, and in the neck near the union of the body wall with that of the suckers. However the cytoplasm of these cells may be modified, they are (except in advanced stages of degeneration) distinguished from the rest of the parenchyma by their smaller, more deeply staining nuclei which are spherical, average 5 μ in diameter and have abundant chromatin, while those of the other cells are ellipsoidal, average 10 μ by 6 μ, and have one nucleolus and a small amount of chromatin. Nuclei transitional in character exist, however, between these two kinds. Some of the subcuticular nuclei are very small, 2 μ–4 μ, but these pass by transition into those of average size. Cells containing these characteristic nuclei are found beneath the subcuticula in all the specimens studied ; they are, as is noted by E. Walter,[6] coextensive with the cuticle and with that modification of it which lines Laurer's duct and the excretory vesicle, ceasing abruptly with the cuticle. The cells described here and by most writers as typical subcuticular cells are small, polyhedral or rounded, and have a finely granular, densely staining cytoplasm, while the cells of the inner parenchyma are large with a vacuolar cytoplasm, but in this Distomum the former pass by transition into the latter. The glandlike modifications of these cells have usually flask-shaped bodies, whose necks are directed toward the cuticle but cannot be traced beyond the muscular layer, and a finely granular cytoplasm containing large, highly refractive granules. These glandlike cells degenerate ; their nuclei shrink, their granules aggregate into irregular, deeply staining masses, lose their refractive power, and finally disintegrate ; the cells become finely vacuolar and neighboring ones may fuse forming larger vacuolar masses which finally lose all trace of a cellular structure. The

typical cells among which these degenerating ones lie usually become somewhat vacuolar and their cell boundaries may partly break down so that they form a coarse reticulum in which, however, examination always shows typical subcuticular nuclei.

The tissues are well preserved, so that these changes in the subcuticular cells can hardly be considered abnormal ; since, however, the assumption of a glandlike form is not accompanied by any apparent change in cuticle or subcuticula and is followed by degeneration, it seems probable that the change is due to age, and the appearance of the other subcuticular cells confirms this idea. One recalls in this connection the suggestion of Looss [1(a)] that the presence of the subcuticular cells as a definite layer may depend upon the age of the specimen studied, and the facts found here suggest that possibly their glandular or non-glandular appearance may depend upon a similar condition. It is difficult to compare the glandlike cells found here with the glandular subcuticular cells noted by others, for descriptions and figures are seldom definite enough on this point; but it seems possible that they correspond to those noted by Blumberg,[14] to some at least of those subcuticular cells described by Monticelli as skin glands (see Looss [1(a)]), and to the glandlike cells described by Poirier.[7] No degeneration stages such as exist in this form are described by others. The transition of the subcuticular cells into the subjacent parenchyma shows that they must be considered peripheral traces of primitive parenchyma as Leuckart, Macé, Ziegler, Schuberg, and Goto suggest; while their coextensiveness with the cuticle and their ability, in some species at least, to assume a glandlike form (although in *D. patellare* this does not seem to be functional in the adult) indicate that, as Looss and E. Walter suggest and Brandes and Blumberg affirm, they may have a glandular function in some way connected with the cuticle.

On the whole, the facts found in connection with the cuticle, subcuticula, and subcuticular cells of *D. patellare* seem to me to support the view that the cuticle is not a true cuticle, a modified epithelium, or a basal membrane, but a pseudo-cuticle formed when the larval epithelium was lost. It seems prob-

able that this pseudo-cuticle was formed, not by the subcuticular cells, but by cells of similar origin of which the subcuticular cells and possibly the subcuticula are traces.

2. *The Relation of the Giant-cells to Nerves and Muscles.* — Monticelli[5] identifies the giant-cells with ganglion cells, showing that they are in some parts of the body directly connected with nerves, and his work is confirmed by Schuberg,[12] although the nervous connection of giant-cells in the posterior part of the body remains undetermined. Blochmann and Bettendorff,[17] however, noting a close connection between giant-cells and muscle-fibers, homologize them with the myoblasts found in Cestodes by Blochmann.[18] Zernecke[19] distinguishes the myoblasts of Cestodes from the ganglion and sense-cells ; the former are muscle-formers, one process of each of which has a secondary nervous connection, the latter generate the nervous system, in whose central or peripheral parts they lie. Finding the giant-cells favorable in *D. patellare*, I have studied their relation to nerves and muscles.

The histology of the muscles and nerves must be briefly given. Each muscle is a bundle of parallel longitudinal fibrils, around which I can detect no sheath. These fibrils insert separately at the end of the fiber into the limiting membrane. The large muscles are often hollow. The dorso-ventral and radial muscles are simple fibers, but the others branch and their branches anastomose, so that each muscle becomes a complex system of parallel anastomosing fibers. The longitudinal and diagonal muscles are especially long and complex. I could not find muscle nuclei.

The cerebral ganglia and nerve-trunks are composed of a finely reticular substance, in whose meshes granules exist; no nuclei are found in it, no cells are connected with it except the giant-cells, and it has no sheath. The ventral nerves, which can be followed with an oil-immersion lens for some distance,

[17] BLOCHMANN UND BETTENDORFF : Ueber Muskulatur und Sinneszellen der Trematoden, *Biolog. Centralbl.*, Bd. xv, No. 6, 1895.

[18] BLOCHMANN, F.: Ueber freie Nervenendigungen und Sinneszellen der Bandwürmern, *Biolog. Centralbl.*, Bd. xv, No. 1, 1895.

[19] ZERNECKE, E.: Ueber den Nervensystem bei Bandwürmern, *Zool. Jahrbücher*, 1896.

break up finally just within the dermal musculature into branches composed of delicate anastomosing fibrils; these branches do not disintegrate into separate fibrils whose endings can be traced, but spread out, forming very delicate plexuses. The ventral nerves were traced farthest, but all the others pass finally to the body wall, and probably end in the same way. The sensory papillae (Fig. 2) resemble those described by Blochmann and Bettendorff [17] as existing over the body and in the suckers of Trematode cercaria. Each granular nervous bulb has, in the body papillae, a dense fibrillar core which terminates at the end of a small prominence at the apex of the papilla. The rest of the bulb has often a fibrillar appearance, the granules being arranged in longitudinal rows, but in a few cases the fine granules are replaced by a few large, deeply staining ones. From the inner end of the bulb a fiber which looks like a continuation of the fibrillar core passes down into the intermuscular reticulum, and this fiber may, by careful focussing upon tangential sections of the body wall, be followed until, just outside of the dermal muscles, it breaks up into delicate anastomosing fibrils which resemble those into which the ventral nerves disintegrate. No cells, except in a few cases the giant-cells, were found connected with the sensory papillae; no sense-cells like those figured by Blochmann and Bettendorff were found.

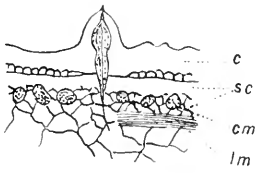


FIG. 2. — Sensory papilla from the body. *c*, cuticle; *sc*, sub-cuticula; *cm*, circular muscle; *lm*, longitudinal muscle.

Small groups of large, deeply staining granules, like those described in some of the sensory papillae, are scattered irregularly in the subcuticula. The granules are solid-looking, and those of a group usually lie close together in a straight line. As they were first seen lying parallel to muscles, close to them, and sometimes between the muscle fibrils, I thought for a time that they might be traces of muscle nuclei, but I afterwards found that they lie beneath the muscles and independent of them. They may be a form of nerve-ending simpler than the sensory papilla.

The body of the bi- or multipolar giant-cell varies from 20 μ to 40 μ in diameter, the processes from 30 μ to 50 μ in length. The ovoid or ellipsoidal nucleus is distinguished by its size (12 μ by 9 μ) and by its one large nucleolus from all the other nuclei of the body save those of the excretory cells, which it closely resembles. The cytoplasm is a fine reticulum whose meshes may elongate so that it becomes fibrillar-looking; usually it looks reticular around the nucleus and more fibrillar toward the cell-periphery and in the processes. Fine granules are scattered in the meshes of the cytoplasm and occasionally a number of large, deeply staining granules occur, like those found in some of the sensory papillae.

Most of the giant-cells are found near the body wall, but they also occur near the cerebral ganglia and nerve-trunks, are scattered through the suckers, and very fine specimens lie upon or near the extrinsic muscles of the ventral sucker. The size of the cells and the winding of their processes prevented me from tracing the distribution of all the processes of any one cell. Processes of these cells may pass (*a*) into the nerve-trunks or ganglia, (*b*) to muscle-fibers, (*c*) to the sensory papillae, (*d*) to the body wall where they spread out as the endings of the ventral nerves do, in a plexus of delicate, anastomosing fibrils.

(*a*) I can confirm on this point the work of Monticelli and Schuberg.

(*b*) Many of the giant-cells are connected by at least one of their processes with a muscle. Most of the processes of the multipolar cells lying near the base of the ventral sucker were traced to its extrinsic muscles; but, contrary to what Blochmann and Bettendorff found, fibers belonging to different anastomosing systems may be supplied with processes from the same giant-cell. This indicates that these cells are not myoblasts. The processes passing to the muscles break up into varicose anastomosing fibrils which can be traced for some distance along the surface of the muscle, developing here and there delicate thickened endings which are closely opposed to the muscle fibrils (Fig. 3).

(*c*) The fibrils of a process passing to a sensory papilla converge until they apparently fuse to form the fibril entering its

base. The different parts of the papillae may be brought into view by focussing on tangential sections of the body wall.

(*d*) The processes which spread out into plexuses cannot be followed far, for after the outline of the process is lost, it is easy to deceive oneself. The same is true in the case of the ultimate fibrils of the ventral nerve and those passing from the sensory papillae. I cannot, therefore, assert that a continuous subcuticular plexus is formed by processes of the giant-cells and ultimate branches of the nerve-trunks, yet the facts suggest this. I hope to test fresh material on this point with special methods. Blochmann and Zernecke find the peripheral plexus in Cestodes within the subcuticular cells, while here the indications are that it lies outside of them, within the dermal muscles. Blochmann and Bettendorff state that a nervous plexus exists within the subcuticular cells in Trematodes, but their preliminary paper gives no satisfactory description or figures of it.

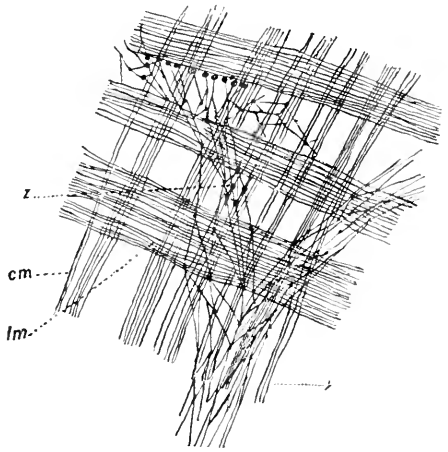


FIG. 3. — Tangential section of body wall. *cm*, circular muscle; *lm*, longitudinal muscle; *y*, process of giant-cell; *z*, process innervating muscle.

These facts seem to indicate that the giant-cells are not myoblasts but true ganglion cells, some of which have retained a primitive character so that they lie at the periphery and innervate both muscles and sense-organs, either directly or through a peripheral plexus. The nervous system is simpler here than in Cestodes, for one cell, the giant-cell, does the work which in Cestodes is assigned to three, — sense-cell, ganglion-cell proper, and myoblast. This slight differentiation in the sensory-motor system reminds one of the instances mentioned by Zernecke where he found in Cestodes a direct connection between sense-cell and myoblast.

3. *Flame-cells and Capillaries of the Excretory System.* — I can confirm the work of Schuberg [12] on these points. The excretory capillaries have a cellular wall of their own, containing large, though widely scattered, nuclei (Fig. 4), and are terminated by closed flame-cells (Fig. 5). No permanent lacunar spaces exist in the parenchyma, the excretory matter being passed to and through the flame-cells by osmosis. The processes of the protoplasmic part of the flame-cell extend between the parenchyma cells. Deeply staining (muscular?) thickenings are found in the capillary walls. There is a marked resemblance between the nuclei of the giant-cells and those of the capillaries and flame-cells, — a resemblance which is, perhaps, partly responsible for the observation of excretory capillaries in the suckers, and for other confusion which has existed between the giant-cells, and cells of the excretory system (see G. Walter,[20] Villot,[21] Macé,[11] Braun [8(a)] upon the peripheral giant-cells, and Wright and Macallum [22] upon the "renal" cells of Sphyranura).

FIG. 4. — Part of excretory capillary, showing nucleus.

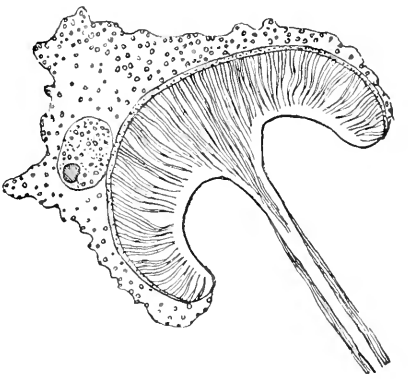


FIG. 5. — Flame-cell.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
April 20, 1897.

[20] WALTER G.: Beiträge zur Anatomie und Histologie einzelner Trematoden, *Arch. f. Naturgesch*, Bd. i, 1858.

[21] VILLOT, A.: Organization et développement de quelques Trematodes endoparasites marins, *Ann. des Sci. Nat.*, tome viii, 1878.

[22] WRIGHT AND MACALLUM: Sphyranura Osteri, *Journ. of Morph*, vol. i, 1887.

A PRELIMINARY ACCOUNT OF THE CLEAVAGE OF ARENICOLA CRISTATA, WITH REMARKS ON THE MOSAIC THEORY.

C. M. CHILD.

I. Cleavage.

The eggs in the jelly in which they were laid were fixed in picro-acetic acid and preserved in alcohol. This treatment renders the jelly perfectly soluble in distilled water, so that the eggs can easily be freed from the jelly by allowing a mass of it containing eggs to stand a few minutes in distilled water. The jelly disappears, and the eggs sink to the bottom. They are stained in dilute Delafield's haematoxylin, cleared and examined in clove-oil.

The individual egg is fairly well filled with small yolk spheres, which are scattered throughout all parts of it and are found in all the cells of the earlier stages. The cleavage conforms closely to the so-called "spiral" or "oblique" type, especially in its earlier stages. Later, as in the other forms of this type, cleavages which follow entirely different laws occur.

Figs. 1, 2, and 3 represent respectively the 2-, 4-, and 8-cell stages from the upper pole. In Fig. 1 both cells are already in division, and the spindles do not lie in the same plane, but are inclined to each other, so that this division is a true oblique segmentation. In Fig. 2 the second division has occurred, producing one enormous cell (*D*) and three much smaller ones (*A*, *B*, *C*). The large cell *D* is in contact with its opposite *B*, at both poles of the egg, *i.e.*, the two cross-furrows are parallel. The cross-furrow at the lower pole is longer than the other and perfectly constant up to a late stage, so that it furnishes an invaluable means of orientation.

It lies at right angles to the future median plane of the adult. The cell *D* is dorsal, *B* is ventral, and *A* and *C* are left

and right. The upper pole represents approximately the anterior end, and the lower pole the posterior end. In other words, the median plane of the adult passes through the blastomere *D* and forms an angle of 45° with the first two cleavage-planes. This orientation does not agree with that given by

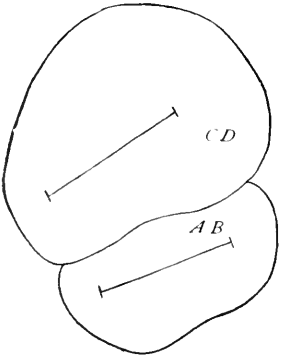


FIG. 1.

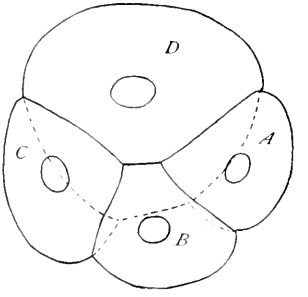


FIG. 2.

Wilson ('92) for *Nereis*, but I am unable to make his figures agree with his orientation. Mead ('94) gives the same orientation for *Amphitrite* that I find in *Arenicola*.

The enormous size of the blastomere *D* is interesting, as it of necessity influences the whole of the cleavage. This is the cell from which the first and second somatoblasts (*X* and *M*) will arise.

In Fig. 3, the 8-cell stage, it is seen that the four so-called micromeres are very large, the two dorsal ones being slightly larger than the ventral pair. The position of the posterior cross-furrow at this stage is considerably ventral to the posterior pole.

The cleavage proceeds in the typical oblique manner up to a stage of fifty-eight cells. During this period, the germ-layers have been separated. The ectoblasts arise as three "quartets" of blastomeres, given off successively and with alternating direction of spindle from the macromeres, the mesoblast (*M*) appears as a single cell, and the entomeres are represented by four cells. The large ectomere *X*, which is to furnish almost the whole ectoderm of the trunk, is seen in Fig. 4. The cells

of the first quartet of ectomeres have divided several times, and sixteen primary trochoblasts have been formed (Fig. 5, *tr* I; Fig. 6, *tr* I). Various divisions have occurred in other blastomeres except the mesoblast (Fig. 5, *M*). Figure 6 shows the passage from the 58-cell stage to the next from the upper pole. The four spindles here represent the first bilaterally symmetrical division and also the formation of the apical cross of eight cells. The second bilaterally symmetrical division in the egg occurs in the largest derivative of *X* at the 70-cell stage. It is followed immediately by the symmetrical division of *M* into right and left halves. At a stage when the egg consists of over a hundred cells, a bilaterally symmetrical division occurs in the entomeres, the last division that they undergo before the blastopore closes. It is an interesting fact that in each of the cases mentioned, *viz.*, the cells of the first quartet, the derivative of *X*, the mesoblast and the entomeres, this first bilaterally symmetrical division occurs in *cells of the same generation*, the eighth, counting the unsegmented egg as the first. The later derivatives of the cells just mentioned all divide symmetrically, though in derivatives of the first quartet, I have often seen what is apparently a partial return to the oblique type in the direction of the spindle. Other cells of the egg

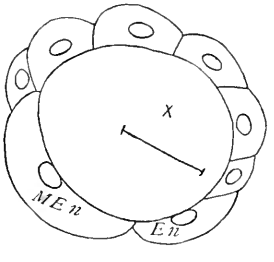


FIG. 3.

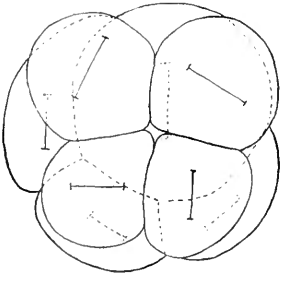


FIG. 4.

continue to divide obliquely up to a stage shortly before the closure of the blastopore, when, I think, all blastomeres are dividing symmetrically or approximately so. The sixteen primary trochoblasts do not divide further, but do not become ciliated until some time after the blastopore closes.

Fig. 7 shows a stage of considerably over 100 cells from the upper pole. The two cells which are lettered *N* are interesting because at this time they become very large, but lie rather deeply, so that they are partially covered by the rosette cells. Each then divides equally, the spindle forming an angle of 45° with the median plane, with its dorsal end nearest the median plane. After a period of rest both pairs divide again, but this time the more nearly median of each pair divides with a vertical spindle, *i.e.*, the ectoderm becomes two-layered here. Although I have been unable to follow these cells farther, it seems probable from their position and size that they may

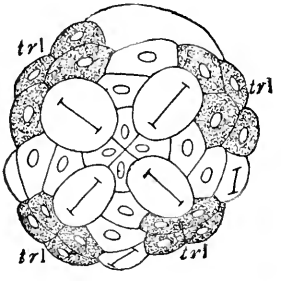


FIG. 5.

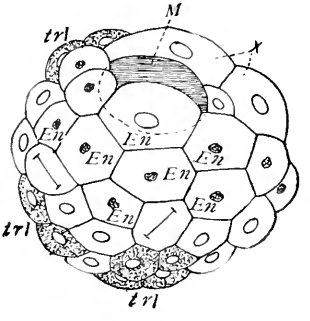


FIG. 6.

form the apical plate and thus the supraoesophageal ganglion or part of it. I do not believe that in *Arenicola* the rosette cells form the ganglion, though Wilson regards it as probable for *Nereis*.

The small cells lettered *K* correspond to the head-kidney cells of *Nereis;* I have been unable to discover a head kidney in *Arenicola*, and these cells remain as small, inconspicuous ectoderm cells.

The gastrulation in *Arenicola* is a combination of invagination and epiboly. The mesoblast begins the process by slowly elongating inward and gradually passing into the segmentation cavity which it just fills. Its bilateral division occurs during its passage. In Fig. 5 it is commencing already to pass inward. The entomeres are not enclosed until some hours later, although their superficial area is constantly decreasing. Opti-

cal sections show that they too are slowly elongating inward, and that their nuclei are sinking farther and farther from the surface of the egg. Finally, these cells extend as a column through the egg to the lower surface of the ectoderm of the upper pole (Fig. 9, *En*). As is seen by this figure, the amount of true overgrowth by the ectoderm is really slight, only the latest stages of the closure of the blastopore being effected in this way. The blastopore is at first nearly circular, but during the later stages of gastrulation it becomes dorso-ventrally elongated. To render this change in shape clear, the growth of the somatic plate must be explained.

The cells of the somatic plate, the derivatives of *X*, are at

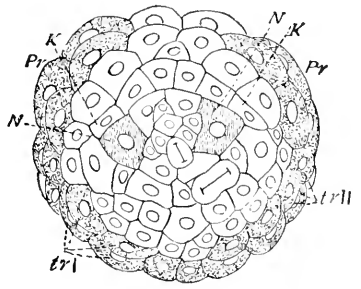


FIG. 7.

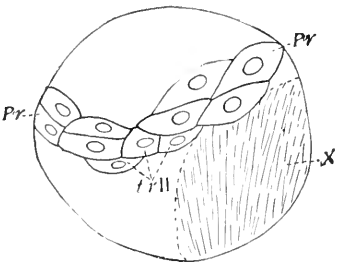


FIG. 8.

this time increasing rapidly in number, and the plate is extending itself posteriorly and laterally, the lateral growth being much more rapid than the posterior. It is this growth of the somatic plate that forces the sides of the blastopore together and finally causes them to meet. Fig. 10 shows the relations of blastopore and somatic plate at a stage shortly before closure. The lip of the blastopore, except the very narrow space dorsally where it is formed by the derivatives of *X*, consists of twelve cells (*St*), four along each side and four ventrally. The cells are all derivatives of the third quartet of ectomeres. I have followed their lineage exactly. At first they alternate with cells of the second quartet, but by a series of divisions they finally pass below these and then, dividing laterally, shut them off entirely from the actual lip of the blastopore. All the other cells shown in Fig. 10 are derivatives of *X*.

The closure of the blastopore begins at the dorsal end. The growth of the somatic plate (*X*) results finally in a concrescence of its two sides along the median line. The first two cells to meet are derivatives of those called *Xr*, *Xl*, in Fig. 10. Just dorsal to their point of meeting lies the proctodaeal region (*Proct* Fig. 10). This, though now filled with small cells, I believe must be regarded as in reality a part of the blastopore. The ventral portion of the blastopore, the portion still open in Fig. 10, later forms the stomodaeum. The distance between the two is continually increased by the ventrally advancing concrescence of the somatic plate. The cells of the

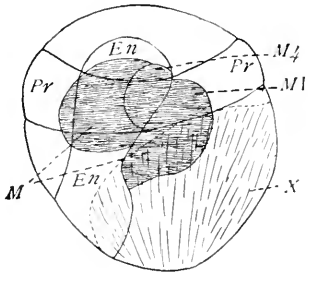


FIG. 9.

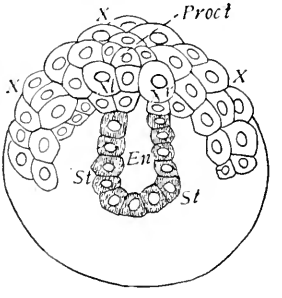


FIG. 10.

blastopore lip are pushed together from behind and from each side, principally the latter, until closure is effected. The somatic plate continues to grow posteriorly, carrying at its tip the proctodaeal region and the cells surrounding it, and thus the line of concrescence between the stomodaeum and proctodaeum increases in length, and growth in length of the larva begins. The area covered by the somatic plate in different stages is shown from the side in Figs. 8, 9, and 12, *X*.

The center of the blastopore from the earliest stages is ventral to the posterior pole, consequently the gastrula is not radially but bilaterally symmetrical, and its main axis does not correspond exactly with any of the future axes. These facts are easily deduced from Figs. 9 and 11.

About as soon as the two mesoblasts are completely inclosed, they begin the formation of the mesoblast bands. The spindle for the first division lies almost dorso-ventrally, the smaller

cell being given off ventrally and somewhat laterally. In each succeeding division, however, the direction of the spindle changes, the daughter cell arising each time more toward the upper pole, until finally at the stage of the blastopore closure the spindles of the mesoblasts are nearly longitudinal. Thus the main axis of the mesoblast band has changed by nearly 90°, but cells given off do not change their own position. Consequently, the anterior ends of the bands are curved ventrally. The position of the right mesoblast and its latest product are shown in Fig. 9, *M* 1 and *M* 4, while the rest of the shaded area shows approximately the extent of that portion of the right mesoblast band already formed. At this stage the change in direction is about half completed. Fig. 11 shows the relations of the layers in optical section just before closure of the blastopore, as seen from the ventral side and slightly anteriorly. The ends of the mesoblast bands (*M*) are seen on each side of the endodermic column.

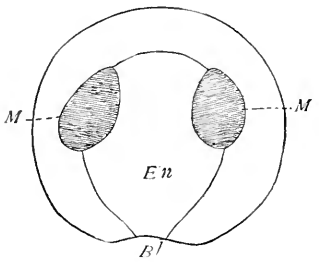


FIG. 11.

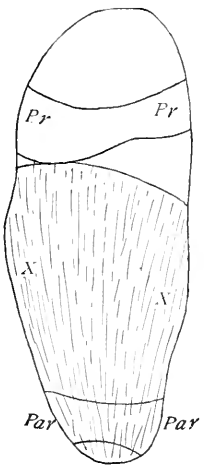


FIG. 12.

As stated above, the sixteen primary trochoblasts (Fig. 6, *tr* I) constitute the first indication of the prototroch. Later these are supplemented by nine cells from the second quartet (Fig. 7, *tr* II), three from each quadrant, except the dorsal, where a gap remains until about the time of the blastopore-closure. Fig. 8 shows the cells of the prototroch of the right side, the three secondary trochoblasts being lettered *tr* II. All the trochal cells elongate greatly, and finally the dorsal gap is closed, but before its closure four cells arising from the first quartet of ectomeres pass through it and are shut off by its closure from all connection with the pretrochal region.

The larva of *Arenicola* possesses a paratroch (Fig. 12, *Par*), but it is not functional until a very late stage.

Dr. Mead has very kindly furnished me with the lineage of the paratroch of *Amphitrite*, and it is interesting to note that the origin of this organ in the two cases is entirely different. The paratroch of *Arenicola* arises from certain derivatives of *X*, but not only is the series of divisions different in direction from that found in *Amphitrite*, but the cells, or a part of them, pass through several more generations than in *Amphitrite*.

The free-swimming trochopore of *Arenicola* already possesses three trunk segments with setae. The pelagic life does not last over two days. The larva[1] sinks to the bottom and crawls slowly about, and the growth of new segments begins. Fig. 12 is an outline of the trochopore in lateral view, the segmentation not being shown. The object of the figure is to show the area of the ectoderm formed by *X*.

Just a word here in regard to the cleavage of *Sternaspis scutata*. My work on this worm was begun at Naples, but, owing to loss of material, I have never yet been able to complete it. I desire to express my sincerest thanks to Professor Agassiz for the privilege of the Agassiz table at Naples, and also to Professors Dohrn and Eisig, as well as the other members of the staff of the Zoölogical Station, for their kindness during my stay. The unsegmented egg of *Sternaspis* is almost completely filled with very large yolk-spheres, but none of them pass into the ectomeres or the mesoblast. All these cells are relatively small, while the entomeres remain as enormous, yolk-packed cells. Yet up to a stage of about eighty cells (as far as I have been able to follow the cleavage of *Sternaspis*) the succession of cleavages is cell for cell the same as that of *Arenicola*. Sixteen cells are formed, corresponding to the primary trochoblasts of *Arenicola*, but *Sternaspis* has no prototroch, and they simply form a part of the ectoderm. On the other hand, the larva never really resembles a trochopore. It is not free-swimming. Vejdovsky ('81) states that the larvae at Trieste

[1] The larvae are very easily kept alive in covered glass dishes with a supply of *ulva*. I have kept them in the laboratory for three months, and thus have been enabled to preserve a complete series of stages of the late larval period and the metamorphosis.

were ciliated, but I have never been able to observe any cilia or any swimming motion in those raised at Naples. The larva simply elongates and bursts the egg membrane and then twists and squirms along the bottom and soon bores into the mud. Vejdovsky's account of the cleavage is also extremely superficial and careless.

To sum up: as regards orientation, *Arenicola* and *Sternaspis* both agree with *Amphitrite*, but not with *Nereis*. As regards the formation of the prototroch and the concrescence of the somatic plate, *Arenicola* and *Amphitrite* agree, but both differ from *Nereis*. *Arenicola* differs from *Amphitrite* in the origin of the paratroch and in various details, and from *Nereis* in the origin of the lips of the blastopore.

The preceding account has dealt only with the cytogenetic side of the question. The study of the cleavage has brought out a number of facts of cytological interest as well. Suffice it for the present, however, to say that cleavages occur which appear to contradict all the so-called laws for direction of spindle, etc.

II. The Mosaic Theory.

Wilson ('93a) regards the cleavage of *Nereis* as "a visible mosaic-work," and further asserts that "the principle of organ-forming germ regions has here a real meaning and value, and this would remain true even if hereafter it should be shown that both of the first two blastomeres of *Nereis*, if isolated, could produce a perfect embryo."

However, if the production of a whole embryo from a half-egg be possible, then the fate of the blastomeres is really after all "a function of their position." Wilson ('93a) suggested the possibility or probability of cellular interaction, thus departing from the true mosaic theory of Roux, of which the fundamental principle is self-differentiation. He still postulated, however, true morphogenetic differentiation in the blastomeres. More lately, as a result of Crampton's ('96a) experimental work on *Illyonassa*, he ('96b) has reaffirmed his views.

Lillie ('95) went so far as to say "the more precocious the differentiation of the organs of the somatoblast, the greater the

difference in the size of the cells (*i.e.*, the first two cells of cleavage). The two cells may be equal in size when the organs in question are not precociously developed. The same principles suffice to explain unequal divisions throughout the cytogeny."

My work on *Arenicola* and *Sternaspis*, together with a comparison of previous work, has led me to somewhat different conclusions. The oblique cleavage does not appear to be so strictly or so simply a mosaic as has been supposed.

First, corresponding cells differ greatly in size and structure in different forms without any corresponding differences in time of differentiation. To mention a few examples: In *Arenicola*, where the unsegmented egg contains much yolk, equally distributed, all the cells of several generations, including ectomeres, first somatoblast, and mesoblast, acquire yolk granules. The ectomeres are very large, and the first somatoblast and mesoblast are the largest cells in the egg, leaving the entomeres quite small. In *Sternaspis*, where the egg is even more closely packed with yolk, it is all retained in the entomeres, and these are enormous, leaving the ectomeres and the mesoblast as small, protoplasmic cells.

Yet in both these cases the cleavage is cell for cell the same up to a late stage, and the differences in the order of cleavage are not great. In general, the cells have the same fate, except that *Sternaspis* has no prototroch.

Comparisons along this line may easily be carried further, but this is perhaps sufficient to show that the appearance of a large blastomere does not necessarily imply that it is packed with precociously differentiated material.

Secondly, as the number of studies of the oblique cleavage increases, it becomes more and more evident that cell homology is not a true homology. *Sternaspis* never possesses a prototroch, but large cells corresponding to the primary trochoblasts of *Arenicola* are formed, and every division up to the differentiation of the trochoblasts in *Arenicola* is exactly paralleled by *Sternaspis*. Again, *Arenicola* and *Amphitrite* both possess a paratroch, but in *Arenicola* the cells forming it arise by a different series of divisions and pass through several

more generations than do the paratrochal cells in *Amphitrite*. A cell in the segmenting egg of *Arenicola* arises in exactly the same manner as the head-kidney cell of *Nereis*, but remains as a part of the ectoderm.

These facts seem to bear more or less directly on the question of the mosaic theory, for, if we have a true mosaic in these cases with so great uniformity of cleavage up to a stage of a hundred cells or so, may we not expect to find a somewhat closely corresponding uniformity in the fate of the blastomeres? Of course the view is possible that each egg is, so to speak, laid out in a different mosaic, even though the forms of cleavage may correspond, but is it probable?

There is, however, a third argument against the mosaic theory as related to the oblique cleavage, which seems more conclusive than either of the preceding.

About a year ago Crampton's ('96a) experimental work on *Illyonassa* appeared. It was followed by an appendix by Wilson ('96b). In the latter paper Wilson asserts that the results of Crampton's work confirm his view that the cleavage of *Nereis* is "a visible mosaic-work." Crampton succeeded in separating the segmenting egg of *Illyonassa* into halves, quarters, and eighths, which were capable of further development. He asserts that the parts, with the exception of a few details, segment exactly as they would if the rest of the egg were present, and that therefore *Illyonassa* possesses no "postgenerative" or "regenerative" power, and that the cleavage is a mosaic. He noticed, it is true, a few changes in direction of cleavage, or in size of the products in the partial embryos, but explains them as the result of lack of pressure, owing to the absence of the other blastomeres. If differences of pressure are able to change the form of cleavage so easily here, it is strange that changes do not occur in the normal cleavage where in many cases, as far as can be seen, the spindles lie nearly or quite in the direction of greatest pressure, and the division pushes whole groups of cells from their positions temporarily. Furthermore, in no case was Crampton able to bring the larvae up to a stage late enough to determine that "regeneration" could not take place. He did, it is true, obtain partial

circles of cilia instead of complete ones, but in only one case, and then indefinitely, does he give the number of ciliated cells. In no case were mesoblast-bands formed, even where they might be expected. This early death shows at any rate the extreme dependence of one portion of the embryo upon another.

Finally, and this is the crucial point, from a study of his paper it appears that "regeneration" has in most cases actually occurred. In no single instance does the partial larva retain the form which the part would have in the whole, though doubtless this is due in part to the rounding out of cells in consequence of the absence of external pressure. But the ectomeres always become more or less changed. They form a rounded cap, not a half or a quarter of such a cap. Lastly the ectomeres or cells which, in the normal cleavage, come to cover the *outer side* of half the entomeres, in the half embryo, *completely overgrow these entomeres, thus forming a gastrula and bringing the blastopore to closure.* In his description of the half embryos Crampton says: "Ectomeric divisions continue, the ectodermic cap grows lower, as shown in Fig. 18, before another division of *A* and *B* takes place. After the fourth division of these viewed from the lower pole there are four cells easily recognizable as entodermic. These are finally completely overgrown by the ectodermic cells, and in one case, sixteen hours after isolation, a partial circle of cilia was developed." Again, in speaking of the ¼ blastomeres, he says: "Throughout later development, two large cells containing yolk matter are plainly seen, inclosed by the clear ectodermic cells." Still again, in another embryo of the same sort: "The two entomeres are still distinctly visible, surrounded by ectoderm cells." In his figures the same point is shown several times.

Now, in order to accomplish this complete overgrowth and inclosure of the entomeres, either the ectodermal cells have changed their method of cleavage and come to lie where normally they would not, or else more cells than the normal number have arisen from the macromeres.

Unfortunately, only the most general statements are given regarding the later development, but in any case the larvae are

not ½ or ¼ larvae, but have become more or less complete wholes. Wilson appears to have overlooked this point entirely, in quoting this work in support of his views. He asserts that "apart from a few unimportant details, blastomeres of the two-cell or four-cell stage, whether isolated singly or in groups, segment precisely as if the missing portions of the egg were present, and the resulting larval fragment is completely devoid, not only of the power of a regulatory rearrangement of its material, but also of regenerative or post-generative power. The cleavage is thus demonstrated to be in the gasteropod precisely what I have asserted that of *Nereis* to be, *viz.*, "a visible mosaic-work."

Even here, then, in this highly specialized type of cleavage, the fate of the cells has been altered by a change of conditions. If we accept Roux's "Reserveidioplasson," it is of course possible to escape from this difficulty.

The oblique cleavage is not, then, a direct division of the egg into so many sets of organs which exist as "Anlagen" in the cells, but the organs are differentiated as the result of processes going on in the egg as a whole, though, later in development, self-differentiation may occur to some extent. The cell is after all no true morphological unit in cleavage, as, indeed, an increasing number of facts is showing us, *e.g.*, the papers of Hammar ('97b) and Andrews ('97a), and it is difficult to believe in the face of these and other facts that it is physiologically isolated.

I believe that any theory of development, proceeding on a strictly cellular basis, must fail in its attempt to explain ontogeny. The organism from the unsegmented egg to the adult is a whole, and at every stage of its existence acts as such ('93b). There is often visible organization in the unsegmented egg, but this, by no means, corresponds to the adult organization. That organization is the final result of the processes constantly going on in the developing egg, and changes occurring in one part are the result of the preceding changes in the other parts and in their turn become causes of others.

Cleavage, however, if not necessarily cell-differentiation, is not merely a mechanical splitting up of the egg, nor is its form

determined, as Hertwig states ('97c) by the arrangement of the yolk. As was pointed out above, the yolk, though distributed throughout the unsegmented egg, may go to all the cells, and yet these cells may exhibit great differences in size, or it may appear in only a small number of them. Both the form of cleavage and the distribution of the egg-substance are determined by some power in the egg, which acts, at least in part, according to laws which are not yet understood. This power constitutes the true organization of the egg. The fertilized egg is not divided into pigeon-holes containing substances for different portions of the adult body, but it is simply a cell possessing the power to initiate certain chemical and physical processes, which in their turn initiate others, until, as the final result, the adult appears. This is epigenesis pure and simple, but it differs from Hertwig's position in that it recognizes a more fundamental organization in the egg than the visible one consisting of protoplasm and deutoplasm.

This organization is in general terms the specific nature of the reproductive cell. It is a phase of the same power that determines that the egg of *Arenicola cristata* shall develop into *Arenicola cristata*. At present we are in the dark concerning it. According to Weismann it is the germ-plasm, but whatever it be called, it is present from the beginning, and the visible cytoplasmic organization, the form of cleavage, and the whole ontogeny are the result of it.

ZOOLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
April, 1897.

LITERATURE.

'97a. ANDREWS, G. F. Some Spinning Activities of Protoplasm in Starfish and Sea-urchin Eggs. *Journ. of Morph.* Vol. xii, No. 2. 1897.

'96a. CRAMPTON, H. E. Experimental Studies on Gasteropod Development. *Arch. f. Entwickelungsmechanik*, Bd. iii, Heft 1. 1896.

'97b. HAMMAR, J. A. Ueber eine allgemein vorkommende primäre Protoplasmaverbindung zwischen den Blastomeren. *Arch. f. mikr. Anat.* Bd. xlix, Heft 1. 1897.

'97c. HERTWIG, O. Zeitung und Streitfragen der Biologie. Heft 2. Mechanik und Biologie. 1897.

'95. LILLIE, F. R. The Embryology of the Unionidae. *Journ. of Morph.* Vol. x, No. 1. 1895.

'94. MEAD, A. D. Preliminary Account of the Cell Lineage of Amphitrite. *Journ. of Morph.* Vol. ix, No. 3. 1894.

'81. VEJDOVSKY, F. Untersuchungen über die Anatomie, Physiologie und Entwickelung von Sternaspis. *Separat. aus dem xliii. Bd. der deutsch. math.-naturwiss. Classe d. kais. Acad. d. Wiss., Wien.* 1881.

'93b. WHITMAN, C. O. The Inadequacy of the Cell-theory of Development. *Journ. of Morph.* Vol. viii, No. 3. 1893.

'92. WILSON, E. B. The Cell Lineage of Nereis. *Journ. of Morph.* Vol. vi, No. 3. 1892.

'93a. WILSON, E. B. Amphioxus and the Mosaic Theory of Development. *Journ. of Morph.* Vol. viii, No. 3. 1893.

'96b. WILSON, E. B. Appendix to '96a. On Cleavage and Mosaic Work. *Arch. f. Entwickelungsmechanik.* Bd. iii, Heft 1. 1896.

CENTROSOME AND SPHERE IN CELLS OF THE OVARIAN STROMA OF MAMMALS.

A PRELIMINARY COMMUNICATION.

C. M. CHILD.

THE presence of centrosome and sphere in the cell has been found in the majority of cases to present some relation to the karyokinetic processes. The number of cases, however, which appear to indicate that the function of the centrosome and sphere may extend beyond their connection with karyokinesis is being multiplied.

Without making any attempt at completeness, a few of the references bearing on this point may be given here. Heidenhain ('94) and Flemming ('91), among others, have studied the centrosome in leucocytes and giant cells; Dehler ('95a) and Lenhossék ('95b) have found them in ganglion cells of the frog; Miss Lewis ('96c) has found them in ganglion cells of an annelid, and they have been demonstrated in various tissue cells besides ('91).

In some of these cases it appears probable that the cells under consideration have completed their karyokinetic history, and that the centrosome is merely a relic of a past stage, or else that it possesses some additional relation to the economy of the cell which is not as yet understood.

Again Morgan's ('96b) recent work on the production of artificial astrospheres in sea-urchin eggs appears to indicate that structures at least very similar to centrosomes and spheres may appear as the result of abnormal environment. Auerbach's ('96d) latest work on spermatogenesis shows the formation of a "Nebenkern," which without doubt corresponds to the sphere in this case in each generation of sperm cells. After its appearance in the last generation at the close of cell-division, the "Nebenkern" has a special function to fulfill in the metamorphosis of this cell into the spermatozoön. These cases

are sufficient to show that the presence of a sphere and centrosome in the cytoplasm may often be due to some other cause than an approaching or just finished mitosis. In this preliminary paper it is desired to describe a case which the writer believes may be included under this head.

Some months since, in the preparation of mammalian ovaries for histological work, the ovary of a pregnant dog was sectioned, and, being double-stained with Delafield's haematoxylin and erythrosin, showed the stroma to consist almost entirely of comparatively large, polyhedral cells with a coarse cytoplasmic network and a round nucleus containing clumps of chromatin of varying size and position. But the most striking feature of the preparation was the fact that each cell apparently possessed a large sphere [1] deeply stained with erythrosin. In fact the whole appearance of the slide reminded one of an amphibian testis. The older corpora lutea showed cells somewhat similar in structure but more vacuolated, and without any trace of the sphere.

Examination of the ovaries of pregnant rabbits revealed the fact that the structure of the stroma cells was almost perfectly identical with that found in the pregnant dog. In this case, however, it was found that these cells appeared *only in the ovary of the pregnant female*. Rabbits which were not pregnant showed the usual structure of the ovarian stroma without any trace of the sphere in any of the cells. One exception must be made to this statement, *viz.*, the case of a rabbit examined during the period of lactation a few days after the young were born. The ovary of this female showed the same structure as was found in the ovaries of pregnant animals.

My observations along this line extend at present no farther than this: I have examined three pregnant rabbits and one in lactation, and in all cases have found the structure referred to in the stroma cells. I have also sectioned the ovaries of three non-pregnant rabbits, and in no case was even a trace of a similar structure to be found. I have had no opportunity to extend my observations on the dog beyond the one case mentioned, that of a pregnant female where the sphere was first seen.

[1] Mr. W. H. Packard, a fellow of the University, who was preparing the sections, was the first to observe this structure.

Ovaries of the white rat and of the cat from both pregnant and non-pregnant animals have been examined, but without finding anything similar to the sphere in the dog and the rabbit, although the cells of the stroma show a very similar structure in other respects. It would appear, then, from the facts cited above that the presence of the sphere has some relation to the period of pregnancy, though of what nature it is impossible to state. Furthermore, a sphere of this kind is found only in certain mammals, though others show changes in the structure of the stroma similar to those observed in the dog and rabbit.

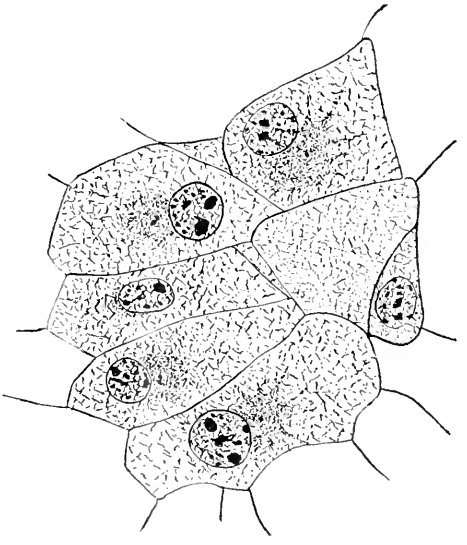


FIG. 1.

Below is given a short description of the finer structure of these cells as found in the rabbit, but both description and figures apply equally well to the cells of the dog as far as sphere and centrosome are concerned.

Fig. 1 [1] shows a group of these cells as they lie in the ovary. The stroma is made up almost entirely of these cells, the spindle-shaped cells of the ordinary stroma being almost wholly absent. Figs. 2 and 3 show single cells. As is readily seen, the nucleus does not usually occupy the center of the cell, but is often pushed considerably toward one side. When the section happens to be in a favorable plane, as in four of the cells in Fig. 1, there is seen on one side of the nucleus — usually on the side toward the greater mass of cytoplasm — a large, con-

[1] A $\frac{1}{12}$ Leitz oil immersion lens was used in the study of these cells, and the figures are all drawn with an Abbé camera. They do not show the relations with anything like the clearness of the actual preparation, which was fixed in acetic sublimate and stained with Heidenhain's iron haematoxylin followed by orange G.

spicuous condensation of the cytoplasm. As stated above, the cytoplasmic network of these cells is in general quite coarse. This statement, however, does not apply to the sphere. Although I have not seen the stages of the formation of the sphere, I believe from its appearance and relation to other parts of the cell that it is without doubt simply a condensation of the cytoplasm. In the figures it is represented as granular, but this appearance is probably due to the condensation. In staining qualities it resembles the cytoplasm, except that it takes the plasma stains much more deeply, and thus stands out very distinctly from the rest of the cytoplasm. Radiating fibers

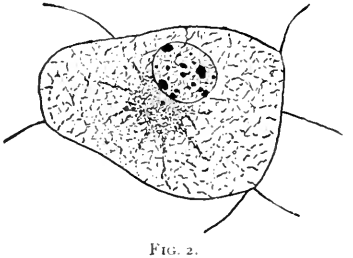


FIG. 2.

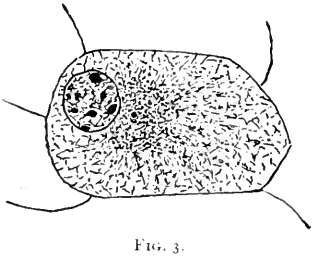


FIG. 3.

like those so common in astrospheres and spindles in the various stages of karyokinesis are not present, but the sphere resembles very closely the structure described by Auerbach ('96d) as "Nebenkern" in the spermatogonia and spermatocytes of Paludina, and, I believe, arises in the same way. But, though no distinct fibers are visible, the structure of the cytoplasm around the sphere proper is more or less distinctly radiate. Two of the cells in Fig. 1 show this to some extent, as does also Fig. 2. Sometimes this radiate arrangement is visible, though indistinctly, nearly to the periphery of the cell, as I have attempted to show in Fig. 3. It is probable that the difference between a sphere of this sort and a distinctly radiate sphere with fibers is one of degree and not of kind.

Perhaps even more striking than the presence of this sphere in these cells is the presence of a perfectly distinct centrosome — or in some cases two — in its center. Two of the cells in Fig. 1 show two centrosomes, the rest in which the sphere is in the plane of section show one.

I have been able to demonstrate this centrosome beyond all doubt by staining with Heidenhain's iron haematoxylin and then with orange G. or Bordeaux red, the best results being obtained with the haematoxylin and orange G. The extraction of the haematoxylin is carried just to the point where the cytoplasm has lost the last traces of the stain. Then after the contrast-stain and mounting, the haematoxylin is seen to be confined to the nucleus and to a single (or double) tiny but perfectly distinct granule in the center of the sphere. Error seems to be impossible here because by proper extraction the centrosome is the only extra-nuclear body in the cell that shows a trace of haematoxylin, and in contrast to the orange about it it is perfectly sharp and well defined. Moreover, the abundance of these cells and the uniformity with which the centrosome is visible renders the sections most striking. The group in Fig. 1 is drawn as seen in the section, and probably a hundred other similar groups could have been selected from the same section. I cannot state positively that every sphere contains a centrosome, but from the very large proportion of cases that I have observed it appears extremely probable that such is the case.

It is possible to obtain fairly good preparations by staining with gentian violet or with Flemming's triple stain, safranin, gentian violet and orange G., but the pictures thus obtained do not compare with those given by iron haematoxylin and orange.

Now the question arises as to the origin of these cells and their relation to the ordinary ovarian stroma. At first I regarded them as belonging to the corpora lutea, but further study has shown that they occupy the position, not of the corpora lutea, but of the stroma itself. Moreover, the cells of the corpora lutea are perfectly distinguishable from them.

As stated above, the details of their origin have not yet been worked out, but the following observations may throw some light on the matter.

In the ovary of the adult non-pregnant rabbit the stroma is composed principally of two kinds of cells. One of these is the elongated fiber cells, some of the shorter of which are shown in the spindle-shaped cells with deeply staining nuclei

at the right of Fig. 4. These cells are especially abundant near the periphery of the ovary. The amount of cytoplasm is small, and the cell boundaries are very difficult to see, so that the general effect of the section through a mass of these cells is that of large numbers of closely crowded, elongated, deeply chromatic nuclei lying in a very faintly stained ground substance. Large strands of these cells extend toward the interior of the ovary and often surround the corpora lutea. Fig. 4 is a group of cells taken from the margin of one of these strands. The remaining portions of the stroma are composed of the cells shown in Fig. 4 to the left and in Fig. 5. The nucleus is scarcely elongated and stains less deeply, the cells

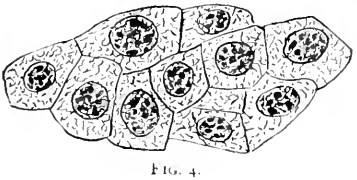


FIG. 4.

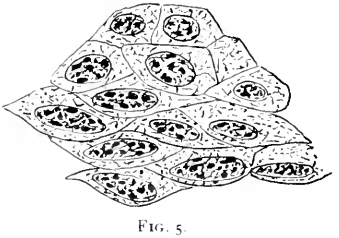


FIG. 5.

are polyhedral in outline, and the cell boundaries are more distinct. As shown in Fig. 4, these two sorts of cells do not appear to be sharply separated, but seem to grade into each other through intermediate forms. Now if the polyhedral cells in Figs. 4 and 5 were to undergo hypertrophy and acquire a sphere and centrosome in the cytoplasm, they would resemble very closely the cells of Figs. 1, 2, and 3, or if these cells were to shrink and lose their sphere and centrosome, they would resemble the cells of Fig. 5. I believe it probable that one or both of these changes occurs, and that the cells of Figs. 1, 4, and 5 represent different stages in the history of the same cell. As to whether the hypertrophy is periodic or whether the hypertrophied cells degenerate it is impossible to state as yet. I have found, however, no definite indications of degeneration.

Although I have never been able to find sphere or centrosome in the ovarian stroma of non-pregnant animals, at present the possibility of their existence cannot be denied. However, I think it improbable that it does exist as such.

I have never found any indications of karyokinesis in any of the stroma cells outside the corpora lutea, so that the presence of the centrosome and sphere is probably not associated with karyokinesis in this case.

If my suggestion that the cells of Fig. 1 arise by hypertrophy from the cells of Figs. 4 and 5 be correct, then it is probable that the appearance of the centrosome and sphere is in some manner connected with this hypertrophy.

In the rat and the cat there is a somewhat similar change in the structure of the stroma cells during pregnancy, but no centrosome or sphere appears. Sobotta ('96a) also mentions nothing of the sort in connection with the mouse. Whether there is less hypertrophy in these cases I cannot as yet state.

The results of my observations thus far are then that in the stroma cells of the ovary of the dog and the rabbit a distinct centrosome and sphere appear during pregnancy, but apparently are not in any way connected with karyokinesis.

The presence of these structures under these conditions opens up a number of problems, *viz.*, Is the centrosome a permanent organ of these cells? How is the appearance and disappearance of the sphere and centrosome in the cytoplasm related to the other changes occurring in the cell? How generally do these structures appear among mammals, and lastly, What is the relation of these changes of the ovarian stroma to pregnancy?

I hope to be able to throw further light on some of these questions, but it seemed that a short account of the results obtained thus far might be of interest.

LITERATURE.

'96d AUERBACH, L. Untersuchungen über die Spermatogenese von Paludina vivipara. *Jen. Zeitschr. f. Naturwiss.* Bd. xxx (N. F. xiii). 1896.

'95a DEHLER, A. Beitrag zur Kenntniss vom feineren Bau der Sympathischen Ganglienzelle des Frosches. *Arch. f. mikr. Anat.* Bd. xlvi. 1895.

'91 FLEMMING, W. Attraktionsphären und Centralkörper in Gewebezellen und Wanderzellen. *Anat. Anz.* Bd. vi. 1891.

'94 HEIDENHAIN. Neue Untersuchungen über die Centralkörper und ihre Beziehungen zum Kern und Zellprotoplasma. *Arch. f. mikr. Anat.* Bd. xliii. 1894.

'95b VON LENHOSSÉK. Centrosom und Sphäre in den Spinalganglien zellen des Frosches. *Arch. f. mikr. Anat.* Bd. xlvi. 1895.

'96c LEWIS, MARGARET. Centrosome and Sphere in Certain of the Nerve Cells of an Invertebrate. *Anat. Anz.* Bd. xii. 1896.

'96b MORGAN, T. H. The Production of Artificial Astrospheres. *Arch. f. Entwicklungsmechanik der Organismen.* Bd. iii. 1896.

'96a SOBOTTA, J. Ueber die Bildung des Corpus luteum bei der Maus. *Arch. f. mikr. Anat.* Bd. xlvii. 1896.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
November, 1896.

PRELIMINARY ACCOUNT OF THE CELL LINEAGE OF PLANORBIS.

SAMUEL J. HOLMES.

THE eggs of Planorbis are deposited in capsules which are usually found fastened by a viscid secretion to stones or aquatic plants. In *P. trivolvis*, the species studied, the period during which eggs are laid extends from early spring until late in the fall. The eggs contain somewhat less than the usual amount of yolk; there is a clear, protoplasmic area at the animal pole, while the lower half of the egg is composed principally of deutoplasm.

In the matter of nomenclature I have followed Conklin in designating the different cell generations given off from the macromeres by coefficients instead of exponents. For instance, 1*a* would represent a cell of the first generation of ectomeres, 2*a* one of the second, and so on.

At the first cleavage the egg is divided into equal blastomeres, and the four-cell stage is produced by the almost simultaneous division of these two blastomeres into subequal cells. This division takes place in a right-handed spiral; two of the cells *B* and *D* meet in a cross furrow at the vegetative pole, while the other two blastomeres, *A* and *C*, come in contact above. The first cleavage furrow is oblique to the future longitudinal axis of the embryo; a plane cutting the centers of *B* and *D* nearly coincides with the future sagittal plane. A cleavage cavity makes its appearance in the two-cell stage, reaching its maximum size immediately before the division into four cells. Small cavities commonly occur between the blastomeres during several subsequent stages of cleavage.

The first quartette of ectomeres is given off in a left-handed spiral, the cells 1*a* and 1*c* meeting in a cross furrow at the apical pole. This division is followed by a dexiotropic cleavage of the macromeres, and soon afterward the first generation divides in a dexiotropic direction, thus giving rise to a

sixteen-cell stage. The lower tier of cells resulting from the latter division are the trochoblasts or turret cells of Conklin. A third quartette of ectomeres is given off in a left-handed spiral, completing the separation of the ectoderm; at the same time a laeotropic division occurs in the second quartette. The form of the egg which now contains twenty-four cells is almost spherical; there is a large cleavage cavity which is not, however, in any way homologous with that of the two-cell stage. The macromeres are much smaller than is usual among the eggs of the mollusca; in fact they scarcely exceed in size the

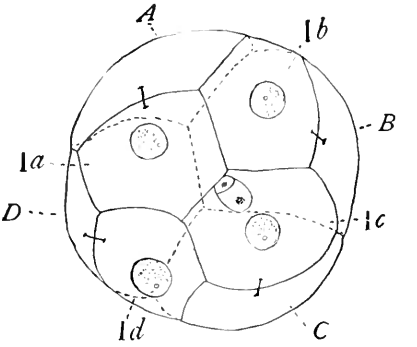


FIG. 1. Egg of eight cells.

cells of the third quartette. They are quite conspicuous, however, on account of the yolk granules they contain which give them a bright golden yellow color.

The resting period which occurs at the twenty-four-cell stage is broken by the dexiotropic cleavage of the upper tier of the second generation of ectomeres ($2a^1$, $2b^1$, etc.); and, at nearly the same time, the posterior macromere *D*, by a dexiotropic division, gives off the cell $4d$ which is destined to produce the mesoblastic bands. The ventral moiety of this division, compared with the mesoblast cell, is small, and, as far as it could be observed, remained undivided. The remaining macromeres do not divide until a somewhat later period. The ventral tier of the second quartette next divides dexiotropically; there are now four groups of four cells each belonging to the second generation of ectomeres, one cell in each group being above and one below, while there is a right and a left cell at the same level.

The divisions of the four cells of the third quartette and both tiers of the first quartette quickly follow. The pairs of cells belonging to the third quartette are arranged radially as in Limax (Kofoid), the one cell lying above the other. After these divisions the cross makes its appearance, becoming a very conspicuous feature of the egg. The cells of which it is composed are the eight cells resulting from the laeotropic division of the apical cells of the first generation of ectomeres and the four upper cells of the second quartette which form the tips of the arms. The angles between the arms of the

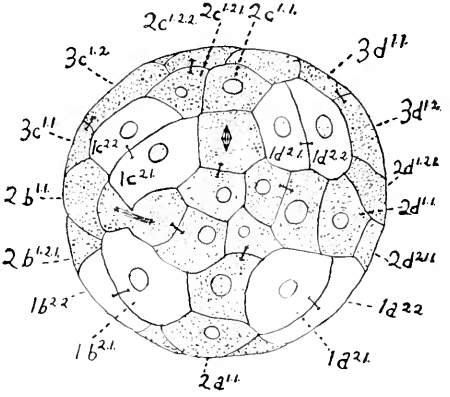


FIG. 2. — Egg of sixty-four cells, seen from above.

cross are occupied by the four pairs of trochoblasts ($1a^{2.1}$, $1a^{2.2}$, $1b^{2.1}$, $1b^{2.2}$, etc.). Compared with Crepidula (Conklin) and Umbrella (Heymons), in which the trochoblasts divide only at a very late period of cleavage, the division of these cells occurs remarkably early. In this respect Planorbis agrees much more closely with Limax (Kofoid), in which the corresponding cleavage occurs at near the forty-cell stage. Both in Crepidula and in Umbrella the trochoblasts are small, but in Planorbis they are conspicuous from their large size and transparency.

The next cells to undergo division are the three entomeres *A*, *B*, and *C*, the corresponding division of *D* having taken place at an earlier period. This cleavage is dexiotropic, the cells at the vegetative pole being much smaller than those of

the upper or fourth quartette. At about this time the large mesoblast cell *M* divides; the two resulting mesomeres lie partly pushed into the cleavage cavity so that only a small portion of each appears at the surface. They become entirely covered over by the ectoderm at about the sixty-four-cell stage. With the division of ectomeres and the mesomere the number of cells has reached forty-nine. This stage marks another resting period. The egg has now seven entomeres, two mesomeres, and forty ectomeres, the first and second generations of ectomeres each containing sixteen cells, while the third quartette is composed of eight. Up to this time all of the ectodermic cells of a quartette have undergone division at approximately the same time. The cleavage of certain cells of the third quartette which now takes place introduces an exception, in this respect, to the previous regularity of cleavage. The anterior cells of the lower tier of the third quartette, $3b^2$ and $3c^2$, divide bilaterally, and, at nearly the same time, the two posterior cells of the upper tier of the same quartette, $3a^1$ and $3d^1$, undergo a division which is likewise bilateral. The large cells $3a^2$ and $3d^2$ remain for a long time undivided. The cells $2a^{1.2}$, $2b^{1.2}$, etc., and $2a^{2.1}$, $2b^{2.1}$, etc., next undergo a laeotropic division, and, at the same time, the three cells of the fourth quartette, $4a$, $4b$, and $4c$, divide in an equatorial direction. Soon afterwards the cleavage of the upper anterior cells of the third quartette, $3b^1$ and $3c^1$, occurs, followed by the division of the cells forming the bases of the arms of the cross ($1a^{1.2}$, $1b^{1.2}$, etc.). The egg now consists of seventy cells, — twenty cells of the first quartette, twenty-four of the second, fourteen of the third, eight of the fourth, and four small cells at the vegetative pole. From this stage on the cells of the third quartette which have not hitherto kept pace with those of the first and second undergo rapid divisions. $3a^{1.1}$, $3a^{1.2}$, $3d^{1.1}$, and $3d^{1.2}$ each give off a small cell toward the ventral pole, and $3b^{2.1}$, $3b^{2.2}$, $3c^{2.1}$ and $3c^{2.2}$ likewise give off a small cell in the same direction at the anterior side of the egg; thus arise four pairs of small cells, two anterior and two posterior. Soon all of the upper cells of the third quartette divide in the same direction as before. There thus results in the anterior

quadrants *b* and *c*, three pairs of cells of the third quartette placed the one above the other, while in the posterior quadrants *a* and *d*, there are three pairs of cells similarly arranged situated above the large cells $3a^2$ and $3d^2$. Before the foregoing divisions of the third quartette are completed the cells $2a^{2.1.1}$, $2b^{2.1.1}$ etc., and $2a^{2.2}$, $2b^{2.2}$, etc., have begun to divide, and there soon follows a cleavage of the four cells at the apical pole ($1a^{1.1}$, $1b^{1.1}$, etc.). Meanwhile the fourth quartette has divided again, making sixteen entomeres in all. With the

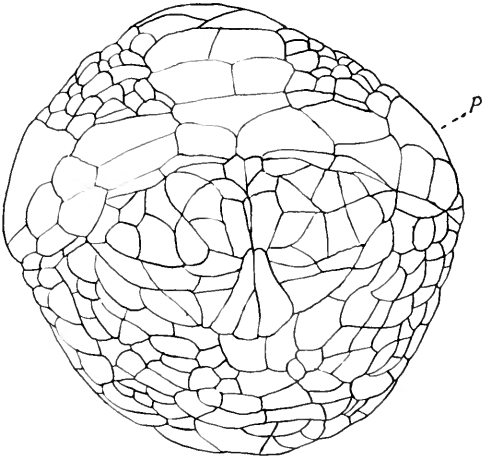


FIG. 3. — Gastrula with the blastopore nearly closed; *P*, prototroch.

cleavage of the basal cells of the arms of the cross the number of blastomeres in the egg reaches one hundred and four. The cells of the third quartette continue to divide quite rapidly, while the cleavage of the second quartette takes place more slowly. The cells of the first quartette whose divisions have hitherto been of the spiral type begin, at the next cleavage, a series of bilateral divisions which result in a longitudinal splitting of the arms of the cross similar to that which was found by Conklin to occur in Crepidula. This splitting first occurs in the anterior arm of the cross; somewhat later the lateral arms begin to divide; the posterior arm, as in Crepidula, remains undivided. The cross develops much more slowly than in the form studied by Conklin. The details of

its history are, in many respects, quite different, and the regularity of its form becomes broken up before it reaches a corresponding degree of development. The cells of the posterior arm enlarge and take part in the formation of the large head vesicle. This enlargement of cells extends to the cells at the center of the cross and finally continues forward to the prototroch; thus the cap of cells lying above the prototroch is cut into two groups of small cells separated by a median band of large, clear cells extending from the head vesicle behind to the prototroch in front. These isolated groups of small cells multiply rapidly and doubtless give rise to the eyes and cerebral ganglia.

The prototroch is formed from the trochoblasts previously mentioned and the uppermost cells of the second quartette which form the tips of the arms of the cross. Possibly other cells of the second quartette may take part in its formation; it is certain that the third quartette has no share in the process. The cells of the prototroch, up to a late stage of development, are few in number, and are arranged in a double row which extends from the ventral side a short distance in front of the blastopore to the head vesicle above. At an early stage they acquire cilia which serve to rotate the embryo in the capsule.

The head vesicle arises mainly from cells of the first quartette lying on the posterior side of the egg. By the enlargement of these cells the apical pole is pushed forward through an arc of 90° so that it comes to lie at the anterior end of the embryo. An apparent effect of this process can be seen in the cells lying in front of the blastopore; many of these cells take on a long, narrow form with their long axes transverse to the median plane of the egg, thus giving the appearance of having been flattened out by pressure from in front.

The cells of the second and third quartettes become so numerous before any organs make their appearance that it is impossible to trace the exact cell lineage of the structures arising from them. All that can be determined is the cell origin of the regions of the body wall from which certain organs are developed. It is quite certain that the cells of the posterior quadrant of the second quartette (derivatives of *2d*)

give rise to the shell gland and the median portion at least of the foot. In fact the cells of the posterior quadrant of the second quartette, with the quadrants *a* and *d* of the third, give rise to the larger part of the body of the embryo.

The cells of the fourth quartette undergo a third division in a more or less equatorial direction and the small cells *A*, *B*, and *C* divide, forming a fifth quartette. The number of entomeres increases still further before gastrulation begins. An embolic gastrula is formed, the blastopore becoming an elongated, slit-like orifice which closes from behind forwards. The lips of the blastopore close for a short time, but the definitive mouth makes its appearance, at a slightly later stage, at the point of closure. Whether or not an actual fusion of the lips of the blastopore occurs cannot at present be stated. The regions in front and behind the blastopore and at the sides are derived from the second quartette, while the cells of the third quartette lie at the angles. In the region around the mouth the cells become large and clear, and this area is continuous posteriorly with a narrow median band of clear cells separating the halves of the fundament of the foot.

The two mesomeres soon after the division of the primary mesoblastic cell come to lie entirely in the cleavage cavity at the posterior side of the egg. Their first division is very unequal and seems to have been overlooked by Rabl. Each mesomere gives off at its anterior end a minute cell, not larger than the first polar body. These cells are clear and lie close together in the cleavage cavity; their further history has not been followed. At the next division of the mesomeres begins the formation of the mesoblastic bands described by Rabl.

Fuller details of the subject of this short sketch will be given in a future paper. It is a source of pleasure to acknowledge the many favors granted me by Prof. C. O. Whitman in connection with this work and the valuable suggestions I have received from Dr. E. G. Conklin.

UNIVERSITY OF CHICAGO,
April 19, 1897.

THE DIGESTIVE EPITHELIUM OF DRAGONFLY NYMPHS.

JAMES G. NEEDHAM.

In the aquatic nymphs of dragonflies the alimentary canal fills a *rôle* of unusual importance. It chews, digests, excretes, and respires. More than its name implies, it is in a broad sense an organ of nutrition, or, rather, it is a series of nutritive organs. Its anterior third is concerned with the comminution of the food; its posterior two-fifths with excretion and respiration, and the small remaining middle portion, the ventriculus, alone digests the food, and, according to Cuénot,[1] also alone absorbs it. Recognizing its small size in proportion to the size of the body, and recalling the voracity of these insects, one is prepared to find in it conditions of exceptional activity.

In the dragonflies the ventriculus (*mitteldarm, medi-intestin*) is a simple tube without caeca or diverticula of any sort, slightly

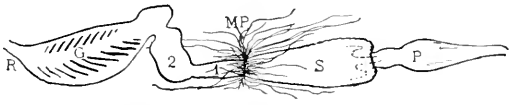


FIG. 1.—Alimentary canal of *Gomphus descriptus* Banks, side view, tracheae removed, × 2. *P*, pre-intestine; *S*, stomach, ventriculus, or mid-intestine; *MP*, Malpighian tubules: 1 and 2 divisions of the post intestine; *G*, gill chamber; *R*, terminal portion of the rectum.

narrowed posteriorly to its junction with the intestine at the origin of the Malpighian tubules. At its anterior end it meets the pre-intestine by a deep circular invagination into which the end of the so-called "gizzard" (*valvule ésophagienne*) descends. Its walls are of nearly uniform structure throughout, consisting of four well-marked layers which are, passing from within outward, (1) a layer of epithelium (not quite a simple layer, as we shall see); (2) a *membrana propria*, or basement membrane; (3) a layer of circular muscles; and (4) an incomplete layer of

[1] CUÉNOT, 1895: Études phys. sur les Orthoptères, *Arch. de Biol.*, xiv.

longitudinal muscle fibers. Sadones[2] has made careful study of all these parts in the nymph of *Libellula depressa* L., and they have been studied in other insects by many investigators.

The part of the ventriculus for which all other parts exist is the epithelium, and although this part has received much attention, there is among investigators little concord of opinion as to the meaning of certain of its structures. The purpose of this paper is to record the results of some additional studies of a combined histological and physiological nature upon this part. Grateful acknowledgment is due Prof. S. H. Gage for kind assistance and advice in the prosecution of these studies.

The nymphs of dragonflies are favorable subjects for studies of this sort, being everywhere common in fresh water, and easily collected, easily kept, and easily fed. A dish of water with some sand and a few plants in it furnished a congenial home for my nymphs so long as I kept them alive; and larvae of *Diptera*, or when these failed, bits of live earthworm, served well for food. The species mostly used were *Leptetrum quadrimaculatum* L., which lives sprawling upon the trashy bottoms of ponds, *Aeschna constricta* Say, which clings to submerged vegetation mostly in streams, and *Gomphus descriptus* Banks, which burrows in the bottom of both streams and ponds. There was found no appreciable difference in structure correlated with their difference of distribution in depth and consequent difference in food; and the species last named was the one used most extensively. It is the one from which the figures have been drawn.

Methods. — For the study of the accumulation of the digestive secretion, some nymphs were kept until wanted in a bare dish of water, where there was no chance of obtaining food. For the study of the discharge of this secretion other nymphs of the same size were fed at regular intervals for a time to bring them into the same condition, and then killed at different intervals after their last feeding. For general morphology, picro-aceto-sublimate (Rath's) was used for fixing, and was fol-

[2] SADONES, 1895: L'Appareil digestif et respiratoire larvaire des Odonates, *La Cellule*, xi.

lowed by paracarmine for staining *in toto*, or by haematoxylin (Gage's) and eosin for staining on the slide. For differentiating functional from nonfunctional parts, Hermann's fluid was used for fixing without subsequent staining. Numerous preparations were made from each of the three species named, at frequent intervals from one to eight hours after feeding. Serial sections of the whole of each preparation were mounted and studied.

I. The Accumulation of the Digestive Secretion.

A well-known type of digestive epithelium in insects consists of more or less elongated cylindric cells which rest upon the *membrana propria* and bear upon their free inner ends a strongly refractive striated border; interspersed among these cylindric cells are little groups or *nidi* of small, roundish cells close to the *membrana propria*, having no communication with the digestive cavity. The cockroach and other *Orthoptera* beautifully exemplify this type. The epithelium of dragonfly nymphs differs from it only by the closer approximation of the *nidi* and the crowding and elongation of the cells between them. Fig. 2 represents the epithelium in its normal resting condition. The digestion of a meal has recently been performed, and the cells have resumed their habitual aspect. The cylindric cells clearly reach the basement membrane. The cells of the nidus are few. The striated border is complete and undulating.[1]

Fig. 3 shows the condition found in a nymph after fasting two weeks. Midway between the nidi the cells have become very much crowded together, their nuclei are flattened as if by pressure, they rise in prominent elevations with very turgid summits from which the striated border has disappeared. The cells of the nidus have also increased in number. An accumulation of granular secretion in the cells between the nidi and their protrusion above the general level are very evident.

I believe that all these cells retain their connection with the basement membrane, although I was unable to trace them to

[1] Compare the single figure by Sadones for *Libellula depressa* L., *loc. cit.*, Pl. II, Fig. 16.

it, or to dissociate them satisfactorily. At *B* in Figs. 3 and 4 are the bases at least of some of them.[1]

Fig. 4 shows the continuation of this process of accumulation as found in a nymph that had been kept in a bare dish of water for two months. I was expecting to find degeneration in the cells of this preparation, but found instead the remarkable condition of things shown in the figure. The cells appeared perfectly healthy. The nidi were full of cells. The striated border had disappeared, or was only faintly discernible in the bottom of the now lumen-like depressions opposite the nidi. The epithelium was about three times its original thickness, and the upper two-thirds of it consisted of the wedge-shaped apices of granular cells crowded above the original level.

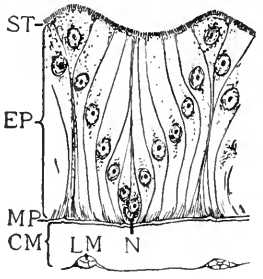


FIG. 2. — Normal resting epithelium soon after the digestion of a meal, × 220. *EP*, the epithelial layer; *ST*, the striated border; *N*, a nidus; *CM*, space filled by the layer of circular muscles; *LM*, longitudinal muscle fibers. Details of the muscles are omitted from all the figures.

Figs. 2, 3, and 4 are from nymphs of the same age and size taken together and kept under identical conditions except as stated above. They are therefore strictly comparable, and together might furnish a morphologist good reasons for taking physiological conditions into account.

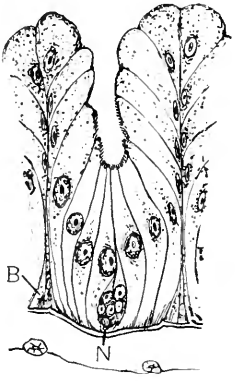


FIG. 3. — Resting epithelium after two weeks' fasting, × 220: parts as in Fig. 2. At *B* are the bases of cells to which the wedge-shaped apices opposite are believed to belong.

II. THE DISCHARGE OF THE DIGESTIVE SECRETION.

After food is eaten it is perhaps an hour before it makes its appearance in the ventriculus. As soon as it enters, the more turgid of the epithelial cells begin to be discharged bodily in whole or in a large part to mix with it. This discharge begins

[1] There is no supporting connective tissue between these cells. In *Corydalis cornuta* L. the basement membrane is produced upward in anastomosing plates with which all the cells are in evident contact.

at the anterior end and continues posteriorly with the progress of the food. When the nymph has gone long without food and there is much accumulation, the discharge is correspondingly great. It is in fact remarkable to see how large a part of the epithelium will thus at once be destroyed. I have chosen, however, to show in the drawings the discharge taking place after moderate or slight accumulation, as being more normal and on the whole more instructive.

Fig. 5 represents a normal discharge from epithelium in which the accumulation had been slight and somewhat irregular. The portion included between two nidi on the right side is discharged; that on the left unaffected. The preparation was made from a nymph fed an hour and a half before fixing and from a region just before the middle of the ventriculus.

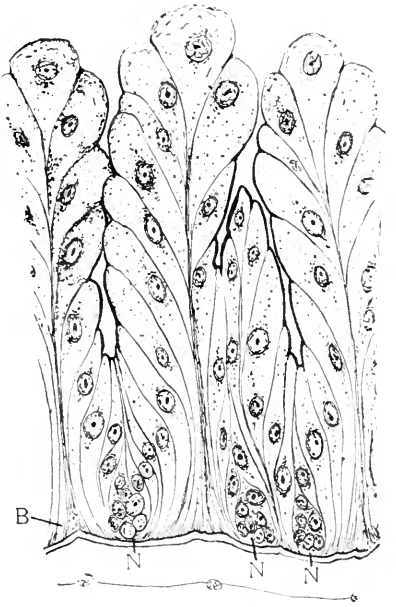


FIG. 4. — Resting epithelium after two months' fasting, × 220: parts as in Figs. 2 and 3.

Each globule of discharge represents the larger part of an epithelial cell, including generally its nucleus. Sometimes the globule appears to be cut off from the cell substance below it by a wall formed previous to its discharge, but much more often it presents the appearance of having been crowded out by the compression of adjacent cells, in which case it is narrowed to a more or less slender point (*B*, Fig. 5). In no case have I seen a small portion of the cell contents protruding through a cleft in the striated border as found by Van Gehuchten[3] in *Ptychoptera contaminata;* but in all cases the discharge involved the whole free end of the discharging cell from which the striated border had disappeared as if by solution.

[3] VAN GEHUCHTEN, 1890: Recherches histologiques sur l'appareil digestif de la larve de la Ptychoptera contaminata. *La Cellule*, vi.

Fig. 6 is from a nymph that was fed three hours before fixing. A large area (*D*) is discharged; groups of cells remain in which the discharge is yet to occur.

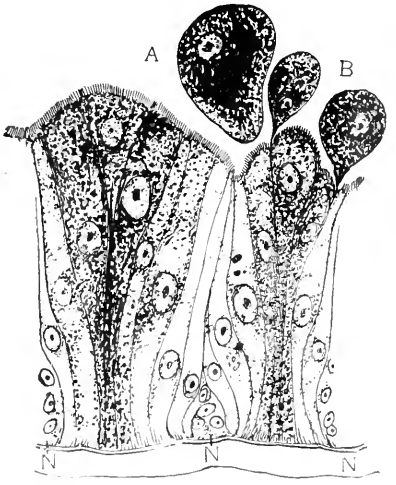


FIG. 5. — The beginning of a discharge in which the left-hand portion shown is not yet involved, two hours after feeding, × 275. *A*, a large detached globule of secretion. *B*, smaller globules still slightly connected by a slender stalk-like portion, compressed between the remaining cells.

Fig. 7 is from the same series, but shows a larger area in which a very moderate discharge is in various stages of progress. The forked summits of the cells at the right seem to indicate that the discharge in them was partial. Two nuclei are found not uncommonly in these cells (see Figs. 4, 6, 8), only one of which appears to be lost at a time.

Fig. 8 is from the same series, but from a point nearer the anterior end, and thus represents a later stage. It shows, in fact, the end of the discharge. The beginning occurred midway between the nidi, but these last rounded globules came from points nearly opposite them. The intervening cells have already acquired a border; they have plenty of room, are now nearly cylindrical, and the prominent internal folds (Figs. 2, 3, 4), due to later compression, have not yet appeared. A study of hundreds of such sections shows the progress of the discharge to be about as follows. The discharge begins in the anterior end of the ventriculus about an hour after feeding simultaneously with

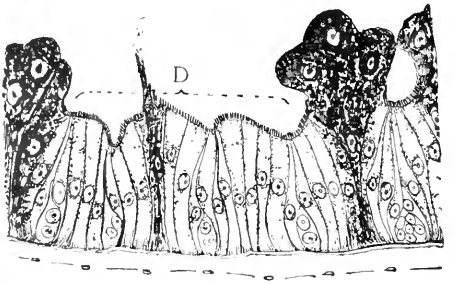


FIG. 6. — An irregular discharge found three hours after feeding, × 150. The discharge is complete throughout the portion *D*, on the point of beginning on either side. These different conditions found side by side afford opportunity for an interesting comparison of the state of the nidi.

the appearance of the food. Here it reaches its height in something less than two hours, and in three hours is about finished. It progresses slowly through the length of the ventriculus, beginning at the posterior end in something less than three hours, and being completed in six to eight hours after feeding. Thus in the posterior end, where the cells are somewhat longer and more numerous, it will be seen the discharge occupies a longer time.

III. The Replacement of the Discharged Cells.

The long cells which extend from the basement membrane to the lumen of the ventriculus and the little round cells nestled together against the basement membrane form two markedly

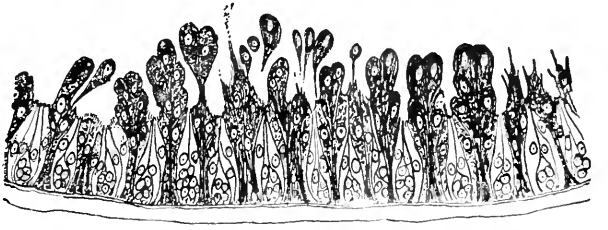


Fig. 7. — A normal discharge of the digestive secretion from the same series as Fig. 6, × 105.

different parts of the epithelium. The latter have been discussed under various names corresponding to the diverse views held as to their function. They have been called *cryptes, drüsen-crypten,* and *drüsen* by Basch,[4] Frenzel,[5] and Faussek[6] respectively. More recently Visart[7] has considered them to be glands. This view has been copied widely.

They have been called *germinal buds* by Miall and Denny,[8]

[4] Basch, 1858: Untersuchungen über Chylopoetische und uropoetische System der Blatta orientalis, *Sitzungsbr. der k. k. Akad. Wiss. Wien, math.-nat. Klasse,* xxxiii.

[5] Frenzel, 1885: Einiges über den Mitteldarm der Insekten sowie über Epithelregeneration, *Archiv. Mier. Anat.,* xxvi.

[6] Faussek, 1887: Beiträge zur Histologie des Darmkanals der Insekten, *Zeit. Wiss. Zool.,* xlv.

[7] Visart, 1894: Contr. &c. tubo digerenti Arthropoda, *Atti d. Soc. Toscano de Sci. Nat.,* xiii. Of this paper I have seen only abstracts.

[8] Miall and Denny: The Cockroach, London, 1886.

and have been discussed as centers of replacement by Balbiani,[9] Bizzozzero,[10] and others. Throughout my work I have used the term *nidus* as a convenient, descriptive, noncommittal name.

Most of the recent investigators have looked upon them as centers of regeneration, with various reasons for taking that view. Van Gehuchten, finding the nucleus to be discharged with the secretion, based his belief largely on the theory that when a cell loses its nucleus it must die and be replaced. Balbiani found nidi made up of cells varying in number and size, and assumed these to represent different stages of development. Bizzozzero and Cuénot attach most significance to the mitotic figures found in the nidi and not found elsewhere in the epithelium, a fact noted by many writers. This evidence is good so far as it goes. The mitotic figures indicate that cell division is in progress here, but tell nothing of what may be doing elsewhere. It is now my privilege to add as a result of experimental studies some additional evidence of the correctness of the view that each nidus is a germinative center.

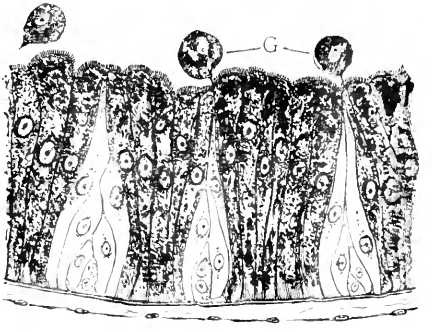


FIG. 8. — The end of the discharge, × 220, from the same series as Figs. 6 and 7, but from the anterior end of the ventriculus. *G*, the last of the globules to be thrown off; these are nearly opposite the nidi.

This evidence is based on observations upon (1) the *source* of the *digestive secretion;* (2) *differentiation by reagents;* (3) the *position* of the nuclei; (4) the decrease in the number of cells in the nidi during digestion; and (5) the encroachment of young cells upon the old.

(1) After tracing the discharge from beginning to end through several series of experiments, it is perfectly evident that the

[9] BALBIANI, 1890: Études anatomiques et histologiques sur le tube digestif des Cryptops, *Arch. de Zool. Exp.*, xi.

[10] BIZZOZZERO, 1893: Ueber die Schlauchförmigen Drüsen, etc., *Arch. Mier. Anat.*, xlii.

digestive secretion is given off from the cells between the nidi, and never any part of it from the nidi themselves. This is as shown in preceding figures. The slender tube Frenzel found connecting the nidus with the digestive cavity (which made it desirable for the *cryptes* of Basch to be rechristened *drüsen cryptcn*) has no existence. What so appeared was doubtless the slender prolongation of a young cell growing toward the ental surface.

(2) After witnessing the discharge of the digestive secretion, there is no question as to the part that is functional in producing it; and when that part is affected by a given reagent while another is not, the second must be of different character. Hermann's fluid blackens all those epithelial cells which are discharging or ready to discharge their secretion, and leaves the small cells of the nidus, the membrana propria, and muscles equally pale and clear. With haematoxylin and eosin, the eosin stains deeply the functional cells, including the nuclei of the more elevated ones, while the haematoxylin with its usual selectivity for germinative protoplasm stains the cells of the nidus most deeply.

(3) In sections favorable for showing the typical structure the nuclei of the cells are arranged in rows extending from the nidus obliquely upward into the folds (see Figs. 2–4); these lines indicate the course of their progress toward the surface. I have whole series of sections of the stage shown in Fig. 3, throughout which the nuclei are beautifully festooned between the elevations of the ental surface. Naturally, the nidus shows as in the figure only when the section is central. An irregularity of common occurrence is shown at the right side of Fig. 4, while the left side of that figure is quite regular. In nearly every case it is easy to follow two outgoing lines of nuclei from each nidus.

(4) When the accumulation of digestive secretion is considerable, the nuclei of the nidus are numerous; after its discharge one finds but few of them remaining. But if one will add to the number remaining the number of the new cells which have suddenly appeared beside them which are not blackened by Hermann's fluid, he will have a number about equal to the

original number of nuclei in the nidus, a fact sufficiently accounting for their disappearance. Within the nidus at the right of Fig. 6, the cells are more numerous than in those nidi within the area from which the secretion has entirely been given off. Compare also Figs. 3 and 8.

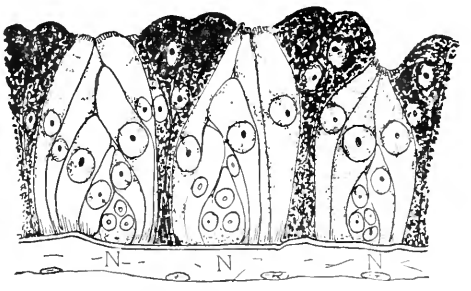


FIG. 9. — Epithelium, six hours after feeding, showing newly formed cells reaching up to the surface, replacing those discharged, × 220.

(5) So far as one may judge, division within the nidus takes place with great regularity. As new cells develop at the base of the nidus, the older ones are crowded upward. The nucleus of each grows rapidly, and soon acquires a spindle-shaped cell body. When preëxisting cells are not being used up in the digesting of food, the young ones thus formed grow slowly, and making their way upward against the compression of the older cells, are crowded into crescentic form. But they continue to increase, nevertheless, as a glance at Figs. 2, 3, and 4 shows. But when the presence of food occasions the removal of the surcharged older cells, the increase in size of the younger ones is remarkably rapid. Just before a discharge, Hermann's fluid blackens all the cells except the spherical ones immediately within the nidus: just after, it leaves several cells on each side entirely clear: and the inference is that these cells have sprung up during that short interval, the lateral compression of the older cells being removed by their discharge, and have attained their growth but have not yet become functional.

FIG. 10. — A dissociation, showing the constitution of the nidus *N*, × 240.

Fig. 9 shows the young cells apparently in the act of crowding their way to the surface. The blackened area represents all that remains of the part that was functional before the last discharge. The clear area consists of cells that were uppermost in the nidus, but which have suddenly grown up to adult and functional proportions. If this

be not actual replacement happening before one's eyes, it comes as near it as the nature of the case will admit.

One naturally asks what becomes of the remnants of the old cells, and whether there is any connection between their disappearance and the rapid growth of the young cells. On these questions I have obtained no light.

Summarizing their history, we may say, then, in a word, the epithelial cells originate by divisions in the cells of the nidus, grow, crowd their way to the surface, acquire a striated border, become functional, secrete, discharge, digest, and die, and then give place to others which will run the same swift course.

Volume I. *October, 1897.* *Number 3.*

ZOÖLOGICAL BULLETIN.

CONTRIBUTIONS ON THE MORPHOLOGY OF THE ACTINOZOA.

IV. ON SOME IRREGULARITIES IN THE NUMBER OF THE DIRECTIVE MESENTERIES IN THE HEXACTINIÆ.[1]

J. PLAYFAIR McMURRICH.

THE discovery of three of the principal features of the morphology of the Hexactiniæ, — the hexamerous arrangement of the mesenteries, their association in pairs, and the occurrence of directives, — we owe to several observers. Ehrenberg ('34) was, I believe, the first to note the hexamerous arrangement; the association of the mesenteries in pairs was first discovered by Erdl ('42) but fully worked out by Hollard ('51) a few years later; while the occurrence of directives was first observed by Thorell ('58), and more fully described by Schneider and Rötteken in 1871. The study of numerous species has demonstrated the general occurrence of these three characteristics, which may be regarded as normal for the group, but departures from the normal arrangement have also been observed. Thus, instead of hexamerism, a decamerous or an octamerous symmetry may obtain; indeed, one of the earliest thorough studies of members of the group, namely that by Hollard, was made upon a decamerous form. So too, although two pairs of directive

[1] Nos. I–III of these Contributions were published in the *Journal of Morphology*, vols. iv and v.

mesenteries are usually present, nevertheless, in a number of cases, a reduction to a single pair has been observed, not only in the Halcampidæ, but also in other less primitive families, and more especially in the Sagartidæ.

This condition, which is associated with a corresponding reduction of the number of siphonoglyphes, was observed by Thorell ('58) and has been studied more recently by A. F. Dixon ('88), Carlgren ('93), and Parker ('97). I have made some observations on monoglyphic specimens of Metridium marginatum, but Parker's observations on the same species have been so much more extensive that a record of my results seems unnecessary, as they throw no additional light on the significance of the abnormality.

The reverse condition, an increase in the number of the directives beyond the normal, is apparently much less frequent, and has, up to the present, been described only by G. Y. and A. F. Dixon ('89) in Bunodes thallia, and by Parker ('97) in Metridium marginatum. Parker found three siphonoglyphes and three pairs of directives in only *one* out of one hundred and thirty-one specimens ; but in four specimens of Bunodes thallia the Dixons found that one had two pairs of directives, one *three* pairs, one *four* pairs, and one only one pair. In the Bunodes as in the Metridium the siphonoglyphes corresponded in number with the directives, and furthermore it is to be noted that an irregular arrangement of the mesenteries was also found in all the specimens which presented what may be termed, following Parker's nomenclature, a polyglyphic condition. An increase in the number of siphonoglyphes has also been found in two other species and probably denotes a corresponding increase of directives ; the Dixons ('91) describe the occurrence of three grooves in a specimen of Metridium dianthus, and Haddon and Shackleton ('93) state that from two to seven (!) siphonoglyphes occur in different specimens of Condylactis Ramsayi.

I have recently had the opportunity of examining a species in which the number of the directives seems to be as a rule increased above the normal. This is the form originally described by Verrill ('83) as Sagartia spongicola, several speci-

mens of which I found in a collection from the West Indian seas made by the Bahama Expedition of the State University of Iowa. I have been able to compare the specimens with some kindly sent me by the U. S. Fish Commission; and in all have examined seven specimens, each of which showed abnormal arrangements of the mesenteries. Three cycles of mesenteries occur, the members of the first cycle alone being perfect; and the third cycle is sometimes incomplete. I give below in tabular form a statement of the arrangement of the perfect pairs of mesenteries in each specimen, those pairs which are directives being indicated by the letter D.

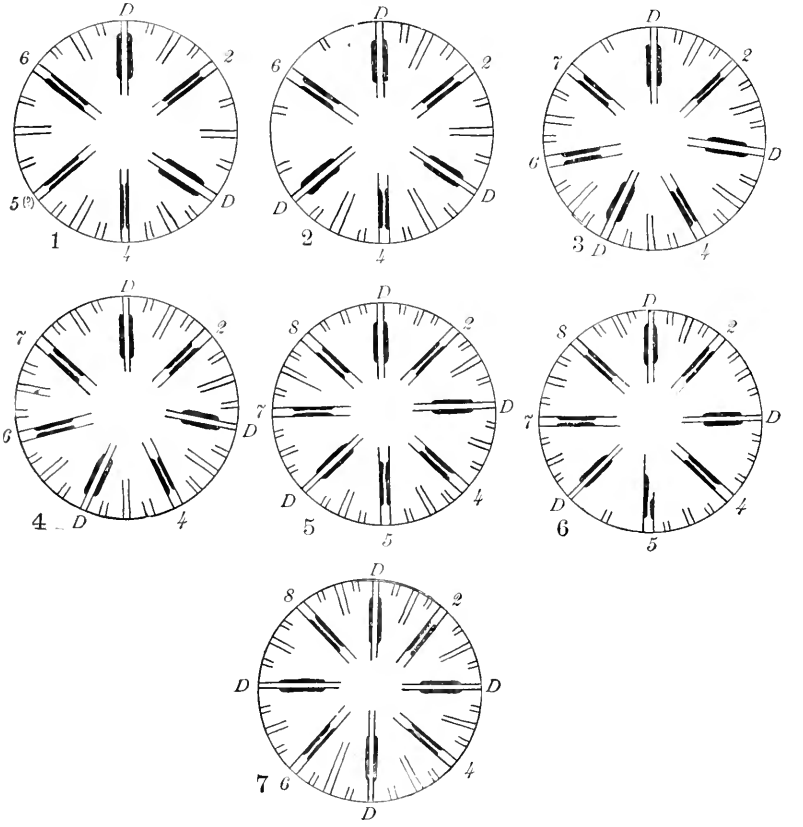
Specimen No. 1	D—2—D—4—5—6
" No. 2	D—2—D—4—D—6
" No. 3	D—2—D—4—D—6—7
" No. 4	D—2—D—4—D—6—7
" No. 5	D—2—D—4—5—D—7—8
" No. 6	D—2—D—4—5 (?)—D—7—8
" No. 7	D—2—D—4—D—6—D—8

In drawing up this table I have in all cases started with a directive, though it is of course impossible to say that in each case the tabulation has commenced with the same mesentery. Specimen No. 6 was received from the U. S. Fish Commission, the rest are from the Iowa University collection.

It will be seen from this table that of the seven specimens two are arranged on the hexamerous plan, two on a heptamerous plan, and three on an octamerous, though with regard to specimen No. 6 it is not quite certain whether the pair numbered 5 belongs to the first or second cycle, its relations to adjacent second and third cycle mesenteries indicating, however, its probable membership in the first. Furthermore it will be noticed that in one specimen there are two pairs of directives, which, however, are not opposite each other but are separated by only a single pair of the first cycle; in five specimens there are *three* pairs of directives, and in one specimen *four*. It is possible that specimen No. 1 should be credited with three pairs of directives, since there is some uncertainty concerning the pair of mesenteries numbered 5, one member

of which appears to have its longitudinal muscles on its endocœlic surface, while in the other they appear to be on the exocœlic side.

In order to show the arrangement of the directives more perfectly, as well as the relations of the mesenteries of the second and third cycles, I give below a diagrammatic representation of a transverse section of each of the specimens examined.



A complete absence of all directives has been described by Boveri ('93) for the genus Gyractis; and for the reception of this form he has proposed an order Holactiniæ, considering the radial arrangement of the mesenteries which the absence of the directives brings about, sufficient to warrant the separation of forms showing it from the "biradial" Hexactiniæ.

Boveri traces these forms back phylogenetically to a stage antecedent to the appearance of the mesenteries in cycles and in pairs, *i.e.*, to a stage where the mesenteries developed, as in the Edwardsiæ, simply and bilaterally. By the development of two mesenteries on each side, the Edwardsia stage was brought into an hexactinian condition, and the formation of another mesentery on each side of one of the Edwardsian directives produced the Scytophorus condition. A further continuation of this process, the development of another mesentery on each side of the other pair of directives, resulted in the Holactiniæ, which, it must be assumed, later acquired a tendency to develop additional mesenteries cyclically and in pairs as do the Hexactiniæ.

Boveri consequently assumes the actual persistence of the Edwardsian directives in both Scytophorus and Gyractis, the absence of one pair in the former genus and of both pairs in the latter being only apparent. With his views as to Scytophorus I fully coincide, but believe that in Gyractis we have to deal with an hexactinian in which both pairs of directives have disappeared, — just as one pair has disappeared in numerous specimens of Metridium marginatum, the mesenteries which really represent them having developed their longitudinal muscles on adjacent faces.

On reading Boveri's paper I recalled the fact that I had been unable to make out directive mesenteries in Ricordea florida ('89), and I again subjected my preparations of that form to a close scrutiny. The stomatodæum of this species is oval, its walls having numerous longitudinal ridges, but there are no siphonoglyphes. Owing to the disc-like form of the column and the moulding of the base over the irregularities of the surface to which the animals attach themselves, it proved difficult to obtain perfectly satisfactory transverse sections of the entire column. In two cases, however, I succeeded in getting sections from which I could be reasonably certain as to the presence or absence of directives ; in one case I found *no* directives and in the other a single pair, which, however, was not opposite the end of the long axis of the stomatodæum, but to one side of it.

With these results I turned to the related genus Rhodactis and examined three specimens of R. Sancti-Thomæ. In my original description ('89) of this form I described one pair of directives as being present, and somewhat hastily assumed the presence of the second pair, though I could not be sure of its existence. A further examination showed that in one specimen but a single pair of directives was present and that pair was situated some distance from the end of the long axis of the stomatodæum; in a second specimen one pair was again present, also to one side of the axis of the stomatodæum, but at the opposite end of the stomatodæum there was some irregularity in the arrangement of the mesenteries, and I could not be certain that a second pair of directives was not present there; and finally in a third specimen I found *no* directives, though here again, owing to certain mesenteries not being cut exactly transversely, it is impossible to affirm their absolute non-existence.

These results, especially those obtained from Ricordea, seemed to me to have considerable bearing on the validity of the proposed order Holactiniæ. Recently two other observations of forms lacking directives have been recorded. Haddon and Duerden ('96) failed to find directive mesenteries in Cystiactis tuberculosa, and Kwietniewski ('97), finding none in Thalassianthus senckenbergianus and T. aster, has established for these forms an order Thalassianthæ. We have, then, four different genera belonging to as many distinct families, according to the usual classification, and yet presenting the essential peculiarity upon which the new order is based. If the order is a valid one, then we must separate Gyractis from the Bunodidæ, which it seems to resemble most nearly. Ricordea must be removed from its association with Rhodactis, to which it is closely related even in its histological peculiarities; Cystiactis must be regarded as belonging to an entirely different group from Alicia; and Thalassianthus must be placed in a new order even though it should prove necessary to separate it from Actineria. But this is not all. We must place one specimen of Ricordea florida in the new order and leave another among the Hexactiniæ, and we must similarly place one specimen of

Thaumactis medusoides (Fowler, '88) in the new order and leave the other out; and so with Sagartia undata (Carlgren, '93). This, it seems to me, is a *reductio ad absurdum.*

The complete absence of directives is not necessarily a phylogenetic peculiarity, but one extreme of the same tendency to an irregularity in the number of the directives of which many examples are now known. There seems to be no more reason for considering the complete absence of directives a peculiarity of ordinal importance than there is for so regarding their reduction to a single pair or their increase to three or four pairs. It is rather a peculiarity which is essentially individual, possibly rising secondarily in some cases to the value of a specific or even a generic character, and to regard it as of ordinal value is, it seems to me, inconsistent with our present ideas as to the phylogeny of the Anthozoa.

In conclusion it may be pointed out that the irregularities which have been described in the arrangement of the mesenteries of Gyractis and Thalassianthus are just what might be expected to occur in connection with the absence of directives, since we usually find considerable departure from the normal arrangement even in forms which lack only one pair (*cf.* Thorell, '58, Carlgren, '93, and Parker, '97).

UNIVERSITY OF MICHIGAN,
June 10, 1897.

LITERATURE.

'34 EHRENBERG, C. G. Die Corallenthiere des rothen Meeres. Berlin. 1834.

'42 ERDL. Beiträge zur Anatomie der Actinien. *Müller's Archiv.* 1842.

'51 HOLLARD, H. Monographie Anatomique du Genre Actinia de Linné. *Ann. Sci. Nat.* 3me sér., xxv. 1851.

'58 THORELL, T. Om den inre byggnaden af Actinia plumosa Müll. *Ofvers Kongl. Svensk. Vet.-Akad. Forhandl.* 1858.

'83 VERRILL, A. E. Report on the Anthozoa, etc. *Bull. Museum Comp. Zoöl.* xi. 1883.

'88 DIXON, F. On the Arrangement of the Mesenteries in the Genus Sagartia Gosse. *Sci. Proc. Royal Dublin Soc.*, N. S. vi. 1888.

'88 FOWLER, G. H. Two New Types of Actiniaria. *Quart. Journ. Micr. Sci.* xxix. 1888.

'89 DIXON, G. Y. AND A. F. Notes on Bunodes Thallia, etc. *Sci. Proc. Royal Dublin Soc.* N. S. vi. 1889.

'89 MCMURRICH, J. P. The Actiniaria of the Bahama Islands, W. I. *Journ. of Morph.* iii. 1889.

'91 DIXON, G. Y. AND A. F. Report on the Marine Invertebrate Fauna near Dublin. *Proc. Roy. Irish Acad.* Third Series, ii. 1891.

'93 HADDON, A. C., AND SHACKLETON, ALICE. Descriptions of some New Species of Actiniaria from Torres Straits. *Sci. Proc. Royal Dublin Soc.* N. S. viii. 1893.

'93 CARLGREN, O. Studien über nordische Actinien. *Kongl. Svensk. Vet.-Akad. Handl.* xxv. 1893.

'93 BOVERI, TH. Das genus Gyractis. *Zoöl. Jahrb. Abth. für Syst.* vii. 1893.

'96 HADDON, A. C., AND DUERDEN, J. E. On some Actiniaria from Australia and Other Districts. *Sci. Trans. Royal Dublin Soc.* Third Series, vi. 1896.

'97 KWIETNIEWSKI, C. R. Actiniaria von Ternate. *Abhandl. Senckenberg. Gesellsch.* xxiii. 1897.

'97 PARKER, G. H. The Mesenteries and Siphonoglyphs in Metridium Marginatum Milne-Edwards. *Bull. Mus. Comp. Zoöl.* xxx. 1897.

ORIGIN OF THE PRONEPHRIC DUCT IN SELACHIANS.

EMILY RAY GREGORY.

A RENEWED study of the origin of the pronephric duct in Selachians was suggested to me by Dr. W. M. Wheeler of the University of Chicago, in whose laboratory the work has been carried on, and to whom I am indebted for the material used and for helpful directions.

There have been different opinions on this subject, which are well represented by the papers of Joh. Rückert,[1] J. W. van Wyhe,[2] and Carl Rabl,[3] to which reference will be made later. The paper by Laguesse,[4] who also used *Acanthias*, was inaccessible. The material used was *Acanthias* embryos, of which there were quite a number, showing different stages of development. Several methods of killing and hardening were employed, but all gave essentially the same results. Cross sections were examined with great care, so that no misinterpretation might occur through slanting sections; and where a question of fusion was involved, a Zeiss $\frac{1}{12}$ immersion lens was employed.

In an embryo of 25 somites the Anlage of the pronephros is seen to consist of segmental outgrowths of the somatic layer of the somites, beginning at the seventh and extending over six segments. There is a slight difference in the development on the two sides of the embryo in respect to the position of the anterior end. These outgrowths are connected with the ectoderm at their outer edge, and in places this layer seems to

[1] Ueber die Entstehung der Excretionsorgane bei Selachiern, Joh. Rückert, *Archiv. für Anat. und Phys.*, 1888.

[2] Ueber die Mesodermsegmente des Rumpfes und die Entwickelung des Exkretionssystems bei Selachiern, J. W. van Wyhe, *Arch. f. m. Anat.*, Bd. 33, 1889.

[3] Ueber die Entwicklung der Urogenitalsystems der Selachier, Carl Rabl, *Morph. Jahrbuch*, Heft 4, 1896.

[4] Sur le développement du Mesenchyme et du pronéphros chez les Sélaciens (Acanthias), *Comptes rendus hebdomadaires des séances et mémoires de la Société de Biologie*, tome iii, série ix, 1891.

have proliferated at the point of union, and to have become more than one cell thick. When this connection with the ectoderm is artificially severed, the appearance of the Anlage and the overlying ectoderm shows that the connection must have been a true fusion. In some cases the Anlage seems to carry away with it a few cells of ectodermal origin. Camera drawings showing these conditions from the two sides of an

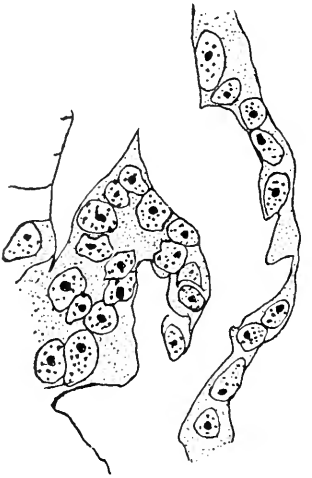


FIG. 1.

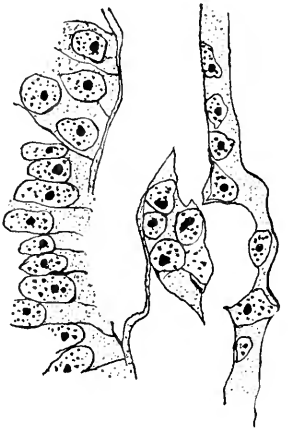


FIG. 2.

embryo are seen in Figs. 1 and 2. This fusion is, however, of very short duration, and there is no sign of it in later stages.

The part of the duct receiving the pronephric collecting tubules is developed with the pronephros from this same Anlage, but the origin of the duct proper, *viz.*, that portion which lies beyond the last pronephric tubule, is more difficult to establish. The preparations made showed in all stages a very constant condition of fusion between the tip of the distally growing duct and the ectoderm. In some cases this fusion extended for a considerable distance, in others over only a few sections, perhaps not more than one or two. There were only one or two exceptions, which could be readily explained by accidental separation or from the short duration of the union. On the other hand, there were cases where the duct existed perfectly free in the space between the somites and the ecto-

derm for such a distance that the sudden turn and fusion with the ectoderm was very marked.

Fig. 3 (*a*, *b*, *c*, *d*, *e*) shows five successive sections at the end of the left pronephric duct of an embryo 5 mm. long (about 33

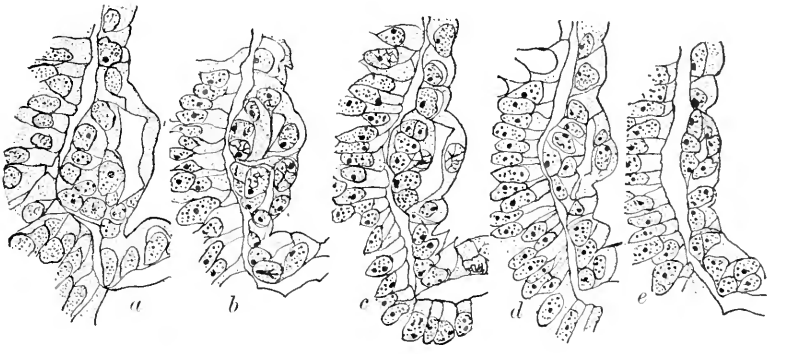


FIG. 3.

somites). Fig. 4 (*a*, *b*) shows two sections near the end of the right duct of the same embryo. Fig. 5 (*a*, *b*, *c*, *d*, *e*, *f*) shows the conditions at the end of the left duct of another embryo 5 mm. long (about 30 somites). Between *e* and *f* ten sections

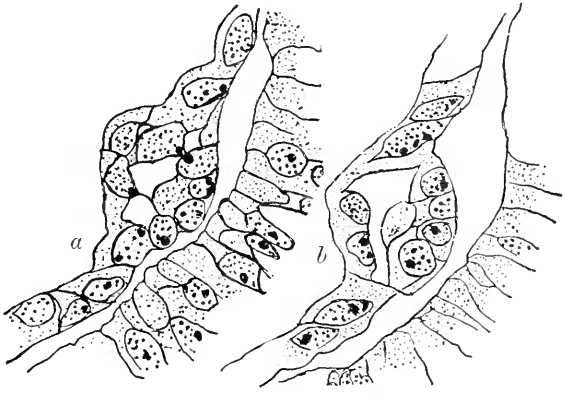


FIG. 4.

similar in character to *e* are omitted. In *f* the two cells which form the end of the duct are deeply stained and readily distinguished from the others. In this case the fusion is of unusually long duration. In addition to many series like the above,

one section was found (Fig. 6) in which it is evident from the deeper stain of the nuclei that karyokinesis has just taken place between the ectoderm and the duct. It is not possible that these are two adjacent cells dividing longitudinally, since the sections immediately preceding and following show no such condition.

While the duct is separating from the ectoderm in the older regions, the point of division is very often marked by projecting points of tissue on both duct and ectoderm. Sometimes

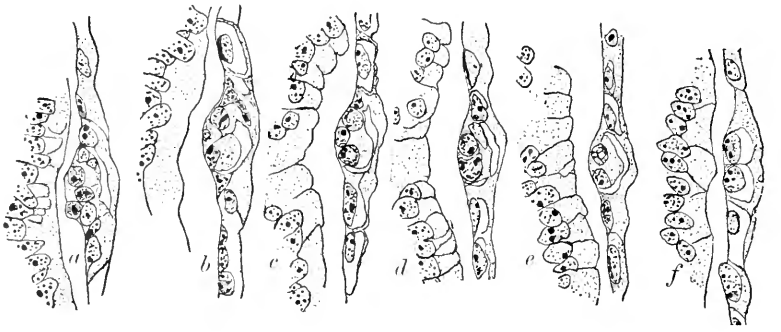


FIG. 5.

this may be noticeable only on the one or the other. Drawings of two such sections are represented (Fig. 7, *a*, *b*).

A very few division figures were found in the duct, but its increase in size while separated from all other tissues would of itself indicate that such growth must take place. Although the duct frequently lies for much of its length close against the somites, there is always a distinct line of separation. The nuclei in the outer mesodermal layer are also very constantly found at the inner end of the cells, as may be seen from the sections figured. In frontal sections the entirely different and independent arrangement of cells in the duct and in the somites is also very marked.

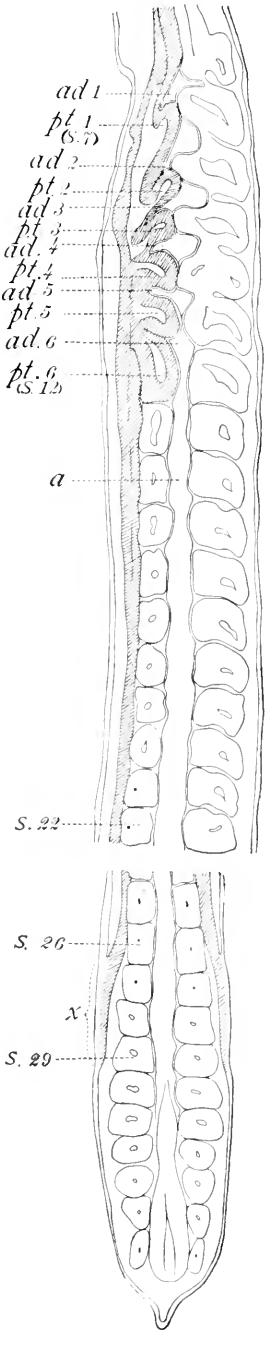
Fig. 8 is a reconstruction from frontal sections of an embryo 6½–7 mm. long, with about 38 somites (two gill slits open). This shows that the pronephros on the right side consists of six tubules, the first of which is developed from the 7th somite, with four aortic branches running between. At either end is

another small diverticulum of the aorta. The duct lies close against the mesoderm as far as the 26th somite, where it bends outwards and fuses with the ectoderm opposite the 27th somite. The fusion continues to the end of the duct opposite the 29th somite. The pronephros on the left side of the same embryo also begins at the 7th somite, but has only five tubules with three aortic diverticula. The end of the left duct may be seen in the figure. The aortic branches on the right side seem to correspond very closely with the descriptions already given by Paul Mayer, Rückert, van Wyhe, and Rabl. On the left there seem to be only wide pockets pushed out from the aorta. In embryos of from 7 to 10 mm. long these reach the wall of the pronephric tubule. Running from one to another (in one case over three successive segments) were found structures which may be interpreted as glomi. These have also been seen by van Wyhe, who says (p. 480): "Es ist klein, sehr vergänglich und besteht aus einem gefässführenden Strang, der von der dorsalen Lippe eines Ostiums in schräger Richtung zur ventralen zieht. Der Strang erstreckt sich in seiner Mitte ringsum vom Peritonealepithel bekleidet, frei durch die Leibeshöhle, und ist an beiden Enden befestigt." Similar rudimental glomi were found on the right side, although the presence of the "Darmgefässe" modifies their position. The objection made by Rabl to this interpretation does not seem to be tenable in the case of a rudimental organ, where one does not expect to find the structure perfect or complete.

FIG. 6.

FIG. 7.

In a number of the embryos the primitive ova containing



granules resembling yolk, mentioned by Balfour and other observers, are very conspicuous. They occur in the somatopleure as well as in the splanchnopleure.

From the facts here given it seems reasonable to conclude that the earliest Anlage of the pronephros fuses temporarily with the ectoderm, and may possibly receive some few cells from it. The first part of the duct proper, lying just beyond the last pronephric tubule, seems necessarily to share to some slight degree in the mesodermal origin of the anterior region. This follows from the fact that there is no line of division between the two regions of the duct, and that Rabl and other observers have shown that karyokinesis occurs throughout the length of the duct. Most important of all, we must conclude that so far as the duct develops distally, the connection of its tip with the ectoderm is maintained. The mere fact of the actual fusion of the tip of the duct with the ectoderm throughout its growth would be sufficient evidence of a genetic relation for those who accept the principle of the teloblastic growth of organs. The position of the pronephros in *Acanthias* seems to be identical with that in *Pristiurus* as found by van Wyhe and Rabl. It will be seen that the results here

FIG. 8. — a = aorta, ad = aortic diverticulum, pt = pronephric tubule, S = somite, x = fusion of duct with ectoderm.

given correspond with those of Rückert (p. 215) in so far as the union of the Anlage of the pronephros with the ectoderm is concerned. Apparently Rabl describes the same thing in his "sichelförmige Masse" or "Strang" (637), but the appearance of the ectoderm in his drawings, though somewhat diagrammatic (XIII, 3, 4, 5, 11, 12), seems to support Rückert's interpretation of the immediate relations rather than his own. The phylogenetic conclusions might be the same. In regard to the genetic connection between the ectoderm and duct the results harmonize with those of Rückert and van Wyhe. It seems impossible, however, to draw so sharp a line as Rückert has drawn between the end of the pronephros and the beginning of the duct, and to say that the duct is derived only from the ectoderm. On the appearance of the paper by Rabl, my preparations were again examined carefully, but without finding any grounds for abandoning the view that there is a genetic connection between the duct and the ectoderm in Selachians.

UNIVERSITY OF CHICAGO,
May 8, 1897.

DR. GADOW AND MISS ABBOTT ON THE VERTEBRAL COLUMN OF FISHES.

O. P. HAY.

In the *Publications of the Field Columbian Museum*, vol. 1, pp. 1–54, the writer issued a paper entitled, "On the Structure and Development of the Vertebral Column of Amia." Before this paper appeared, but after it had passed out of my control, there was printed in the *Philosophical Transactions, Royal Society of London*, vol. 186, pp. 163–221, a paper by Dr. Hans Gadow and Miss E. C. Abbott, the title of which is, "On the Evolution of the Vertebral Column of Fishes." This is in many respects an excellent treatise, partly on account of the attempt to define accurately many expressions which have been used rather loosely, and to introduce other terms which are more concise than those hitherto employed. The confirmation of Klaatch's results regarding the manner of development of the vertebral centra of sharks is important, as is also the authors' determination of the origin of the elastica externa. The results of their study of Amia are, of course, of interest to myself, and in some respects they anticipated my own conclusions. On the other hand, the paper is somewhat marred by a number of typographical errors, by the apparent transposition of paragraphs (p. 175), and by rather unsatisfactory text figures. Aside from these minor defects, the work, it seems to me, is pervaded by an erroneous theory concerning the origin of the skeletogenous tissue and the perichordal cartilages.

I will first of all make a remark regarding the definitions of the two kinds of vertebral centers recognized by Dr. Gadow and Miss Abbott. They animadvert on Kölliker's terms "chordal centra" and "perichordal centra"; but the chordal sheath, being a product of the notochord, might not improperly be regarded as a part of the notochord itself; and then Kölliker's names would be appropriate. On the other hand, our authors proceed to say that the centra of the higher Vertebrata,

Kölliker's "perichordal centra," are more properly chordal than are the "chordal centra" themselves; nevertheless, they adopt Kölliker's term in the sense employed by him. If in this connection it is necessary to keep up the distinction between the notochord and its sheath, it would be better to adopt the proposed term, "auto-centra."

One of the most interesting propositions advanced by Gadow and Abbott is that relating to the origin of the skeletogenous tissue. According to them, cells are given off from the ventral portion of the protovertebra, which cells form a pyramidal mass whose base lies against the lower border of the notochord, but whose apex rises to its upper border or higher. It has hitherto been held that all, or nearly all, of the skeletogenous layer has been thus derived. But these authors have discovered another source of skeletogenous cells, and, to judge from the diagrams presented on page 188, at least one-half of these cells originate there. This new fountain of formative materials is found in the upper portion of the protovertebræ. Cells are there emitted which form an inverted pyramid, the apex of which extends to the lower border of the notochord. The dorsal pyramid grows downward in front of the ventral pyramid belonging to the same protovertebra. The bases and the apices of these two sets of pyramids are capable of developing into cartilage, an intermediate zone not attaining this capability. The result is that the bases give rise to the neural and hæmal arches (basidorsals and basiventrals), while the apices give origin to the "intercalated cartilages" (interdorsals and interventrals). From this it will be seen that the two dorsal ridges of the embryo, from which arise the neural arches, are in small part only derived from the lower portion of the protovertebræ while the two ridges below the notochord receive some materials from the upper portion of the protovertebræ.

It may appear remarkable that so great a mass of cells is contributed to the skeletogenous layer by the upper ends of the protovertebral plates without its having been discovered long ago. The production of the dorsal contingent proceeds not earlier than the other, and many observers have witnessed the upward growth of the ventral cells.

Considering too that this discovery is of so much importance, it is unfortunate that the authors did not illustrate their paper with better and more convincing figures. Several text figures representing cross sections are given on page 181; but they are hardly satisfactory. Taking into consideration the curvature of the protovertebral plates it must be exceedingly difficult, in cross sections, to distinguish the limits between two such interdigitating masses of cells. One would suppose that sagittal sections would furnish better evidences, but the authors have not given such a section through the proper portion of the tail.

It has been my fortune to be permitted to examine several well-prepared series of embryos of *Squalus acanthias;* and for this opportunity I am indebted to Dr. W. M. Wheeler, of the University of Chicago. One series consists of sections of a specimen only 5 mm. in length; two other series belonged to embryos 6 and 7 mm. long. I have studied these with the view of testing the statements of the authors referred to, and I trust that I have done this without prejudice. I know no reason for objecting to the theories of the learned authors, in case they are well founded.

In the tail of the 5 mm. *Squalus* cells from the lower border of the protovertebra are seen to be given off toward the notochord. They lie against the latter on each side, and the cluster extends above the notochord so as to reach the lower border of the spinal cord. More anteriorly the skeletogenous cells rise still higher. It was observed that, as the interprotovertebral spaces are approached, the cells appear to drop to a lower level, and may then rise not higher than the middle of the notochord; but this condition lasts for only the thickness of about two sections. It seems to me possible that this may furnish an explanation of some of the appearances presented in the text figures given by the authors on page 181.

In no sections of specimens 5 mm. and 6 mm. long did I find any indications of the emission of cells from the upper ends of the protovertebræ. This was first seen in sections of the embryo 7 mm. long and at about the thirtieth segment behind the head. In this series each body segment comprises

about nine sections. The emission of cells begins at about the fourth section from the front, is seen most distinctly in the sixth, and is not seen in the seventh. Hence, the cells are given off from about the middle third of the upper edge of the protovertebra. A string of these cells can be followed over the spinal cord, where they join those from the other side. They lie above other cells which have come from the ventral border of the protovertebral plate. I have nowhere found any evidences that cells derived from the upper edge of the protovertebral plate grow downward toward the notochord. The sections represented on page 181 of Gadow and Abbott's paper are, in my opinion, in too advanced a stage to reveal clearly the origin of the skeletogenous cells. The evidence of this is to be found in the great number of cells which crowd the space above the spinal cord. These have undoubtedly come from both the ventral and dorsal borders of the protovertebræ. In the sections which I have examined the two sets of cells are caught before they have commingled.

It is to be noted that, according to the authors, all the interdigitating dorsal and ventral masses of cells are formed during the development of the skeletogenous tissue. Also, the colonies of cells are planted which give rise to the interdorsals and the interventrals. Furthermore, each ventral sklerotome fuses with the dorsal sklerotome of the succeeding protovertebra to form a skleromere. But a stage at once ensues in which every trace of segmentation is lost in the sheath of cells surrounding the notochord, and "metamerism is then shown only by the nerves and by the cavities in the dorsal portion of the myotomes." This being true, it would be interesting to learn in what way the fusion of a ventral sklerotome with the dorsal of the next segment differs from its fusion with the dorsal of its own segment.

But the cells which constitute the dorsal and the ventral sklerotomes later give origin to the cartilaginous pieces, eight in number, which are called basalia and interbasalia. How, in view of the fact that in one stage all the boundaries between the sklerotomes disappear, we are to know that the base of the ventral sklerotome is converted into the basiventral cartilage,

and its apex into the interdorsal, we are not told. We can, it seems to me, know this only in case the two structures occupy the same area. But I do not believe that the basiventrals do occupy the same areas that the ventral sklerotomes occupied. It seems to be accepted that the arches, upper and lower (basidorsals and basiventrals), are placed, primitively at least, opposite to or in the septa between the myomeres, while the intercalated cartilages (interdorsals and interventrals) lie opposite the myomeres themselves; but if the views of Gadow and Abbott are correct, the very opposite is true. The basalia would be myomeric; the interbasalia, intermyomeric. (See diagrams on page 188.) The authors hold that the sklerometes alternate with the myomeres, and that the ribs, like the intermuscular septa, are intermyomeric, and they account for this by the peculiar manner of fusion of the sklerotomes; but their explanation assumes the myomeric position of the bases of the sklerometes and their resultant basalia. We have no reason for believing that the neural arch grows out of the upper anterior angle of the sklerometes and the rib out of the lower posterior angle.

On the whole, since the materials composing the sheath of skeletogenous cells that surround the notochord do certainly all fuse, so as to be devoid of all segmentation, it is easier to believe that, when differentiation leads to the formation of cartilages, the basalia are constituted out of cells that were derived partly from the protovertebra in front, partly from the one next behind.[1] It is difficult to see why the adjacent edges of two dorsal sklerotomes should not be as likely to remain in permanent fusion as the ventral sklerotome of one protovertebra with the dorsal of another.

In their study of Amia, Dr. Gadow and Miss Abbott have come to conclusions in many ways different from those reached by myself. Indeed, I am obliged to antagonize most of the statements made by these writers. As regards materials, my own appear to be much more complete. So far as I can discover from their paper, they possessed no specimens smaller

[1] We must keep in mind the possibility that the cartilage cells are, or are derived from, immigrants from some outside region.

than 56 mm. in length. At this stage the vertebral column is far advanced in its development.

From the fact that one of the arch-bearing discs of the middle of the tail had united with the archless disc behind it, the authors came to the conclusion that in all parts of the vertebral column the archless discs form the posterior halves of the definitive vertebræ. However, in the unions occasionally formed between the arch-bearing and the archless discs of the tail the one without arches is probably more commonly in front. Besides this, the text of my former paper and its Fig. 10 give proof that the elements of the archless disc belong to the anterior portion of the centrum. This conclusion is confirmed by many other preparations in my possession. Hence, the terms "precentrum" and "postcentrum," employed by Gadow and Abbott, are to be understood in just the opposite sense. If, however, as appears probable, the archless discs in the Stegocephali unite with the arch-bearing disc in front, it would be better to employ terms applicable to both groups. I have been obliged to propose the rather inconvenient terms pleurohæmacentrum and epi-hypocentrum.

Gadow and Abbott also claim to have found, in the trunk region, the lower intercalated cartilages. While admitting the possibility of the correctness of their identification, I am inclined to believe that they are mistaken. What they regard as these cartilages have, in my former paper, been designated as "aortal supports," and appear to be merely extensions downward from the bases of the lower arches. They are, wherever I have seen them, continuous with these arches, except in the hinder dorsal region, where they seem to be cut off from connection with the down-growth of the lower arches by the rapid development of the bone. Somewhat similar developments of the lower cartilages are found in Acipenser for the protection of the aorta, and in this fish the intercalated cartilages also occur. These so-called interventrals do not occur in the anterior portion of the tail of Amia, where we might expect to find them, in case they were such.

It is affirmed by the authors that the upper and lower arches, basidorsals and basiventrals, of the tail of Amia do not lie in

the same transverse plane, but that the basiventrals lie in the plane of the interdorsals. This I regard as a palpable error. Fig. 10 of my paper already referred to was drawn under the camera from a sagittal section near the mid-line. It shows at the right hand that in the middle of the tail the homotype pieces do lie quite accurately in the same transverse plane. Sections of other specimens confirm this view. The authors are correct in their statement that the basidorsals of the trunk region rest on the summits of the interdorsals, but each basidorsal rests on the interdorsal which was originally in front of it, not on the one behind. I can discover no reason whatever for supposing that the basiventrals have been pushed backward.

On page 202 of their paper Gadow and Abbott proceed to explain the arrangement of the arcualia and the manner of their consolidation into the definitive vertebræ. They say there is, outside of the elastica, a thick zone of connective tissue, which forms a layer of bone on its inner suface, and that, in this zone of connective tissue, cartilage cells from the basal portions of the arcualia grow round the chordal sheath preparatory to the formation of the central discs. In the tail, all the arcualia rest on the thin layer of bone which is just outside of the elastica, and are themselves, for the most part, surrounded by bone. In the trunk region, as already stated, the basidorsals are out of contact with the notochord. The outgrowth from the anterior half of the basiventral grows upward in front of the downwardly directed outgrowth from the posterior half of the interdorsal just above it; while the interventral becomes united in a similar way with the basidorsal just above it. These contiguous semi-rings then fuse to produce "complete rings of cartilage, hyaline, and perichondrally ossifying in the arcualia, more fibro-cartilaginous in the newly formed belt." In the tail, of course, there are two such rings for each skleromere. But not all of the outgrowth from each arcuale develops into cartilage, only the anterior half of it. The hinder half becomes connective tissue and then is changed directly into bone. It results from this that there are for each skleromere of the tail two belts of cartilage and

two of bone. The latter develop so cunningly that they unite not the same elements that were united by the cartilage, but the basiventral with the basidorsal of the next myotome behind, and the interventral with the interdorsal of its own myotome.

The scheme of development worked out by the authors is a complex one, and difficult to comprehend; but after having given, I believe, sufficient attention both to it and to a comparison of their statements with actual sections, I feel prepared to make the following remarks.

The cartilaginous arcualia do not rest on any layer of connective-tissue bone. They come into direct contact with the elastica externa and remain in contact with it.

There are from the arcualia no downward and upward outgrowths of cartilage, not even of fibro-cartilage, which meet and fuse to form rings around the notochord outside of any layer of connective-tissue bone. The earliest bone formed starts apparently as a ring around the base of each arcuale where it rests on the elastica. From its place of origin it spreads in all directions. In any section it will be seen rising in one direction against the surface of the cartilage and spreading in other directions over the notochordal sheath. Any outgrowth of cartilage from the arcuale such as described would have to break through this sheath of bone. So far as I can discover, all the bone of the centrum is derived from this earliest stratum by increase in thickness and later by the sending out of anastomosing plates into the surrounding connective tissue. After careful comparison of the figures of the paper of Gadow and Abbott with sections of specimens of various sizes up to 125 mm., I have come to the conclusion that those alleged outgrowths are little, if anything, more than dense masses of connective tissue which occur in the region of the articular ends of the vertebræ. I can discover no cartilage cells outside of the early formed layer of bone. In a specimen 125 mm. long every portion of the original cartilage is yet distinguishable, and I find no trace of such outgrowths or even room for them. We are told that it is the bone belts arising in these outgrowths which bind together the various dorsal and ventral pieces and which constrict

the growth of the chorda. But the arcualia are bound together by the bone which I have described, and it is this bone too which leads to the constriction of the chorda. All this happens before a spicule of other bone is visible.

It might be supposed that the bone which has been described by Gadow and Abbott and that described by myself are identical. I have carefully considered this possibility, but I cannot reconcile their views and my own.

The renewed study of my preparations in the attempt to test the statements of the authors referred to has led to the recognition of some things to which I had attached perhaps too little importance, and which I at one time thought might offer an explanation of the views of those writers. There do exist what appear to be outgrowths from the arcualia, and these outgrowths extend from one arcuale to the other, connecting all of those of each disc; *but these outgrowths lie against the elastica, between it and the first formed bone.* In the anterior dorsal region of a specimen 44 mm. long there is observed such a layer of cartilage, although it may be seen in younger specimens. It is thickest near the articular ends of the centra, while in the intermediate regions it becomes very thin, a single layer of cells. Immediately between the bases of the various arcualia of each vertebral centrum or disc it seems to be wholly wanting, thus producing gaps in what forms practically a sheath of cartilage around the notochord. This reminds us of the sheath of cartilage which surrounds the notochord of the young Lepidosteus, except that in Amia the cartilage is not continuous from one vertebra to those adjoining.

As to the origin of the cartilage, it undoubtedly develops after the layer of bone has been deposited and spreads from the bases of the arcualia. When the bone is first laid down over the elastica it either lies close against this sheath or there are to be seen between the elastica and the bone, a very few nuclei which closely resemble those of the bone-cells already enclosed. A little later the cells lying under the bone increase in number; and gradually they take on the appearance of cartilage cells surrounded by their matrix.

It seems to me that this thin layer of cartilage represents

the tardily developing remains of a more complete sheath of this substance, which must have enveloped the notochord of the ancestors of this interesting fish.

This cartilage is far from occupying the position assigned by Gadow and Abbott to the outgrowths of cartilage described by them, and far from having the thickness of those outgrowths. Nevertheless, in sections taken near the ends of the vertebræ, where, on account of the flaring of the centrum, the cartilage is struck very obliquely, it has the deceptive appearance of being very thick. Furthermore, in such sections, it may have the still deceptive appearance of being mixed up with the dense connective tissue there found. That such sections will explain the figures presented by Gadow and Abbott I am far from affirming; but I can hardly resist the conclusion that the layer of cartilage which I have described has something to do with the outgrowths which they have described and figured.

Figure 1 given on page 204 of their paper presents a familiar appearance, although the notochordal sheath appears unusually thin. The section has evidently been taken near the anterior end of the centrum. The bone there represented belongs to the early formed layer which I have mentioned. Between this bone and the elastica externa is seen a layer of cells which the authors have not referred to. These belong to the layer of cartilage of which I have previously spoken. Below the bone is a collection of cartilage cells marked C. C. and said to belong to the cartilage which surrounds the notochord. It is nothing, however, but the anterior ends of the "aortal supports," the interventrals of Gadow and Abbott. They appear to lie below the bone because the cartilage has grown downward and forward under the first formed layer of bone. In Fig. 2 the supports are still excluded from contact with the notochord for the same reason; but in a section very close behind this the supports would touch the notochord and would at the same time become confluent with the cartilage of the lower arch. That is, neither the cartilage C. C. nor the "supports" *I. V.* are in reality separated by bone from contact with the notochord. The relation of the "supports" to the lower arches is shown in Fig. 9 of my former paper.

In case the outgrowths described by Gadow and Abbott are really present, we should have around the notochord two more or less complete sheaths of cartilage and perhaps two distinct layers of bone.

It seems to me that the essential feature of the theory advocated by Gadow and Abbott, is to be found in their claim that the basidorsals and the basiventrals which unite to produce the definitive vertebra have had their origin from different protovertebræ, and hence correspond to different myomeres. Through the overlap of the myomeres these cartilages are brought into contact and become consolidated. In this way the writers seek to explain the alternation of the myomeres with the skleromeres. But I do not believe that sufficient evidence has been produced to establish this position. An examination of sagittal sections of Amia at various stages of development fails to show that the ventral arches belong to the anterior myomere, the dorsal arches to the posterior myomere. Nor do I find any proofs of any such slant of the intermuscular septa as the authors affirm and represent in their diagrams. The septa take a course across the vertebral centra which is better represented by this figure ≶, the angle on the right hand being directed backward and placed near the middle of the height of the centra.

Certainly the theory proposed by Dr. Gadow and Miss Abbott is not needed in order to escape the acceptance of the hypothesis of a resegmentation, or "transverse splitting" of the skeletogenous sheath which surrounds the notochord. At a certain stage this sheath has lost all traces of its original segmentation. Why should it regain this segmentation? The cells proceed to differentiation. Some of those lying opposite the intermuscular septa are transformed into the cartilages of the arches. Of the cells opposite the myomeres, some develop into the cartilages which constitute the interbasalia, others into connective tissue which binds the more solid elements together. All this might happen without any transverse splitting and in an organism in which the myomeres do not overlap. The earliest segmentation around the notochord was due to the independent origin of the various sklerotomes; the later segmentation, to differentiation in a mass of similar embryonic cells.

THE NEURAL GLAND IN ASCIDIA ATRA.

MAYNARD M. METCALF.

JULIN and Herdman have described in *Phallusia mammillata* a very interesting condition of the neural gland. Instead of having a single large anterior opening (the ciliated funnel), the gland in this species has many small lateral openings connected by small branched tubes with the main duct. The ciliated funnel is always small, and frequently the duct of the gland does not extend far enough forward to open into it. The many small lateral openings lead into the peribranchial cavity.

Roule has described a similar condition in *Ascidia Marioni*. This species has numerous lateral openings from the duct of the gland into the peribranchial chamber, and also has a reduced ciliated funnel, the latter, however, always being connected with the duct of the gland.

I have recently found somewhat similar relations in *Ascidia atra*, from Jamaica, W. I. I have had for examination only two specimens of this species, but as they agree in the character of the parts to be described, there is no room to doubt they are normal characters.

Ascidia atra is a large black ascidian, shaped much like *A. mentula*. Its ganglion and subjacent gland lie far back of the ciliated funnel, as is the case in all the *Ascidias*. The gland is of enormous size, underlying the whole of the much-elongated ganglion and projecting in front of the latter a distance equal to seven-eighths the length of the ganglion (*cf.* Fig. 1). The gland is also of great width and of still greater depth, pushing down into the dorsal lamina, whose upper part is enlarged to accommodate the glandular tissue (*cf.* Fig. 2). None of the sixty species of tunicates I have studied show a gland whose size in relation to that of the body is so great.

The duct of the gland shows several features of special interest. The main duct extends from near the posterior end of the gland, over its dorsal surface, and forward to the ciliated funnel,

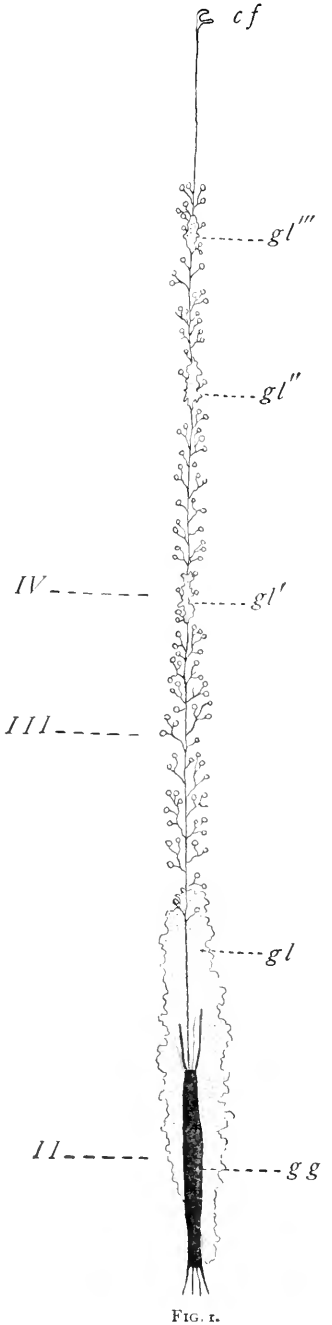


FIG. 1.

which is of normal size and presents the common horseshoe-shaped appearance. At three distinct points the main duct is associated with a considerable mass of glandular tissue, as shown in the accompanying figures (1 and 4) at *gl'*, *gl''*, and *gl'''*. The larger neural gland lies ventral to the duct, opening into it dorsally by very numerous openings; in fact the ventral wall of the duct in this region can hardly be said to be present at all. The three accessory glands, on the other hand, surround the main duct, opening into it from all sides (*cf.* Fig. 4).

Between the anterior end of the large gland and the most anterior of the accessory glands the duct gives off numerous branches, which usually rebranch from one to five times, each twig ending in a little ciliated funnel that opens into the peribranchial chamber (*cf.* Figs. 1, 3). There are one hundred and sixteen of these accessory funnels in one specimen, and nearly the same number in the other specimen. Each funnel has a round aperture with flaring lips, the whole appearance being much like that of the accessory funnels in *Phallusia mammillata*, except that the red pigment connected with the accessory funnels in the latter species is wanting in *A. atra*.

It is interesting to note that the

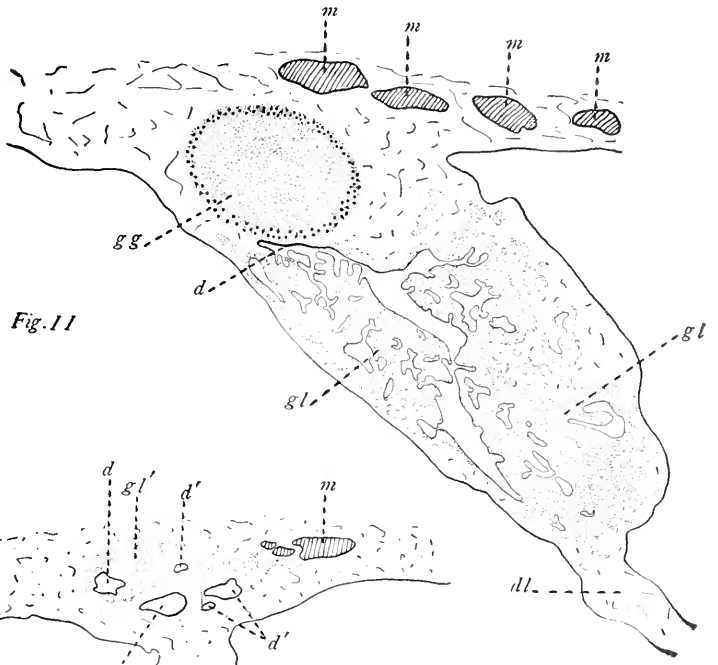


Fig. II

Fig. IV

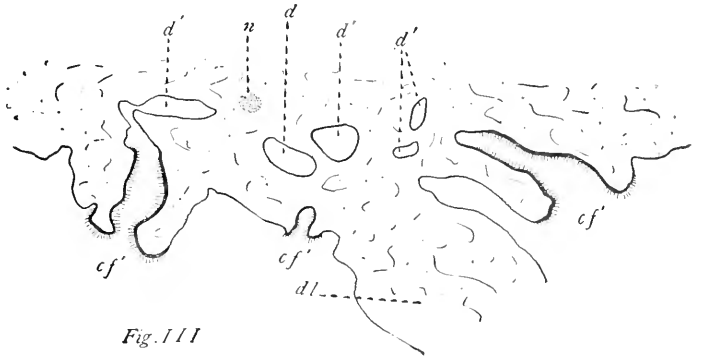


Fig. III

EXPLANATION OF FIGURES.

FIG. 1. — Dorsal view of ganglion and neural gland with its duct.
FIG. 2. — Cross section through ganglion and neural gland at the point marked *II* in Fig. 1.
FIG. 3. — Cross section through the duct of the neural gland at the point marked *III* in Fig. 1.
FIG. 4. — Cross section through one of the accessory glands at the point marked *IV* in Fig. 1.
Fig. 1 is a reconstruction from serial sections, and is magnified about nine times.

dorsal tubercle of *A. atra* is well developed, being of large size and presenting the usual horseshoe-shaped appearance. In *A. Marioni* and *Ph. mammillata*, the only other species having accessory funnels, the dorsal tubercle is much reduced. In *Phallusia* it may even be wholly absent, or at least so small as to be indistinguishable from the accessory funnels. I have not studied *A. Marioni*, but according to Roule's description the tubercle in this species can always be distinguished by its size, though not by its shape, from the round accessory funnels.

A. atra is unique in the following points: (1) in the great size of its neural gland; (2) in the possession of three accessory glands along the duct of the neural gland; (3) in the association in the same individual of a well-developed dorsal tubercle with numerous accessory funnels. This species agrees with *A. Marioni* and *Ph. mammillata* in the possession of numerous lateral branches of the neural duct, each opening by one or more ciliated funnels into the peribranchial chamber.

The discovery of the character of the neural gland and its openings in *A. Marioni* and *A. atra* I believe argues strongly against the isolation of *Ph. mammillata*. The agreement between these three species in so remarkable a character indicates, I believe, that the three are more nearly related to each other than either is to the other *Ascidiae*. If, then, classification is to express relationship, we should either return *Phallusia* to the genus *Ascidia*, or separate *A. atra* and *A. Marioni* from the other *Ascidiae*, including them under the genus *Phallusia*.

From the Biological Laboratory of
The Woman's College of Baltimore,
May 31, 1897.

REFERENCE LETTERS

cf = ciliated funnel.
cf' = accessory ciliated funnel.
d = duct of neural gland.
d' = branch of duct of neural gland.
dl = dorsal lamina.
gg = ganglion.
gl = neural gland.
gl', *gl''*, *gl'''* = accessory glands.
m = longitudinal muscle of intersiphonal region.
n = nerve.

NOTES ON THE ANATOMY OF NAUTILUS POMPILIUS.

LAWRENCE E. GRIFFIN, B.A., B.PH.

SCHOLAR IN THE DEPARTMENT OF ANIMAL BIOLOGY OF THE UNIVERSITY OF MINNESOTA.

[The valuable material upon which these notes are based was presented to the Department of Animal Biology of the University of Minnesota by Mr. Louis Menage, and I am indebted to Professor Nachtrieb for placing it at my disposal, and for the aid he has given me during the progress of the work.]

ATTACHMENTS OF THE MANTLE TO THE SHELL.

THE body of the Nautilus is held within its shell mainly by means of two large shell-muscles, which are attached to the shell at each side over large crescentic areas, nearer the dorsal than the ventral side of the animal. Between the ends of the muscles and the shell are thin plates of a chitinous substance which are sometimes spoken of as tendons. These tendons are light brown, and are composed of a large number of very thin layers. The long axis of the muscle attachment is directed dorso-ventrally.

The mantle also is attached to the shell along three separate lines, which extend between corresponding points of the shell-muscles. Huxley ('58) named these "aponeurotic bands." This general name I have found it convenient to retain, while naming each separate band according to its position as follows:

The dorsal aponeurotic band, extending between the dorsal ends of the shell-muscles over the dorsal surface of the body.

The anterior ventral aponeurotic band, extending between the ventral ends of the shell-muscles around the ventral surface of the body.

The posterior ventral aponeurotic band, extending between the dorsal ends of the shell-muscles around the ventral surface of the body, posterior to the anterior ventral band. Owen ('32) described the two first-mentioned bands, the dorsal and anterior ventral, and other writers have since noted them. Of the third band, the posterior ventral, I find no mention in any of the literature on the subject. Thin bands of the mate-

rial which exists between the ends of the muscles and the shell also extend along the aponeurotic bands. The posterior ventral band is narrower and more difficult to recognize than the others. Each band and muscle leaves a slight, though often distinct, mark upon the inner surface of the shell.

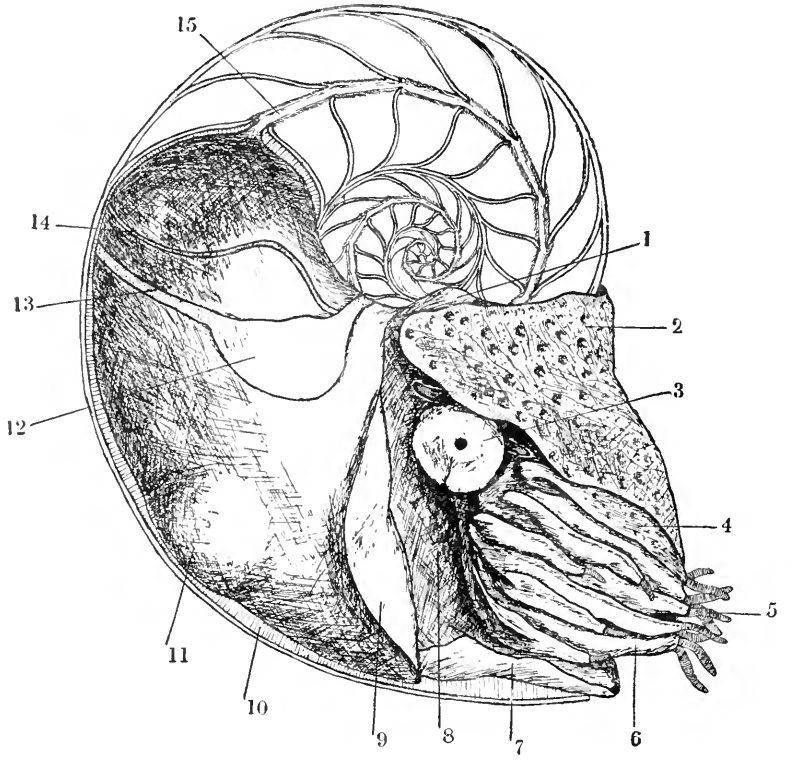


FIG. 1. — Nautilus pompilius, female. 1. Dorsal flap of mantle. 2. Hood. 3. Eye. 4. Second tentacle, showing papillated surface. 5. Cirrus of tentacle. 6. Tentacle forming sheath for cirrus. 7. Funnel. 8. Crus of funnel (M. collaris). 9. Reverted edge of mantle. 10. Occupied chamber of the shell. 11. Nidamental gland. 12. End of shell-muscle. 13. Anterior ventral aponeurotic band. 14. Posterior ventral aponeurotic band. 15. Siphuncle.

These attachments of the mantle to the shell can do little toward holding the Nautilus in its shell ; but they must be of the greatest importance by preventing water from passing between the mantle and shell to the siphon, thus penetrating the chambers and destroying the hydrostatic function of the shell.

Upon examining a large series of shells (more than one hundred) and the sixty-six specimens of Nautilus in the collection, I found that the dorsal and posterior ventral aponeurotic bands limit the formation of the septa. The entire area of the mantle posterior to these bands is active in the secretion of the lime forming the septa. But at the boundary formed by these bands the formation ceases. As the animal grows away from the last-formed septum the bands also move forward, maintaining a constant relation to the shell-muscles. A slight deposition of lime upon the inside of the shell extends forward from the edge of the septum to the above-mentioned limiting bands. It is evident that during what are called the resting periods of the Nautilus an intense secretory activity is maintained (at least in certain areas), and that during growing periods the secretion is very slight.

The Salivary Glands.

The presence of salivary glands in Nautilus has been considered doubtful. Near the œsophagus, upon the floor of the mouth, are two papillose processes, one at each side of the tongue. Owen ('32) described these as containing glandular cavities. Valenciennes ('41) more correctly described glandular bodies within the processes. From studies made upon serial sections of these glands I am convinced that they are true salivary glands, homologous, at least, with the anterior salivary glands of the Octopoda.

The glands are ovoid in shape, situated within the lower third of the processes. As a rule, each process contains but one gland ; in one case, in which serial sections were made of the process, an accessory gland was found opening beside the main gland. The glands are compound tubular, all the tubules being separated by septa of connective tissue which extend in from the body of the process, and all opening into a common central cavity. In some cases this cavity opens into the mouth cavity directly, while in others a very short duct leads from the gland to the mouth cavity. The opening of the duct into the mouth is upon the inner side of the process, somewhat below the center.

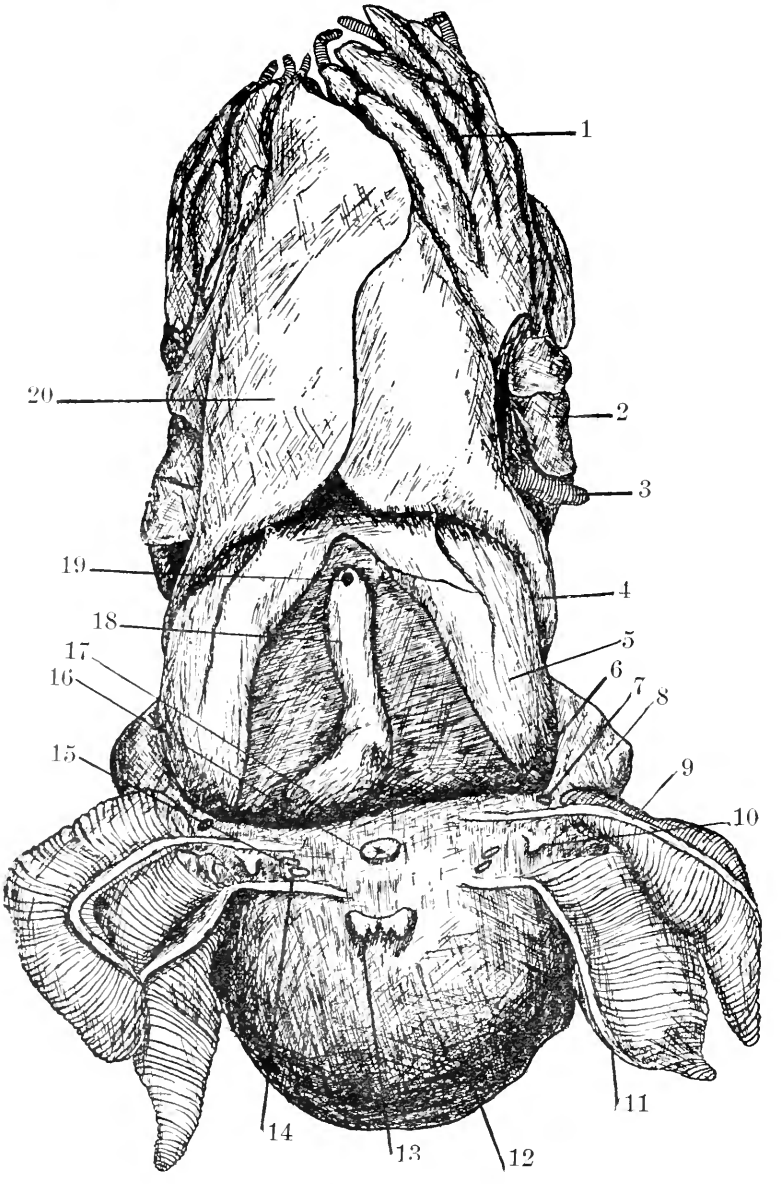


FIG. 2. — A male Nautilus pompilius seen from beneath, the mantle being folded back over the posterior portion of the body. 1. Tentacles. 2. Eye. 3. Infra-ocular tentacle. 4. M. collaris. 5. Shell-muscle. 6. Opening of the rudimentary reproductive organs of the left side. 7. Outer nephridial pore. 8. Mantle. 9. Branchial vein of superior gill. 10. Superior osphradium. 11. Inferior gill. 12. Mantle. 13. Inferior osphradium. 14. Inner nephridial pore. 15. Viscero-pericardial pore. 16. Anus. 17. Needham's sac. 18. Penis. 19. Opening of penis. 20. Funnel.

The tubules and the central cavity are lined by a columnar epithelium. The epithelial cells are very slender, their height being about twenty times the other diameters. Each cell contains an oval nucleus situated near its base.

The processes which contain the salivary glands are mainly composed of connective tissue through which run strands of longitudinal and longitudinally oblique muscle fibers. Blood lacunae penetrate the process. Toward the upper end of it the lacunae become so numerous that the connective tissue

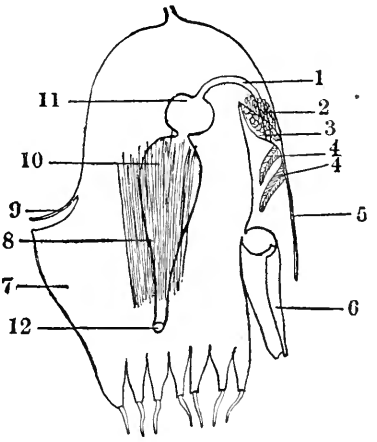


FIG. 3. — Diagram of Nautilus pompilius, modified from Lang. 1. Intestine. 2. Venous appendages. 3. Kidney chamber. 4, 4. Gills. 5. Mantle. 6. Funnel. 7. Hood. 8. Shell-muscle. 9. Dorsal flap of mantle. 10. Crop. 11. Gizzard. 12. Mouth.

forms merely a network around them. The processes are supplied with blood by an artery which comes from the buccal branch of the anterior aorta. There is no closed capillary system either in the salivary glands or in the processes. A nerve from the buccal ganglion accompanies the artery. The processes are clothed with a fine columnar epithelium.

THE OTOCYST.

The otocysts of Nautilus pompilius lie in hollows upon the front side of the cartilage to which the great muscles of the head and body are attached. They are situated one at each side, below and behind the junction of the cephalic, pedal, and pleural ganglia. The otocysts are ovate in form, and about

3.5 mm. in the direction of their long diameter. The end of the auditory nerve spreads out fan-wise upon their surfaces. The auditory nerve has its origin in the cerebral ganglion. In its course it passes over the pedal ganglion, where the sheaths of the nerve and the ganglion are so closely united as to make it appear that the nerve has its origin in the pedal ganglion.

The otoliths are white calcareous bodies, 2 mm. by 1½ mm. by 1 mm. These dimensions are average. In form, the otoliths are roughly ovate ; though the shape varies to a slight extent, not always being exactly the same in the two otoliths of one Nautilus. Each otolith consists of an immense number of small elliptical crystals, solidly cemented together. The crystals vary in size from .0011 mm. to .0066 mm. in thickness, and in length from .0033 mm. to .014 mm. The crystals are composed of calcium carbonate, giving characteristic chemical and light reactions. They all have the shape which would be assumed by a perfect crystal of dog-tooth spar if all the angles were rounded. Very frequently cases of the twinning of two or more crystals are seen. In cases where two crystals are twinned the angle between their axes is usually 78°, any divergence from this angle being quite small, so far as observed. In cases of twinning the ends of each crystal are as perfect as in single crystals. These unions of several crystals form the cross and star-shaped bodies, "etc.," mentioned by Macdonald ('55). The bright points observed by Macdonald are seen only when the focal plane of the lens is above the plane of the crystal.

Macdonald seems to have been the only one who has dissected the otocysts of Nautilus. He dissected a fresh specimen of Nautilus umbilicatus, and was the first to rightly locate the otocysts of Nautilus. He, however, describes the otocyst as "filled with a cretaceous pulp consisting of minute, elliptical otokonia." As far as regards the "otokonia" I agree perfectly with Macdonald. But in every case in which I have dissected the otocyst of Nautilus pompilius I have found a single large otolith, and not in any case a cretaceous pulp. When this mass was boiled in concentrated solution of caustic

potash no change occurred, either in the shape of the mass or in its hardness. Drawings were made of the masses before treatment, and with these the masses were afterwards compared. But the crystals were broken down, according to the well-known action of caustic potash upon calcium carbonate. This experiment tends to prove that the crystals were not cemented together after the death of the Nautilus by the coagulation by alcohol of some fluid in which the crystals normally are immersed. Upon treating the masses with dilute sulphuric acid they were broken down, and characteristic crystals of selenite were formed. If these masses which I find in the otocysts were produced by coagulation processes they would neither be so regular in shape as they are, nor solid, but would abound in cavities filled with a coagulate. In only one case have I found any organic matter with these masses, and in this the quantity was very small and the material was found as a thin layer upon the outside of the mass. From these considerations I judge that the otolith of Nautilus pompilius is single.

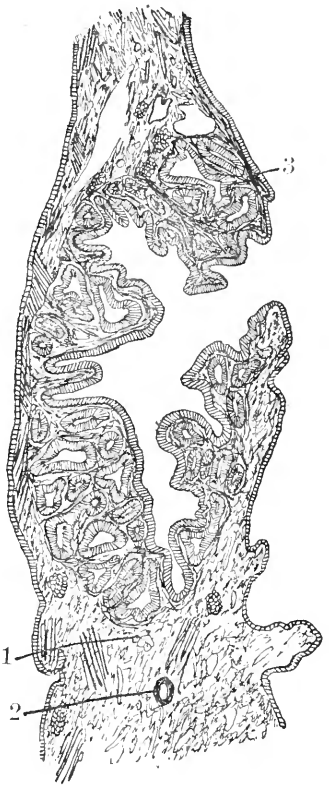


FIG. 4. — A section of the salivary gland of Nautilus pompilius. Outlined with the camera lucida.

THE PALLIAL COMPLEX.

The pallial complex of Nautilus pompilius presents several points in which it differs from that of other Cephalopoda. The mantle cavity extends completely around the body. It is deepest ventrally and shallowest at the sides, and about a third as deep dorsally as ventrally. The gills are four in number,

as are also the nephridia. The gills, moreover, are not situated upon the body wall, as in other Cephalopoda, but upon the inner side of the mantle-fold, near its junction with the body. If the mantle be split along the mid-ventral line it is plain that the gills hang from the mantle, while the sacs of the nephridia lie wholly within the mantle-fold ; a small portion of the posterior venous appendages hanging into the body cavity back of the mantle. The heart lies just back of the mantle-fold. The branchial veins run for some distance in the mantle-fold. The position of the venous appendages within

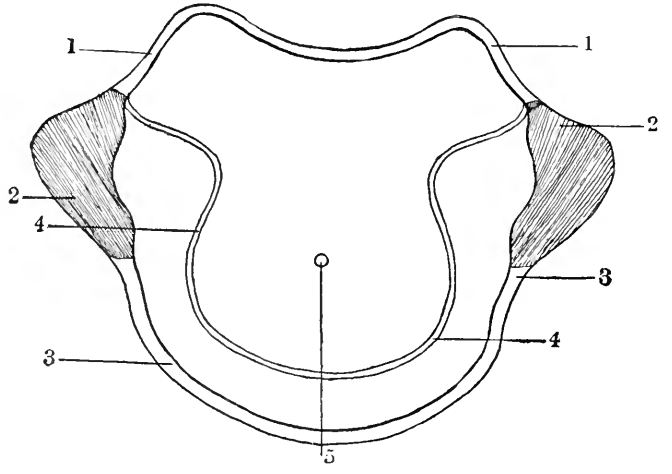


FIG. 5. — The lines of attachment of the mantle to the shell spread upon a flat surface. 1. Dorsal aponeurotic band. 2. End of shell-muscle. 3. Anterior ventral aponeurotic band. 4. Posterior ventral aponeurotic band. 5. Position of siphuncle.

the mantle-fold necessarily causes the posterior portion of the mantle to be very much thickened. Owing to this position of these organs the posterior part of the mantle-fold has also lost its musculature to a great extent. When the mantle is reverted these parts sink into the body cavity, and the gills appear to be situated upon the body wall. It is probably due to these conditions that previous observers have failed to see the true relations of the parts noted. The gills are, however, plainly situated upon the mantle, since they are at the sides of the nephridia, where the mantle is thin and very muscular.

The nephridial and pericardial openings, the anus, osphradia, and nidamental gland are also all upon the inner side of the mantle. The only parts of the pallial complex which are situated upon the body wall are the openings of the reproductive organs.

Thus there is in the Nautilus pompilius the same arrangement of the parts of the pallial complex as in many Gasteropoda. Opening in the median line upon the inner side of the mantle is the anus. Symmetrically disposed upon either side of the anus, also upon the inner side of the mantle, are the pericardial and nephridial pores, gills, and osphradia. Within the mantle, near its base, are the nephridia. The heart is close to the base of the mantle, but not within it. The reproductive openings, as in the Gasteropoda, are upon the body wall, one at each side of the body.

These relations of the parts of the pallial complex are very evident in all my specimens (sixty-six in all), and must be very much more distinct in living specimens, in which the mantle has not been shrunken by reagents. From the manner in which Huxley ('58) speaks of the positions of the nephridial pores, I judge that he recognized the same relation of the parts of the pallial complex which I have here.

In none of the papers listed in the bibliography at the end of this paper have I found mention of the points which I here note, and which seem important enough to justify publication in advance of a more extended account of the anatomy of Nautilus.

BIBLIOGRAPHY.

1894. APPELLÖF, A. Die Schalen von Sepia, Spirula und Nautilus. Studien über den Bau und das Wachstum. *Svenska Akad. Handl.* Bd. xxv, No. 7, 106 pp. 3 figures. 12 plates.

1887. BATHER, F. A. The Growth of Cephalopod Shells. *Geol. Mag.* Dec. 3. Vol. iv, pp. 446–449. 3 figures.

1888. BATHER, F. A. Professor Blake and Shell-growth in Cephalopoda. *Ann. Mag. Nat. Hist.* Vol. i, pp. 421–427.

1831. BENNETT, GEORGE. The Inhabitant of the Pearly Nautilus. *London Medical Gazette.* viii, p. 729. Figs.

1859. BENNETT, G. Notes on the Range of some Species of Nautilus, on the Mode of Capture, and on the Use made of them as an Article of Food. *Proc. Zoöl. Soc. London.* Pt. 27, pp. 226–229.

1878. BENNETT, G. On the Habits of the Pearly Nautilus (Nautilus pompilius). *Report 47th Meet. Brit. Ass. Adv. Sc.* (1877). p. 101.

1825. BLAINVILLE, H. D. DE. Art. "Nautile" in *Diction. des Sciences natur.* Tome 34. Paris. pp. 285–296.

1834. BLAINVILLE, H. D. DE. Anatomie des Coquilles polythalames siphonées récentes, pour éclaircir la structure des espèces fossiles (Anatomie du Nautile flambé, du Nautile ombiliqué et de la Spirule). 2 plates. *Nouvelles Ann. de Mus.* Tome 3, pp. 1–27.

1879. BLAKE, J. F. On the Homologies of the Cephalopoda. *Ann. Mag. Nat. Hist.* Vol. iv, No. 22, pp. 303–312.

1888. BLAKE, J. F. Remarks on Shell-growth in Cephalopoda. *Ann. Mag. Nat. Hist.* Vol. i, pp. 376–380.

1856. BOOGAARD, J. A. Recherches microscopiques sur les spermophores du Nautile. *Ann. Sci. Nat.* 4e sér., Zoöl. Tome 6, pp. 314–318.

1856. BOOGAARD, J. A. Berigt omtrent mikroskopische onderzoekingen van de spermaphoren van Nautilus pompilius. Published with van der Hoeven, 1856.

1883. BOURNE, A. G. The Differences between the Males and Females of the Pearly Nautilus. *Nature.* Vol. xxviii, p. 580.

1879. BROCK, J. Studien über die Verwandtschaftverhältnisse der dibranchiaten Cephalopoden. *Habilitationsschrift an der Universität Erlangen.* Erlangen. 8vo, 29 pp.

1888. BROOKS, HENRY. Preliminary Remarks on the Structure of the Siphon and Funnel of Nautilus pompilius. *Proc. Bost. Soc. Nat. Hist.* Vol. xxiii, pp. 380–382.

1891. BRUHL, C. B. Cephalopoden-Anatomie. i. Wien. 8 plates.

1839. CHARLESWORTH, EDW. On a Young Shell of Nautilus pompilius. *Proc. Zoöl. Soc. London.* vii, p. 123.

1889. Foord, A. H., and G. C. Crick. On the Muscular Impressions of Coelonautilus cariniferous J. de C. Sowerby, sp., compared with those of the recent Nautilus. *Geol. Mag. London.* Dec. 3. Vol. vi, pp. 495–498. 2 figures.

1857. Gould, Aug. A. On the True Nautilus umbilicatus of Lister. *Proc. Zoöl. Soc. London.* xxv, pp. 20, 21.

1850. Gray, J. E. On the Animal of Nautilus. *Ann. Nat. Hist.* Second Series, vol. vi, pp. 268–270.

1884. Grobben, C. Morphologische Studien über den Harn und Geschlechtsapparat sowie die Leibeshöhle der Cephalopoden. *Arch. Zool. Inst. Wien.* Bd. v, pp. 179–252.

1895. Haller, B. Beiträge zur Kenntniss der Morphologie von Nautilus pompilius. *Denkschr. Med. Nat. Gesellsch. Jena.* Bd. viii. Z. Forschungen von R. Semon. Bd. v, pp. 189–204. 2 figs. Plates XI, XII.

1847. Hoeven, J. van der. Ueber die Structur des Nautilus pompilius. *Fror. Not.* 3. Reihe. Bd. iv, No. 69, pp. 33–35.

1847. Hoeven, J. van der. On the Structure of Nautilus pompilius. *Rep. Brit. Assoc. Adv. Sci., 17th Meet. Trans. Sect.* pp. 77, 78.

1848. Hoeven, J. van der. Eenige afwijkingen in de vorm van het hoofd waargenomen by een mannelijk voorwerp van Nautilus pompilius. 1 plate. *Tijdschr. voor de wis- en natuurk. Wetensch.* D. 1, pp. 67–73.

1848. Hoeven, J. van der. Sur la structure du Nautilus pompilius. *L'Institut.* xvi, No. 739, pp. 74, 75.

1850. Hoeven, J. van der. Contributions to the Knowledge of the Animal of Nautilus pompilius. 4 plates. *Trans. Zoöl. Soc. London.* Vol. iv, pt. 1, pp. 21–29. Abstract in *Proc. Zoöl. Soc.* xviii. 1850. pp. 1–9.

1856. Hoeven, J. van der. Bijdragen tot de ontleedkundige kennis aangaande Nautilus pompilius L., vooral met betrekking tot het mannelijke dier. *Uitgegeven door de koninglijke Akademie van Wetenschappen, Amsterdam.* C. G. r. d. Post. 4to. 29 pp. 5 plates.

1856. Hoeven, J. van der. Recherches anatomiques sur le Nautilus pompilius, et particulièrement sur le male. Traduit du hollandais par M. Brullé. 1 plate. *Ann. Sci. Nat.* 4e sér., Zoöl. Tome 6, pp. 290–314.

1857. Hoeven, J. van der. Beitrag zur Anatomie von Nautilus pompilius L., besonders des männlichen Thieres. Aus dem Holländischen übersetzt von F. H. Troschel. *Archiv f. Naturg.* 23. Jahrg. Bd. i, pp. 77–103.

1857. Hoeven, J. van der. Contributions to the Knowledge of the Anatomy of Nautilus pompilius L., especially with Reference to

the Male Animal. 2 plates. *Ann. Nat. Hist.* Second Series, vol. xix, pp. 58–73.

1872. HOFFMANN, C. K. Ueber die Stäbchen in der Retina des Nautilus. 1 plate. *Niederl. Arch. f. Zoologie.* Bd. i, pp. 180–183.

1870. Hydrostatische toestel van Nautilus. *Album der Natur.* pp. 331–334.

1858. HUXLEY, T. H. On some Points in the Anatomy of Nautilus pompilius. 2 woodcuts. *Journ. Proc. Linn. Soc. London.* Vol. iii, pp. 36–44.

1880. JHERING, H. VON. Ueber die Verwandtschaftsbeziehungen der Cephalopoden. *Zeitschr. f. wiss. Zool.* Bd. xxxv, pp. 1–22.

1890. JOUBIN, L. Recherches sur la morphologie comparée des glandes salivaires. *Arch. Zoöl. Expér.* Tome 5. Mém. No. 3, 66 pp. 3 figures. 3 plates.

1890. JOUBIN, L. Recherches sur l'appareil respiratoire des Nautiles. *Revue Biol. Lille.* 2e année. pp. 409–428. Figs. Pl. VII.

1892. JOUBIN, L. Recherches sur la coloration du tegument chez les Céphalopodes. *Arch. Zoöl. Expér.* Tome 10, pp. 277–330. 2 figures. Pls. X–XII.

1865. KEFERSTEIN, W. Beiträge zur Anatomie des Nautilus pompilius. *Götting. Nachricht.* pp. 356–376.

1866. KEFERSTEIN, W. Cephalopoda, in Bronn's *Klassen und Ordnungen.*

1866. KEFERSTEIN, W. *Ibid.*, in *Malakozool. Blätt. f.* 1866. Bd. xiii, pp. 21–38.

1895. KERR, GRAHAM J. On some points in the Anatomy of Nautilus pompilius. *Proc. Zoöl. Soc. London.* pp. 664–686. 6 figures. Pls. XXXVIII, XXXIX.

1892. LANG, ARNOLD. Versuch einer Erklärung der Asymmetrie der Gasteropoden. *Vierteljahrsch. Nat. Gesellsch. Zürich.* 36. Jahrg. 33 pp. 22 figures.

1892. LANG, ARNOLD. Lehrbuch der vergleichenden Anatomie der wirbellosen Thiere. Heft 3, Mollusca. pp. 567–870. Figs. 385–603. Jena.

1883. LANKESTER, E. R. Mollusca in *Encyclopaedia Britt.* Ninth ed. Vol. xvi, pp. 632–695. 153 woodcuts.

1883. LANKESTER, E. RAY, AND A. G. BOURNE. On the Existence of Spengel's Olfactory Organ and of Paired Genital Ducts in the Pearly Nautilus. *Quart. Journ. Micr. Sci.* Vol. xxiii, pp. 340–348. 4 figures.

1888. LENDENFELD, R. VON. Bemerkungen zu Riefshahl's Wachsthumstheorie der Cephalopoden-Schalen. *Zool. Jahrb.* Bd. iii, Abth. Syst. pp. 317, 318. Pl. IX.

1855. MACDONALD, JOHN DENIS. On the Anatomy of Nautilus umbilicatus, compared with that of Nautilus pompilius. 2 plates. *Phil. Trans.* pp. 277–288. Abstract in *Proc. Roy. Soc. London.* Vol.

vii. 1856. pp. 311–314. Abstract in *Ann. Nat. Hist.* Second Series, vol. xv. 1855. pp. 361–363.

1857. MACDONALD, J. D. Further Observations on the Anatomy and Physiology of Nautilus. *Proc. Roy. Soc. London.* Vol. viii, pp. 380–383. Abstract in *Ann. Nat. Hist.* Second Series, vol. xix, pp. 397, 398.

1869. MEIGEN, WILHELM. Ueber den hydrostatischen Apparat des Nautilus pompilius. 1 plate. *Jahresbericht d. Gymnas. u. höher. Bürgerschul. Wesel.* (1868–1869). 4to, pp. 1–18. 1870. *Arch. f. Naturgesch.* 36. Jahrg., Bd. i, pp. 1–34.

1848. NAUMANN, K. FRDR. Ueber die logarithmische Spirale von Nautilus pompilius und Ammonites galeatus. *Berichte über d. Verhandl. d. k. Sächs. Gesellsch. d. Wiss.* Bd. ii, pp. 26–34.

1832. OWEN, RICHARD. Memoir on the Pearly Nautilus (Nautilus pompilius Linn.), with illustrations of its external form and internal structure. 8 plates. *Published by the Royal College of Surgeons in London.*

1833. OWEN, RICHARD. Mémoire sur l'animal du Nautilus pompilius. 4 plates. *Ann. Sci. Nat.* Tome 28, pp. 87–158.

1833. OWEN, RICHARD. Der Nautilus pompilius. Figs. *Fror. Not.* Bd. xxxviii, No. 815, pp. 1–8.

1835. OWEN, RICHARD. Abhandlungen über das Perlboot (Nautilus pompilius) mit Betrachtungen seiner äussern Gestalt, etc. 4 plates. *Isis*, pp. 1–56.

1842. OWEN, RICHARD. On a Specimen of Nautilus pompilius. *Proc. Zoöl. Soc. London.* Vol. x, p. 143. *Ann. Nat. Hist.* Vol. xii, p. 132.

1843. OWEN, RICHARD. On the Structure and Homologies of the Cephalic Tentacles in the Pearly Nautilus. *Ann. Nat. Hist.* Vol. xii, p. 305–312.

1888. PELSENEER, PAUL. Sur la valeur morphologique des bras et la composition du système nerveux central des Céphalopodes. *Arch. Biol.* Tome 8, pp. 723–756. Pls. XXXVII, XXXVIII. 5 figures.

1894. POMPECKJ, J. F. Ueber Ammonoideen mit "anormaler Wohnkammer." *Jahr. Hefte Ver. Vat. Naturk. Stuttgart.* 50. Jahrg., pp. 220–290. 4 figures. Plate IV.

1855. PROSCH, V. Om fangsten af levende Nautiler ved Hvalfangerskibe. *Forhändlgr. skand. Naturforsk.* 6. Möte, pp. 243, 244.

1830. QUOY ET GAIMARD. Description d'un fragment de Mollusque inconnu, présumé être celui du Nautile flambé (Nautilus pompilius Linn.). Illustrated. *Ann. Sci. Nat.* Tome 20, pp. 470, 471.

1834. QUOY ET GAIMARD. Stück eines unbekannten Weichthieres, wahrscheinlich von Nautilus pompilius. Illust. *Isis.* pp. 1146, 1147.

1843. REEVE, LOVELL. History and Observations on the Pearly Nautilus,

involving a new theory to account for the camerated construction of its shell by the aid of the siphonic membrane. *Ann. Nat. Hist.* Vol. xi, pp. 119–125.

1705. RUMPH, G. E. Nautilus major sive crassus in "Amboinsche Rariteitkammer." Amsterdam. Fol., pp. 59–62. Pl. XVII.

1859. SANDBERGER, G. Kurze Betrachtungen über Sipho und Siphonaldute, sowie über Eizelle und andere äussere und innere Merkmale der Schaale des gemeinen Schiffbootes (Nautilus pompilius L.): nebst einigen Vergleichungen mit analogen Stücken der Gattungen, Clymenia, Goniatites u. Ammonites. 1 plate, 7. *Bericht d. Oberhess. Ges. f. Natur. u. Heilk.* pp. 75–80.

1889. SIMROTH, H. Ueber einige Tagesfragen des Malacozoologie, hauptsächlich Convergenzerscheinungen betreffend. *Zeitschr. Naturw. Halle.* Bd. lxii, pp. 65–97.

1887. SMITH, EDGAR A. Note on the Pearly Nautilus. *Journ. Conch. London.* Vol. v, pp. 226, 227.

1889. THIELE, JOHANNES. Die abdominalen Sinnes-organe der Lamellibranchier. *Zeitschr. wiss. Zool.* Bd. xlviii, pp. 47–59. Pl. IV.

1843. Ueber Nautilus pompilius. (Abbildung mit Erklärung.) *Fror. N. Not.* Bd. xxvi. No. 572. pp. 344, 345.

1841. VALENCIENNES, A. Nouvelles recherches sur le Nautile flambé. 4 plates. *Archives du Muséum.* Tome 2, pp. 257–314.

1841. VALENCIENNES, A. Nouvelles recherches anatomiques sur le Nautile (Nautilus pompilius). *Compt. Rend. Acad. Sc. Paris.* Tome 12, pp. 174–179.

1841. VALENCIENNES, A. On the Anatomy of Nautilus. *Ann. Nat. Hist.* Vol. vii, pp. 241–245.

1841. VALENCIENNES, A. Ueber Nautilus pompilius. *Fror. N. Not.* Bd. xvii, No. 369, pp. 265–266.

1841. VALENCIENNES, A. Untersuchungen des Thieres von Nautilus pompilius. *Berlin. Monatsber.* pp. 55–58.

MÜLLER, JOH. Bemerkungen hierzu. *Ibid.* pp. 58, 59.

1895. VAYSSIÈRE, A. Sur le dimorphisme sexuel des Nautiles. *Compt. Rend.* Tome 120, pp. 1431–1434.

1896. VAYSSIÈRE, A. Etude sur l'organization du Nautiles (caractères zoölogiques, dimorphisme sexuel, tentacules et spadice). 4 plates. *Ann. Sci. Nat.* 62e année. 8e sér., tome ii, pp. 137–183 (186).

1879. VIGELIUS, W. J. Bijdrage tot de Kennis van het excretorisch System der Cephalopoden. *Academisch Proefschrift. Leiden.* 8vo. 175 pp. 2 plates.

1879. VIGELIUS, W. J. Over den bouw der nieren bij Cephalopoden. *Tijdschr. Nederl. Dierk. Vereen.* D. 4. 2. Afl. Versl. pp. lix–lxiii.

1880. VIGELIUS, W. J. Über das Excretionssystem der Cephalopoden. *Niederländisches Arch. f. Zool.* Bd. v, pp. 115–184. 3 plates.

1843. VROLIK, W. Sur l'anatomie du Nautilus pompilius. *L'Institut*, xi, No. 518, p. 414.

1843. VROLIK, W. On the Anatomy of the Pearly Nautilus. *Ann. Nat. Hist.* Vol. xii, pp. 173–175.

1848. VROLIK, W. Over het maaksel van den Nautilus pompilius. *Aanteek. Utrecht. Genootsch.* pp. 34, 35.

1849. VROLIK, W. Brief aan — J. J. Rochussen — over het ontleedkundig zamenstel van den Nautilus pompilius. 2 plates. *Tijdschr. voor de wis- en natuurk. Wetensch.* D. 2, pp. 307–327.

1855. VROLIK, W. Lettre sur quelques points de l'organisation de l'animal du Nautile flambé, addressée à M. Eudes-Deslongschamps. 1 plate. Caën. Hardel. 4to. 20 pp. *Extrait du X^e volume des mémoires de la Société Linnéenne de Normandie.*

1895. WILLEY, ARTHUR. In the Home of the Nautilus. 2 figures. *Nat. Sc. London.* Vol. vi, pp. 405–414.

1896. WILLEY, ARTHUR. Letters from New Guinea on Nautilus and some other Organisms. *Quart. Journ. Micr. Sci.* Vol. xxxix, pp. 145–180. 24 figures.

1896. WILLEY, ARTHUR. On some Variations in the Shell of Nautilus, with Description of a new Variety (N. pompilius, var. Moretoni, nov. var.). 3 figures. *Quart. Journ. Micr. Sci.* Vol. xxxix, pp. 227–231.

1897. WILLEY, ARTHUR. Oviposition of the Nautilus. *Proc. Roy. Soc. London.* lx, pp. 467–471. 6 figures. *Nature.* Vol. lv, pp. 402, 403. 6 figures.

1897. WILLEY, ARTHUR. The Pre-ocular and Post-ocular Tentacles and Osphradia of Nautilus. 1 plate. *Quart. Journ. Micr. Sci.* Vol. xl, pp. 197–201.

1870. WOODWARD, HENRY. The Pearly Nautilus (pompilius). Cuttle-fish and their Allies. *Student and Intell. Obs.* Vol. iv. Pt. 1. 2 plates. pp. 1–14. Pt. 2. 2 plates. pp. 241–249.

1871. WOODWARD, HENRY. On the Structure of the Shell in the Pearly Nautilus. *Report 40th Meet. Brit. Ass. Adv. Sci.* (1869). pp. 125–129.

1838. WRIGHT, THOMAS. Observations on Dr. Buckland's Theory of the Action of the Siphuncle in the Pearly Nautilus. *London and Edinb. Philos. Mag.* N. S., vol. xii, pp. 503–508.

Volume I. *December, 1897.* *Number 4.*

ZOÖLOGICAL BULLETIN.

THE AMPULLAE OF LORENZINI OF THE SELACHII.

JAMES E. PEABODY.

I SHALL give in this paper an abstract of some of my results obtained from work on the smooth dogfish (*Galeus canis*, Mitchill), reserving the plates and a fuller description of the lateral-line system, ampullary organs, and Savi's vesicles (of Torpedo) for a more extended paper.

The most casual observation of any of the elasmobranch fishes shows the presence of a large number of pores opening upon the surface. When the skin is pressed a thick, gelatinous, transparent substance is seen to ooze from them, which on mixing with the water covers the surface of the fish with slime. A closer observation and a careful dissection is necessary to establish the fact that these pores must be classed in two distinct groups : to the one group belong the pores of the lateral-line system, and to the other the pores of the ampullary tubes. It is well-nigh impossible in many genera to distinguish superficially the one class of openings from the other.

In tracing the course of the lateral-line canals I have employed the following method. The canals were cleared from their mucous contents by forcing ether through them by means of a syringe and fine canula. When the ether had made its way through all the canals and tubules, an injection mass com-

posed of thick celloidin colored with Prussian blue was used until it appeared at all the pores belonging to this system. The specimen was then put into 20% nitric acid and left for one to three days, until the skin and tissues were softened enough to be easily removed with needle and forceps. A careful dissection left a deep blue cast of the canals with their finest ramifying tubules. Maceration is stopped and the preparation kept indefinitely in a 2% formalin solution. The course of the ampullary canals was readily followed by probing with black bristles.

Since 20% nitric acid softens bone, muscle, and connective tissue, at the same time hardening more or less the nerve tissue, specimens which have been thus macerated for a day or two furnish favorable material for tracing nerve trunks and fibers to their finest ramifications.

For general histology Hermann's fluid and corrosive sublimate have proved the best fixing agents. Iron haematoxylin, with orange G as a counter stain, has given very satisfactory histological results.

Method employed in using Methylene Blue. — After considerable experimentation I have come to adopt the following as a method of procedure for the successful application of methylene blue to selachian material. The head of a live dogfish is severed from the trunk, the snout cut open, and a solution of methylene blue injected with a hypodermic syringe into the gelatinous tissue in which the ampullae are imbedded. I have obtained equally good results with $1\frac{1}{2}\%$, 1%, $\frac{1}{10}\%$, $\frac{1}{20}\%$ solution of the blue, but have found the $\frac{1}{10}\%$ the most convenient. After the stain has been allowed to act for an hour and a half, a small clump of the ampullae is removed to a slide and teased out so that each ampulla is isolated and thus exposed to the air. The slide is then examined under a low power.

One of the most important factors in securing a successful stain is that of exposure of the tissue in the air. I have often found on looking over an apparently worthless series of ampullae a second time that the fibers have become well stained, this result being due apparently to the few moments of further

exposure. Indeed, by careful watching one can see the stain creep along the nerve trunks and the fibrillae out to the finest ramifications. If the stain has worked successfully the fine fibrillae will stand out as sharp blue lines all over the surface of the ampulla, all the rest of the tissue remaining colorless.

Fixation of the blue is accomplished by removing the ampullae into a solution of ammonium molybdate. I have modified somewhat Bethe's original formula (*Archiv f. mik. Anat.*, 1895, Bd. xliv, pp. 579–588) in substituting osmic acid for hydrochloric, using the following solution:

Ammonium molybdate . . .	1 gr.
Distilled water	10 cc.
Peroxide of hydrogen . . .	1 cc.
Osmic acid, 1%	1 drop.

This mixture seems to fix the blue stain more perfectly than any other I have tried, and has two additional advantages: first, that of differentiating the medullary sheaths of the nerves; secondly, that of better histological preservation of the tissue. I have not found the use of ice, recommended by Bethe, necessary for the preservation of the blue.

After remaining in the fixing fluid for one to two hours the ampullae are washed in water for ten minutes, then hurried through the grades of alcohol, cleared in toluol and imbedded in paraffin. Since these organs are so small (about 1 mm. in diameter), twenty minutes in each fluid and an hour in paraffin I find to be sufficient. An injection of the blue may therefore be made at 10 A.M. and by 5 P.M. one may cut a series of sections. The histological character of the cells after such treatment is not very perfect, but the nerve fibers with their branches stand out very sharply defined.

Since alum carmine does not impair the blue it has given the best results as a nuclear stain for sections.

Many facts of nerve relations can best be determined by treating the tissue in the following way. Ampullae well stained with the methylene blue are fixed in ammonium picrate, and frozen by the use of carbon dioxide on a freezing microtome attachment. Freehand sections are cut with a razor

and mounted in a mixture of dilute glycerine and ammonium picrate. If the slide is sealed with asphaltum the preparation may be kept for several weeks. The sections are best studied at once, however, as the blue tends either to fade or to become granular, the fibrillae losing their continuous appearance.

My experience with methylene blue as applied to selachian material has led me to the following conclusions: (1) The tissue must be alive when the blue is applied. I have never had a successful impregnation unless the head is removed from a live animal and injected at once. (2) The strength of solution seems unimportant, since I have obtained a good stain with such extremes as $1\frac{1}{2}\%$ and $\frac{1}{20}\%$. (3) The most important facts to be learned to obtain success with a given tissue are, first, the time which the stain should act on the tissue before exposure to the air, and second, how long an exposure is necessary. The time limits which give success with dogfish do not give good results with skates. (4) The best results are obtained from ampullae which seem scarcely tinged with the blue. When the tissue is deeply stained one finds on examination with the low power of the microscope that the stain is located in the cells, not in the fibers. (5) It is impossible to carry the methylene blue through the paraffin bath unless the tissue is completely dehydrated, hence the importance of securing genuine absolute alcohol. (6) I have been unable to preserve the stain in alcohol or in the clearing oils for any length of time. I have cut paraffin blocks, however, a year after the objects were imbedded, and the blue seems perfectly preserved. This seems the surest means of preservation of the stained tissue till one is ready to use it. Sections mounted in balsam or dammar in August, 1895, have not lost a bit of their clearness.

Groups of Ampullae. — Following Ewart's nomenclature I shall designate the groups of ampullae according to their innervation as supra-orbital, buccal, hyoid, and mandibular. In Galeus it seems impossible to distinguish the supra-orbital and buccal.

In order to determine the number in each group the ampullae were carefully dissected out and counted in two speci-

mens, one a female 1.07 m. long, the other a pup 26 cm. long. The following are the results of my computation:

	IN ADULT.	IN PUP.
In supra-orbital-buccal groups . .	1029	860
In right hyoid group	270	273
In left hyoid group	245	287
In right mandibular group . . .	25	19
In left mandibular group . . .	26	19
Totals . . .	1595	1458

On comparing the two columns of figures it will be seen that the number of ampullae probably does not increase after the birth of the fish, as in some groups the number is larger in the pup. The difference in the total number is probably due to individual variation.

The ampullae are imbedded in a matrix of clear gelatinous tissue, through which run the nerves supplying these organs. The whole mass is surrounded by fibrous connective tissue which is pierced by the tubes as they run out from the ampullae.

Gross Anatomy of an Ampulla. — At its inner end each ampullary tube from the surface opens into one of the so-called ampullae of Lorenzini (Fig. 1, *a.*). Viewed from the side an ampulla is seen to be a sac more or less spherical in form, with slight outpocketings (Fig. 1, *a.pkt.*) which vary in number from eight to twelve. If one looks at the proximal end of the ampulla, the pockets are clearly seen as a circle of protuberances. The internal structure is best made out when the ampulla is sectioned transversely in a plane just above its largest diameter. Looking into the organ one sees the out-pocketings noticed on the outside, while partitions also appear running from the divisions between these pockets

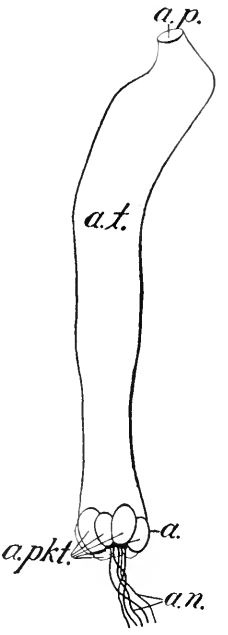


FIG. 1. — Outline drawing of tube and ampulla: *a.*, ampulla; *a.n.*, ampulla nerve; *a.p.*, ampulla surface pore; *a.pkt.*, ampulla pocket; *a.t.*, ampulla tube. × 12.

toward the center. The lumen at the base of the ampulla is thus divided into the above-mentioned eight to twelve compartments (Fig. 3, *a.pkt*). A columnar structure (Fig. 2, *a.c.*) to which the partitions run, is formed in the center of the ampulla. To this structure Boll has applied the name "centrum." The centrum increases in diameter considerably above, so as to form a slightly concave, plate-like cap (Fig. 2, *a.c.c.*), which covers over a portion of the compartments beneath. In longitudinal sections of the ampulla taken through a partition (Fig. 2), it is seen that the edge of the partition curves upward as it passes from the centrum to unite with the outer wall.

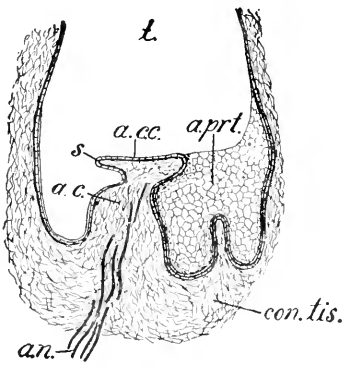


Fig. 2. — Longitudinal section of ampulla through centrum, plane of section passing through an ampullary pocket on the left side of the figure and through a partition wall on the right: *a.c.*, ampulla centrum; *a.c.c.*, ampulla centrum cap; *a.n.*, ampulla nerve; *a.prt.*, ampulla partition; *con.tis.*, connective tissue; *t.*, ampulla tube. × 51.

The average diameters of the ampullae of a dogfish 1.02 m. long are as follows:

In the supra-orbital-buccal groups . .	$\frac{9}{10}$ mm.
In the hyoid groups	$\frac{7}{10}$ mm.
In the mandibular groups . . .	$\frac{3}{10}$ mm.

Histology of Tube and Ampulla. — In histological structure the tubes are comparatively simple. They are lined with a single thin layer of epithelial cells. In surface view the outlines of the cells, most clearly made out from a silver nitrate impregnation, are seen to be polygonal. The oval nuclei have a diameter about one-third that of the cells. In cross sections (Fig. 4, *t.c.*) the nuclei appear as thin ellipsoid bodies, staining deeply. Nucleoli are visible. The shape of the cell body is more difficult to determine, since the nucleus occupies so large a part of its thickness. But after using several methods of fixing and staining I am confident the section of the cell is spindle-shaped, the thickness decreasing from the center toward the periphery, until at the point where two cells are

contiguous the cytoplasm appears like a single thin line (Fig. 4, *z.*). Outside this layer of cells is a sheath of connective tissue of varying thickness constituting the larger portion of the wall of the tube (Fig. 4, *con. tis.*).

The histology of the ampulla is best studied in a longitudinal section. Two regions, distinguished by the character of the lining cells, are to be recognized. First, the centrum cap is covered on its upper surface with a single layer of cells usually

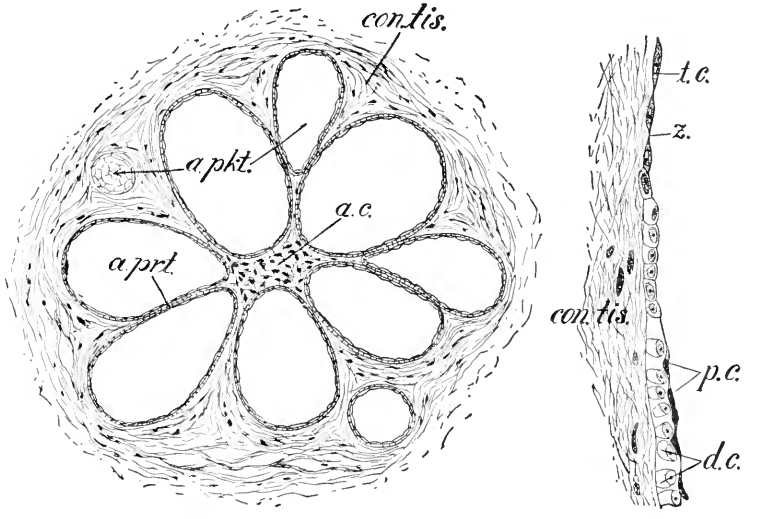


FIG. 3. — Cross section of ampulla below plane of centrum cap: *a.c.*, ampulla centrum; *a.pkt.*, ampulla pocket; *a.prt.*, ampulla partition; *con.tis.*, connective tissue. × 63.

FIG. 4. — Longitudinal section of upper portion of ampullary pocket and of tube: *con.tis.*, connective tissue; *d.c.*, deeper layer of cells; *p.c.*, peripheral layer of cells; *t.c.*, tube cells; *z.*, point where two cells of tube wall meet. × 465.

almost cubical in shape (Fig. 5, *c.c.*[1]). The oval nuclei are large. In many sections the cytoplasm toward the lumen of the tube pushes out into a blunt process, the line of cells in the section then having the appearance of irregularly placed saw-teeth. This same single layer of cells covers the under side of the centrum cap (Fig. 5, *c.c.*[3]), where the latter arches over the ampullary compartments. At the edge of the cap the cells become more elongated, often spindle-shaped (Fig. 5, *c.c.*[2]). The partition walls near the centrum are likewise cov-

ered on either side with a single layer of cells. In the second region, *viz.*, that of the walls of the pockets themselves, two layers of cells are always found. The peripheral layer, that is, the thin sheet of cells next the lumen (Figs. 4, 5, *p.c.*), has an appearance almost exactly like that of the lining of the tube. The cells are flattened, each being almost entirely filled by an ellipsoid nucleus. Nucleoli are prominent. Beneath this thin epithelial layer is a second layer composed of short cylindrical cells (Figs. 4, 5, *d.c.*). The nuclei, large and spherical in shape, are situated at the very ends of the cells, next the peripheral layer. The nuclei of the two layers can be easily distinguished, not only by their difference in form, but also by a difference in their reaction to stains, that is, those cells next the lumen take a deeper stain.

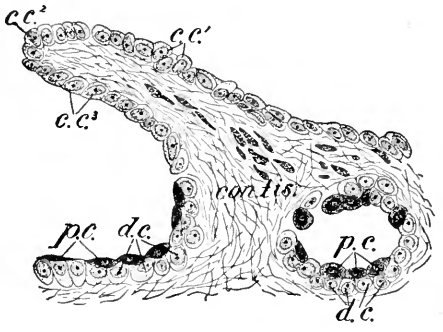


FIG. 5. — Region of Fig. 2 marked *s.: c.c.*¹⁻³, centrum cell; *con.tis.*, connective tissue; *d.c.*, deeper layer of cells; *p.c.*, peripheral layer of cells. × 465.

Innervation of Ampulla. — Five to ten nerve fibers run to each ampulla (Fig. 1, *a.n.*). I have never seen the fibers branch or unite with one another after leaving the nerve trunks. In branching, the nerve trunks simply give off some of their component fibers.

In studying the course of the nerves in the ampulla I have employed the methylene blue method altogether, and this method has given very satisfactory results. When the blue is fixed in the ammonium molybdate with the addition of osmic acid, a longitudinal section in a median plane shows that a medullary sheath encloses the axis cylinder of the nerve along its course up the centrum till just beneath the centrum cap, where it disappears rather abruptly (Fig. 6, *n.m.sh.*). In freshly stained specimens, or where the stain is fixed without the osmic acid, the blue-stained nuclei of Schwann's sheath are distinctly visible.

After the loss of the medullary sheath the axis cylinders pass

upward for a short distance, then divide more or less dichotomously, sending out branches at right angles to their former course (Fig. 6, *n.ax.cyl.*). By the interlacing of these axis cylinders a plexus is formed, occupying nearly the whole area of the centrum cap. All the best results of previous workers were obtained by the use of osmic acid. Since this reagent differentiates the medullary sheath only, their observations could be carried only to the point where this sheath disappears. The fibers were then pursuing a perpendicular course, and the assertion was but natural that this course was continued for a

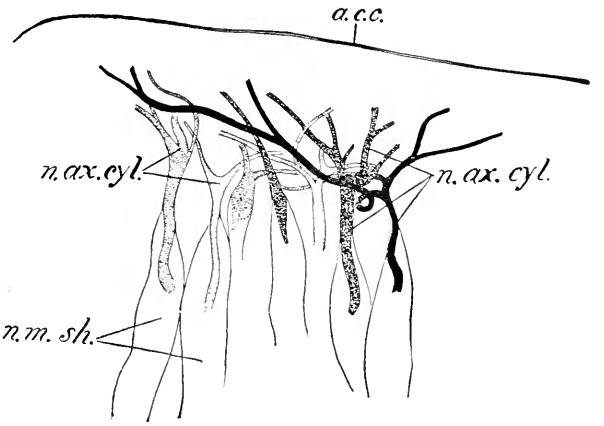


FIG. 6. — Nerve fibers in longitudinal section just below centrum cap : *a.c.c.*, ampulla centrum cap ; *n.ax.cyl.*, axis cylinder of nerve ; *n.m.sh.*, medullary sheath of nerve. × 465.

very short distance, the fibers then coming into connection with the cells at the top of the centrum. As we have seen, this is not at all true. From the plexus already described bundles of axis cylinders collect at the periphery of the centrum cap, and pass out into the connective tissue of the partitions which separate the ampullary pockets. Thus far the nerves have, so to speak, avoided all relations of contact or of continuity with cells.

Having passed into the region where the double layer of cells is found, the nerve fibrils begin to divide, sending out branches which play over the bases of the deeper layer of cells (Fig. 8, *n.ax.cyl.*). The manner of giving off branches is interesting. In well-stained specimens (Fig. 7) the larger axis cylinders are

seen under the highest powers of the microscope to be made up of minute fibrillae. For a distance along their course these fibrillae are separated more or less from one another, but at certain points they are gathered tightly together (Fig. 7, *v.*). When branches are given off, the individual fibrillae may be traced continuously from the larger axis cylinders out along the smaller branches (Fig. 7, *n.ax.cyl.*). On these finer branches is seen the same appearance of collecting and loosening out of fibrillae as has been described above. Such a fact

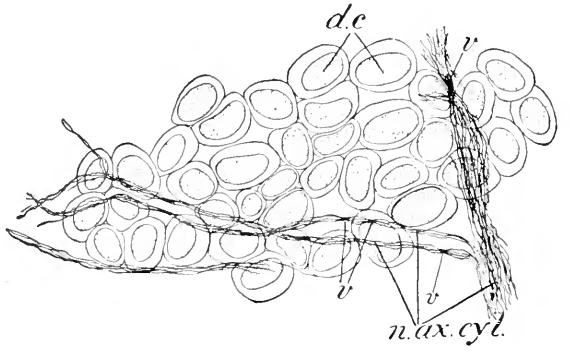


FIG. 7. — Method of branching of axis cylinders of nerves: *d.c.*, deeper layer of cells; *n.ax.cyl.*, axis cylinder of nerve; *v.*, points along course of axis cylinders where the nerve fibrillae are gathered closely together. × $\frac{1}{12}$ oil immers. 2 in. occular.

suggests that the finest individual nerve fibril may be continuous from its peripheral termination back through the bundle of fibers in which it has come, to its ganglion cell near or in the brain.

Along the course of the finest fibrillae little thickenings are seen just at the base of the cells of the deeper layer (Figs. 8, 9, *n.k.*). Thus far I have never seen any branches running up between the cells. If such branches were present I believe they would be stained as the others are by the methylene blue. The distribution of the nerves as described above has been seen in many series of sections.

In thick sections obtained by freezing and fixing the blue with ammonium picrate the cells are stained yellow, the fibers assuming the purple color characteristic of this treatment. If one examines with very high powers the outer surface of the

ampullary pockets, the nuclei of the thin lining layer of the pockets are clearly seen on deep focusing; at a higher focus the outlines of the cells and nuclei of the deeper layer appear; at a still higher focus the nerve fibrils are sharply differentiated (Fig. 7). Since these nerve fibrils appear at neither of the two first-mentioned optical levels the observations made from sections of the ampullary walls appear to be confirmed, namely, that the nerve fibrils ramify only over the bases of the cells of the deeper layer.

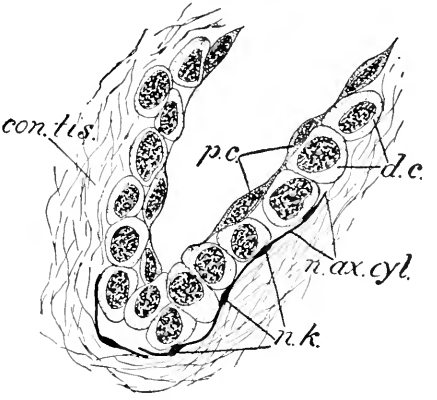


FIG. 8. — Longitudinal section of wall of ampullary pocket: *con.tis.*, connective tissue; *d.c.*, deeper layer of cells; *n.ax.cyl.*, axis cylinder of nerve; *n.k.*, nerve knob; *p.c.*, peripheral layer of cells. × $\frac{1}{12}$ oil immers. 2 in. occular.

In frozen sections prepared as described above, the cells may be isolated from one another by tapping the cover glass. I have seen in several cases a fine, deep-blue fibril running along the surface of the deeper cells and ending

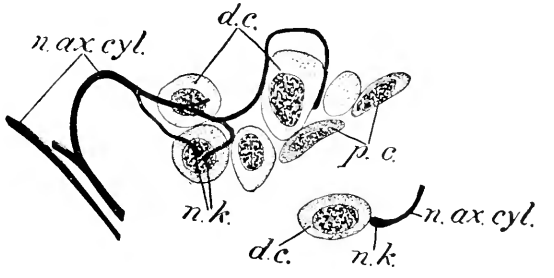


FIG. 9. — Nerve fibrils running over the surface of the deeper layer of cells to end in knobs on the outside: *d.c.*, deeper layer of cells; *n.ax.cyl.*, axis cylinder of nerve; *n.k.*, nerve knob; *p.c.*, peripheral cell. × $\frac{1}{12}$ oil immers. 2 in. occular.

in a slight enlargement (Fig. 9, *n.k.*). These fibers and endings are always *outside the cells*, and it is apparent that the protoplasm of the two structures is not in continuity, since the cells are usually of a yellow color, while the nerve fibrillae are

deeply stained with the blue, the line of separation between the two appearing quite distinct.

To recapitulate the facts of innervation as given by the methylene blue : Five to seven medullated fibers coming from the (seventh pair of) cranial nerves enter each ampulla from below and pass up the centrum. Just beneath the centrum cap the sheath disappears. The axis cylinders, continuing on their course, send out lateral branches, which after division and ramification beneath the centrum cap run out along the partitions to the outer walls of the compartments. Here minute fibrils play over the bases of the deeper layer of cells, ending in slight enlargements on their surfaces. This much of the innervation of an ampulla has been given sharply and clearly by the use of the methylene blue. Since nothing further in the way of nerve structure has been suggested by the method, it seems to me the theories of the function of the ampullary organs must conform to these facts.

Physiology. — Of the theories thus far suggested to explain the function of the ampullae of Lorenzini, but two are supported by anatomical facts: either they are sense organs, or they are glands for the secretion of mucus.

The wealth of nerve supply for these organs almost compels one to believe that they must be sensory in character. This theory was first advanced by Jacobson (1813), and in most of the important papers on these organs since that time, including those of Muller ('51), Leydig ('68), Boll ('68), Merkel ('80), Ewart ('92), the writers have maintained the view that the ampullae were sense organs.

The histological structure of the epithelium lining the pockets does not, however, show the elements ordinarily associated with sensory epithelium. There is no differentiation into sensory and supporting cells, sensory hairs are absent, and indeed specialized sensory cells seem to be altogether lacking. The nerve fibrils end freely on the lower surface of the cells of the deeper layer. The centrum has been regarded as the most important part of the ampulla, in which the nerves were supposed to end. As we have seen, this is not the case. The single layer of cubical cells covering the centrum cap seems to

furnish no support for the suggestion made by Fritsch ('90) that the centrum represents a sense organ which has lost its sensory function because of the pressure of the overlying mucus.

It therefore seems improbable that the ampullae serve as organs for the reception of stimuli which result in sensations of taste or audition, since the structures in vertebrates endowed with such functions have a characteristic epithelium consisting of supporting cells and sensory cells with sensory hairs. If the ampullae prove to have sensory function their structure (free nerve endings beneath an overlying epithelium) makes it more probable that they are of the nature of tactile sense organs.

Facts in support of the glandular theory are less easily attainable. The mucus is present in large quantities, it is true, filling ampullae and tubes; but I have never seen any indication of glandular activity in the cells, nor have any observations pointed to the fact that mucus is discharged from the cells. The very large size of the nucleus leaves little room in the cell for the processes of secretion, and the position of the nucleus at the peripheral end of the cell, where we should expect the mucus if made to be aggregated and discharged, is another fact opposed to the theory that we are dealing with a typical glandular structure.

An interesting correlation has been observed between the habits and the number of ampullae found in three of the selachians common at Woods Holl. As is well known, the shark is very active in its movements, the skate is less active, while the Torpedo is comparatively sluggish. The following table gives the result of a careful counting of the ampullae in the three forms above mentioned.

Number of ampullae in dogfish (*Galeus canis*) .	1595
Number of ampullae in skate (*Raja erinacea*) . .	779
Number of ampullae in Torpedo (*Torpedo occidentalis*)	220

In the structure of the individual ampullae one is struck with the complexity exhibited in Galeus, with the ten to twelve ampullary pockets, as compared with the relative simplicity of the six pockets of the Torpedo ampulla. In the skate the number of pockets is usually seven or eight.

In the lateral-line system of the same selachians there is a decreasing complexity observed in the animals less active in their habits. Galeus has a much-branched, dendritic system of tubules; in Raja the tubules are almost entirely simple, running in a direct course from the lateral-line canal to the surface pores; in Torpedo the same simple plan is observed on the dorsal surface, while on the ventral surface of the fish the lateral-line canal system has wholly disappeared, its place being taken, according to some writers (Garman, '88, Fritsch, '90), by Savi's vesicles.

Whether this simplicity of structure of lateral-line and ampullary organs is always correlated with sluggishness of habit is a question which can only be answered by a careful study of many different genera of the selachians.

Attention should be called, in the discussion of the glandular theory of the ampullae, to the fact that in Galeus, where the ampullae are most numerous, the skin is free from slime, while in the skate the slime is very abundant. It is highly improbable, therefore, that this slime is produced in the ampullae. On the other hand, an increase of sensory function would be expected to accompany a wider range of body movement.

Some experiments in cutting the nerves and studying the effect on the ampullae have been begun which may throw some light on the physiology of these organs. But at the present time no hypothesis yet suggested seems to me to explain the function of the ampullae in the economy of the life of the selachians.

My work on the ampullae of Lorenzini of the selachians was begun at the Marine Biological Laboratory at Woods Holl, Mass., in the summer of 1892. The original object of this research, as suggested by Dr. Howard Ayers, was to determine the relation (whether of contact or continuity) which exists between the nerve fibers and the cells of this so-called sense organ. The methylene blue stain seems to have given decisive evidence on that point. The problem has, however, gradually assumed a larger interest from the standpoint of comparative anatomy and physiology.

In conclusion I wish to express my appreciation of the help received from Dr. Howard Ayers and of the generous resources afforded me at the Williams College room in the Woods Holl Laboratories. A considerable portion of this investigation has been carried on at Harvard University and in the Williams College laboratories. I am especially indebted to Prof. E. L. Mark for his direction of my work during the year at Harvard; and to Dr. James I. Peck of Williams College for assistance in preparing my work for publication. The final copies of the drawings for this paper were made in ink by Dr. Arnold Graf.

Marine Biological Laboratory,
Woods Holl, Mass.
July 15, 1897.

LIST OF THE MORE IMPORTANT PAPERS ON THE AMPULLAE OF LORENZINI.

(*A complete bibliography of the subject will be given in a later paper.*)

1868. BOLL, FRANZ. Die Lorenzinischen Ampullen der Selachier. *Archiv f. mikr. Anat.* Bd. iv, S. 375–391, Taf. xxiii.

1892. EWART, J. C. The Sensory Canals of Laemargus. *Trans. Roy. Soc. Edinburgh.* Vol. xxxvii, No. 5, pp. 59–85. Pl. i, ii.

1892. EWART, J. C., and MITCHELL, J. C. The Sensory Canals of the Common Skate. *Trans. Roy. Soc. Edinburgh.* Vol. xxxvii, No. 6, pp. 87–105. Pl. iii.

1890. FRITSCH, GUSTAV. Die electrischen Fische. Pp. 87–92. Leipzig.

1888. GARMAN, SAMUEL. On the Lateral Line Canal System of the Selachia. *Bull. Mus. Comp. Zoöl.* Vol. xvii, No. 2, pp. 57–119. Pl. i–liii.

1813. JACOBSON, LOUIS. Extrait d'un Mémoire sur un Organe particulier des Sens dans les Raies et les Squales. *Bull. d. Sci. de la Société philomatique de Paris.* Tome 3, pp. 332–337.

1868. LEYDIG, FRANZ. Ueber Organe eines sechsten Sinnes. Dresden.

1678. LORENZINI, STEPHAN. Osservazioni intorno alle Torpedini. Florence.

1880. MERKEL, FR. Ueber die Endigungen der sensibeln Nerven in der Haut der Wirbelthiere. Pp. 39–48. Rostock.

1785. MONRO, ALEX. The Structure and Physiology of Fishes. Edinburgh.

1851. MULLER, H. Ueber den nervösen Follikel-Apparat der Zitterrochen und die sogenannten Schleimkanäle der Knorpel-Fische. *Verhandl. Phys.-Med. Gesellsch. in Würzburg.* Bd. ii, Nr. 8, S. 140–144.

1880. SAPPEY, PH. C. Etudes sur l'appareil mucipare et le système lymphatique des Poissons. Paris.

ON THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF ANIMALS.

FRANK R. LILLIE AND F. P. KNOWLTON.

PROTOPLASM exercises its functions only within a certain range of temperature; the range extends from a minimum temperature where its functions may be said to begin[1] to a maximum above which occurs heat rigor and death, through an optimum which lies nearer the maximum than the minimum.[2] The intensity of any function increases from the minimum to the optimum temperature and then, as a rule, decreases to the maximum, though never to the level of the minimum. This temperature influence is especially well marked in the case of the *formative functions* of protoplasm, and although the number of authors who have noted this is very great, and although use is often made of it to facilitate observation, yet we have no adequate *quantitative* study of its effect in animals. The influence of temperature on growth in plants has, on the other hand, been carefully determined.

The effect of temperature on development in animals is twofold, — on the *rate* and on the *form*. The latter effect is produced only by temperatures above the maximum or below the minimum. *In this sense, then, the minimum temperature for the whole development is the lowest and the maximum the highest at which normal development may occur.* The terms maximum and minimum will be used in this sense throughout this paper.

The effect of temperature on the rate of development involves two variants, time and extent, which are directly proportional to each other. By making either of these a fixed quantity we can measure the effect of the different temperatures in terms of the other. It is generally advisable to fix the *extent* and to measure the times at different temperatures, because it is not possible, as a rule, to express the differing

[1] We do not mean to imply that there is absolutely no metabolism at subminimal temperatures.

[2] See Davenport, Experimental Morphology, vol. i, p. 227.

extents of development in the same time at different temperatures in terms of each other: if for instance we discover that at a certain temperature the egg of the frog develops in twenty-four hours to the blastula and at another temperature to a later stage of gastrulation, we cannot express the effect of the increase in temperature quantitatively; but if, on the other hand, a fixed extent of development takes two days at one temperature and one day at a higher temperature, the effect of the increase in temperature is given quantitatively. However, in the case of mere growth in bulk or length, the *extents* at different temperatures within the same period of time can be directly compared.

In our study of the effects of temperature on development we have included (1) the *regeneration* of *Planaria torva;* (2) the cleavage and gastrulation of Amblystoma and the frog; and (3) the rate of growth of the tail of tadpoles of the frog and toad. The observations made have been much more numerous and have taken up much more time than the tables would lead one to suspect; this is due to the necessity of repeating observations to secure averages so as to eliminate the factor of individual variation, and to the great mortality at high temperatures. The temperatures below 22° C. are in all cases averages, owing to the impossibility of securing perfectly uniform low temperatures during the time occupied by the experiments. The average temperature was calculated from daily readings in the longer experiments, and from more frequent readings in the shorter ones; the variation from the average was rarely more than one degree either way.

1. *Planaria torva.*

a. Normal Temperature Range.—The animals were cut transversely through the middle of the body, thus dividing the pharynx. The time of regeneration of a complete head on the posterior half at different temperatures was then measured.

From the table it is seen that the lowest average temperature at which regeneration took place was 3° C.: of six specimens at this temperature only one regenerated at all, and in

Average Temperature.	Average Time of Regeneration.	Number of Observations.
3° C.	180 days (incomplete)	1
9°	46.6 "	5
13°	21.5 "	6
14°	17.8 "	6
21°	7.3 "	6
26°	6 "	4
28°	5 "	16
29.7°	4.6 "	8
31.5°	8.5 "	9

Table I. — Table showing the time of regeneration at different temperatures of the posterior half of *Planaria torva*.

six months the eyes and brain were still incomplete. Three of the others died before the six months were up; two lived and showed no signs of regeneration, though they were responsive to stimuli all the time. These two were then put at room temperature, and one regenerated fairly normally, while the other showed no sign of regeneration, though it remained alive for several days.

The optimum temperature is 29.7° C., and the time of regeneration at this temperature 4.6 days, the fortieth part of the time at 3° C. At 31.5° C. regeneration was slower, 8.5 days.

The curve which follows (Fig. 1) shows the law of rate of increase.

Between the minimum and the optimum the rate of decrease in time (increase in rapidity) of development diminishes with each degree rise in temperature. Let x equal the decrease in time for each degree increase of temperature, then:

From	3° to 9°	C., x =	22.23	days.
"	9° " 13°	C., x =	6.275	"
"	13° " 14°	C., x =	3.7	"
"	14° " 21°	C., x =	1.5	"
"	21° " 28°	C., x =	.329	"
"	28° " 29.7°	C., x =	.235	"

Table II.

b. Subminimal and Supramaximal Temperatures. — Very little effect was observable in the case of the subminimal temperatures. Some of the anterior halves showed a slight ten-

dency to form a bifid tail at 3° C.; but the process was slow and never went very far.

At temperatures above the maximum no abnormalities were found; at 32° the planarians regenerate partly; small eye-spots

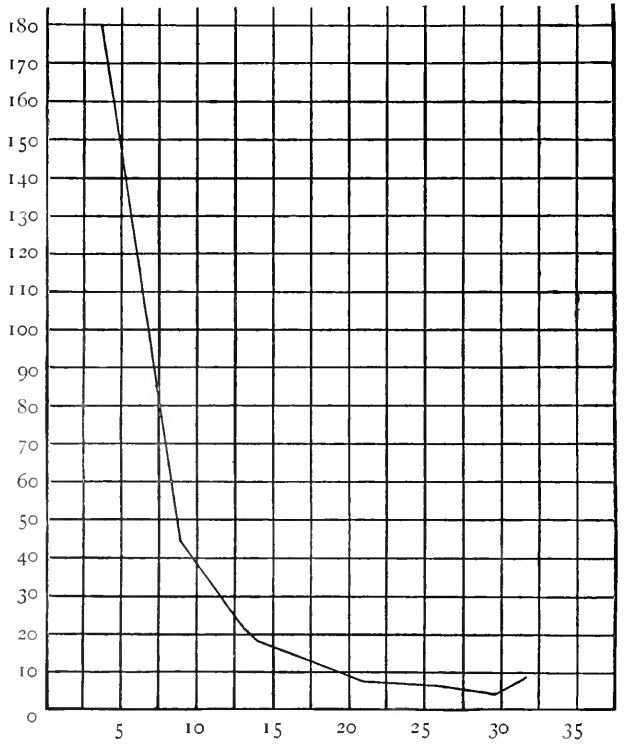


FIG. 1. — Curve of regeneration of the posterior half of *Planaria torva* at different temperatures. The ordinates measure time in days and the abscissae temperature in degrees Centigrade.

may appear, and death occurs in about six days; at 33° there was very slight regeneration, the animals dying within three days. At 34° and above there was no regeneration, and death came on rapidly.

2. *The Early Development of Amblystoma tigrinum and the Frog (Rana virescens).*

The eggs of Amblystoma are laid in March, often before the ice is entirely out of the ponds, when the temperature of the

water is not more than 3° or 4° C. The weather usually remains cool for at least a month after the laying of the eggs, so that during the early development the water never becomes very warm. The eggs of *Rana virescens* are not laid until the water is much warmer; thus they are never exposed to such low temperatures as Amblystoma.

a. Normal Temperature Range. — The extent of the early development measured in both forms was from the first or second cleavage to the last stage of disappearance of the yolk plug; these periods were selected as being most sharply marked ones; and the time occupied even at the optimum is sufficiently long to reduce the amount of error to a very small proportion of the total time involved.

Average Temperature.	Average Time of Development.	Number of Observations.
4° C.	288 hours	2
8°	210 "	4
9.5°	139.2 "	5
13°	96 "	2
14°	90 "	2
18°	60 "	3
22°	40 "	6

Table III. — Table of the time of development of the ova of *Amblystoma tigrinum* from the first cleavage to the last stage of disappearance of the yolk-plug. One observation does not mean necessarily a single egg, but often all the eggs of a bunch.

Average Temperature.	Average Time of Development.	Number of Observations.
4° C.	471 hours	1
8.75°	192 "	1
12.12°	126 "	2
16°	60 "	3
22°	27.5 "	2
24°	25.5 "	2
26°	21.5 "	1

Table IV. — Table of the time of development of the ova of *Rana virescens* from the first, second, or third cleavage to the last stage of disappearance of the yolk-plug. Each observation means at least 30 eggs. The period between the first and third cleavages is relatively so short that it may be ignored in comparison with the whole time involved.

It will be noticed that the form of the curve is similar to the temperature regeneration curve of Planaria. The angle in the curve of Amblystoma would probably disappear with a sufficiently large number of observations ; that is, it is prob-

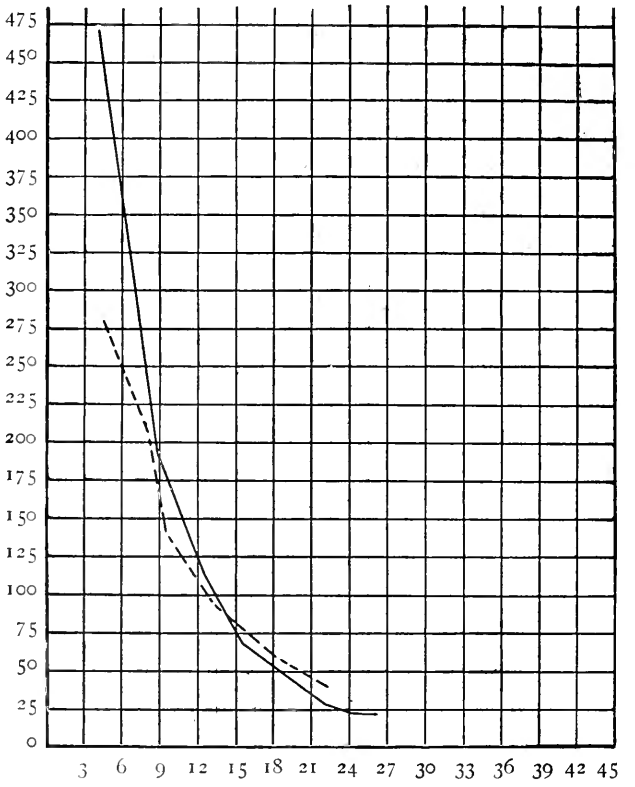


FIG. 2. — Curves showing the effect of the normal temperature range on the early development of Amblystoma and the frog constructed from the above tables The broken line is the curve of Amblystoma. The ordinates give the time in hours, 25 hours for each abscissa; and the abscissae represent degrees Centigrade, 3° for each ordinate.

ably due to individual variation in the eggs observed. It is important to notice that the optimum and maximum temperatures actually coincide. This is due to the difference in temperature range of the dark and light hemispheres ; the cells of the light hemisphere are much more easily affected by low and high temperatures than those of the dark (see section on subminimal and supramaximal temperatures). The cells of the

dark pole would probably show a maximum above the optimum, and similarly for the white ; but we are not able to prove this. It is also conceivable that the coincidence of optimum and maximum is due to the complexity of the processes involved, the temperature range possibly differing for the different factors concerned, such as cleavage, invagination, differentiation, etc.

Let x equal the decrease in time for each degree increase in temperature; the next table shows its value for Amblystoma and the following one for the frog.

4°	to 8° C.,	x = 19.5	hours.
8°	" 13° C.,	x = 22.8	"
13°	" 18° C.,	x = 7.2	"
18°	" 22° C.,	x = 5	"

TABLE V. — Amblystoma.

4°	to 8.75° C.,	x = 58.7	hours.
8.75°	" 12.12° C.,	x = 23	"
12.12°	" 16° C.,	x = 16.5	"
16°	" 22° C.,	x = 5.4	"
22°	" 26° C.,	x = 1.5	"

TABLE VI. — Frog.

The same law holds as for Planaria : between the minimum and the optimum the *rate* of decrease in time of development diminishes with each degree rise in temperature.

Below 14° eggs of Amblystoma develop more rapidly than those of the frog, and above less rapidly ; *see curves* (Fig. 2). The eggs of the frog also develop above the maximum for Amblystoma. This is undoubtedly an inherited effect of the different temperatures to which the eggs are normally exposed, as noted before.

b. Subminimal and Supramaximal Temperatures. — Subminimal temperatures have the same effect on the cleavage of the eggs of both forms. At 0° C. cleavage is entirely inhibited. If the eggs of Amblystoma were put in a vessel containing ice and the vessel placed on ice in the refrigerator, cleavage was inhibited entirely or to a great extent in the white hemisphere, though it went on slowly and fairly normally in the dark hemisphere ; death occurred before gastrulation, the temperature being about 1° C. The same effect was produced in eggs of the

frog at 2° to 3° C. At this temperature some of the eggs of the frog developed further, but always abnormally. The abnormalities produced were of the same nature as those described by Morgan and Tsuda ('94), by Hertwig ('94a) and by Gurwitsch ('96) in their studies on the effect of solutions of halogen salts of sodium and lithium; that is to say, abnormalities in the region of the blastopore, principally *spinae bifidae*. No anencephalic monsters were found.

Supramaximal temperatures have much the same effect, but the abnormalities are much more pronounced, owing partly to the fact that the embryo could be reared to a much later stage. *In both cases the effect is undoubtedly due to the greater sensitiveness both to subminimal and supramaximal temperatures of the cells of the white hemisphere*, as both Hertwig and Gurwitsch have remarked. The greater sensitiveness of the white cells is due to the relatively greater amount of yolk in them.

3. *The Growth in Length of Tadpoles of the Frog (Rana virescens) and Toad (Bufo lentiginosus) at Different Temperatures.*

a. Normal Temperature Range. — The tadpoles of the frog and toad were taken as soon as hatched (at the temperature of the room, 18–20° C.), and two measurements were made of each with the ocular micrometer under a very low power of the compound microscope (13.5 divisions = 1 mm.); the first measurement was the length of the tail *from the anus*, the second the total length. By subtracting the first measurement from the second we could get the length of the head and trunk, but this has not been included in the tables. At the end of 24 hours the measurements were repeated. The tables give the difference between the first and second measurements, that is, the growth in length in 24 hours. The growth in length of the trunk is very variable, owing to the fact that it is increasing at the same time in breadth as well as in depth, and more so than in length. The growth of the tail, on the other hand, is almost purely growth in length, so it alone is represented in the form of a curve. The tadpole is

subsisting entirely on its yolk supply during the time of the measurements, therefore the factor of variable food supply is eliminated. Thus the figures for growth in length of the tail represent only the effect of the different temperatures, excepting naturally individual variations which are partially eliminated by the averages.

Average Temperature.	Average Growth.		Observations.
	Tail.	Total.	
9.27° C.	2.27 units	4.5 units	2
11°	3.16 "	5.33 "	3
14.62°	3.75 "	4.27(?)"	2
18°	6.5 "	9.5 "	1
20.83°	11.16 "	19.83 "	3
25.27°	22.75 "	31.5 "	2
28°	30 "	40 "	1
29°	33.5 "	48 "	1
30°	38 "	47 "	1
31.3°	30.8 "	40.16 "	3
33°	28 "	43.5 "	1

Table VII. — Growth in length of frog tadpoles in 24 hours at different temperatures. 13.5 units equal 1 mm.

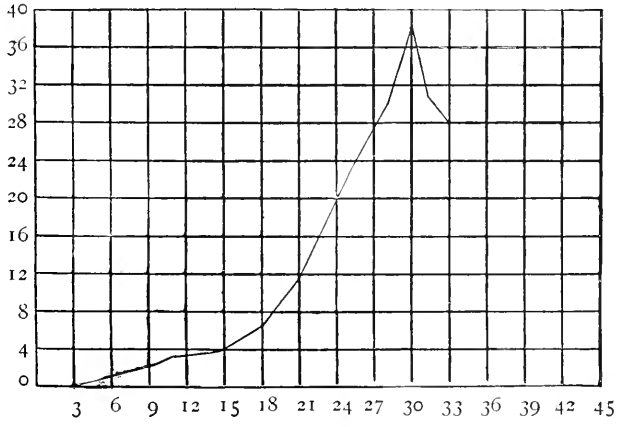


Fig. 3. — Curve of growth of the tail of frog tadpoles constructed from the above table. The ordinates represent units (13.5 = 1. 1 mm.) of growth and the abscissae degrees Centigrade.

Comparison of the curves and of the tables shows that the growth of the frog tadpole begins at a lower temperature than

AVERAGE TEMPERATURE.	GROWTH OF TAIL.	TOTAL GROWTH.	OBSERVATIONS.
9° C.	.5 units	3 units	1
11.6°	2.66 "	5.33 "	3
14°	6.5 "	15.5 "	1
16.3°	7.8 "	16.3 "	3
19.9°	10.16 "	21.16 "	3
24.8°	28 "	41.3 "	2
26°	34 "	39 "	1
29°	41 "	56.7 "	5
30.1°	41.6 "	56.8 "	5
31°	44 "	55 "	1
32°	37.5 "	55.5 "	1
33°	41.5 "	58 "	1
35°	44 "	57 "	1

TABLE VIII. — Growth in length of toad tadpoles at different temperatures. 13.5 units equal 1 mm. The last three readings are from one lot of tadpoles, and their difference from the others represents individual variation. They are not included in the curve.

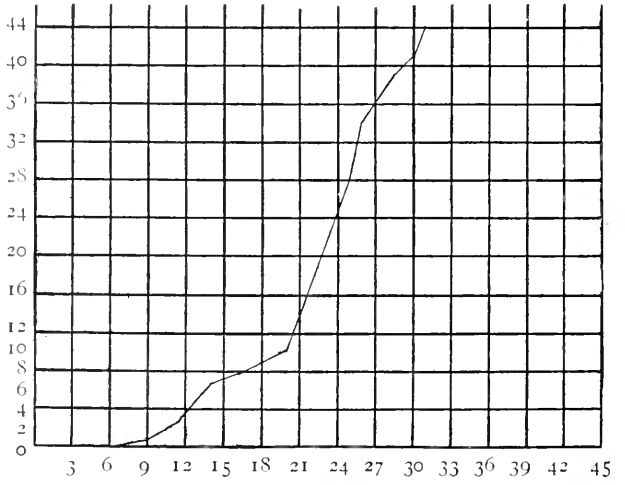


FIG. 4. — Curve of growth of the tail of toad tadpoles constructed from the above table. The ordinates represent units (13.5 = 1. 1 mm.) of growth and the abscissae degrees Centigrade.

the toad tadpole and that the latter continues to grow at a higher temperature (see table, not shown in curve) than the former. This is correlated with the fact that the eggs of the toad are laid later than those of the frog and generally in smaller, shallower pools, the temperature of which is relatively high; this has caused acclimatization to a higher range.

There is a slight decrease in the rate of growth beyond the optimum in the case of the tadpole of the frog. This is probably also the case with the toad, but our observations are not sufficiently extensive to show it, for the material gave out before it could be tested.

In both the frog and the toad tadpoles the rate of increase is relatively slow up to about 20°, when the rate becomes suddenly much more rapid. This is very marked in the tables; for instance, the elongation of the tail in the frog tadpole is 11.16 units at 20° in 24 hours, at 25° it is 22.75, or more than twice the amount at 20°; in the toad tadpole the figures are 10.16 and 28 at approximately the same temperatures, an even more striking increase. The curves show this too; see especially the curve for growth in length of the tail of the toad tadpole. Why there should be this sudden change in rate between 20° and 25° is difficult to determine.

b. Subminimal and Supramaximal Temperatures. — As was to be expected, we found that very low temperatures — that is, below 3° in the case of the frog and 6° in the case of the toad — entirely inhibited growth. But we obtained one result which was entirely unexpected: *at about 2° C. there was an actual shortening in the tadpoles of the frog in twenty-four hours;* this decrease in length was very slight, but sufficiently well marked to be plainly discernible, varying from .5 to 2 units. It was most plainly shown in the youngest tadpoles. We have come to the conclusion that the decrease is due to a diminution in the turgor of the cells, caused by diminished endosmosis dependent either on the low temperature directly, or on the non-production of the active endosmotic substance within the cell, or on both factors combined. In this connection the observation of Davenport ('97) that "the immense increment in weight which accompanies the outlining of the form of the larva (tadpoles of frog and toad) and its organs is due almost solely to absorbed water" is of importance.

Temperatures above the maximum for normal growth resulted as a rule very quickly in death of the tadpoles. There was, however, a marked tendency in the case of those that survived a sufficiently long time for the tail to grow out at an

angle with the axis of the trunk generally inclined ventrally, rarely dorsally, never laterally.

4. *General.*

This is the first time, I believe, that an attempt has been made to represent in the form of a curve the effect of temperature on the rate of development in animals, or that sufficiently extensive observations have been made to render this possible. The literature on the subject is mostly old and of historic interest only; the principal papers are cited in the list of literature. A good and detailed discussion of it is to be found in chapter V of Preyer's *Physiologie des Embryo* ('85).

But there have been more extensive observations on the influence of temperature on the rate of growth in plants. The following table, taken from Vines ('91), p. 293, shows the increments in length of hypocotyls of various plants in forty-eight hours after Köppen and de Vries.

	KÖPPEN.			DE VRIES.		
TEMPERATURE.	LUPINUS ALBUS.	PISUM SATIVUM.	LEA MAIS.	SINAPIS ALBA.	LEPIDIUM SATIVUM.	LINUM USITATISSIMUM.
14.1° C.	9.1 mm.	5.0 mm.				
15.1°	.....	.....	.....	3.8 mm.	5.9 mm.	1.5 mm.
18°	11.6	8.3	1.1 mm.			
21.6°	.....	.....	.....	24.9	38.0	20.5
23.5°	31.0	30.0	10.8			
26.6°	54.1	53.9	29.6			
27.4°	.....	.....	.....	52.0	71.9	44.8
28.5°	50.1	40.4	26.5			
30.2°	43.8	38.5	64.6			
30.6°	.....	.....	.....	44.1	44.6	39.9
33.5°	14.2	23	69.5			
33.9°	.....	.....	.....	30.2	26.9	28.1
36.5°	12.6	8.7	20.7			
37.2°	.....	.....	.....	10	0.0	9.2

TABLE IX. — Increment in length of hypocotyls in 48 hours, from Vines ('86), p. 293.

These figures are strikingly different in the relation of the maximum to the optimum from those which we have found

for animals. In all cases the maximum lies 6 to 8° above the optimum, and there is a very considerable decrease in the rate of growth from the optimum to the maximum. This is best seen in the following curve constructed from the above figures for *Lupinus albus:*

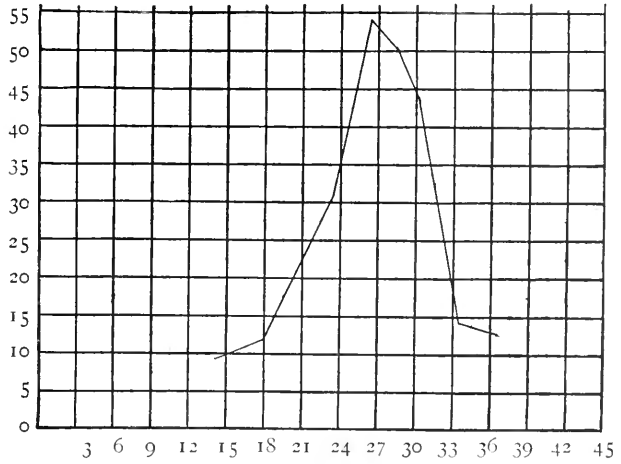


FIG. 5. — Curve of growth in length of the hypocotyl of *Lupinus albus* at different temperatures constructed from figures in Table IX. The ordinates represent growth in millimeters in 48 hours and the abscissae degrees Centigrade.

The ascending limb of the curve is very similar to the curves in Figs. 1–4. But the descending limb is much longer ; in animals, indeed, it may be entirely wanting. This indicates that while the general law of increase in rate of growth is the same for animals as for plants, plants have in general become adapted to resist temperatures farther above the optimum temperature of development. May this not be due to the fact that animals in general can and do retreat from the higher temperatures of direct sunlight, while plants are of necessity subjected to them? Even developing embryos of animals which possess no power of locomotion of their own are placed so as not to be subjected to these high temperatures.

It is rather interesting to find that the curves given above resemble in general form the curves measuring the rapidity of known chemical processes at different temperatures. See Freer and Dunlap.

We do not think it possible to state any general law as to the effect of supramaximal and subminimal temperatures on the *form* of development. The effect differs surprisingly in different organisms. So far as we know, most observations on this point have been made by subjecting the form or eggs to be experimented on to the abnormal temperature for a short time only, and then allowing development to continue at a normal temperature. Our observations, on the other hand, were on the *continuous* effect of abnormal temperatures.

Hertwig ('94b) found that in twenty-four hours at 0° C. no development took place, and that when the eggs were restored to the room temperature, in some of them "a larger or smaller part of the vegetative half of the egg was permanently injured, so that it could not undergo cleavage and had to be gradually excluded from the healthy developing parts." Schulze ('94) doubts this, and concludes from his own observations "that the eggs of *Rana fusca* in the gastrula stage can withstand complete inhibition of the development (by cold) for fourteen days without any sort of injury." However, it is quite certain from our own observations that many eggs develop abnormally at 3° C., as already noticed.

Hertwig ('94a), p. 314, has also noticed that by raising the temperature of the water in which eggs of the frog are developing there is a certain point at which the lower pole is first injured and divides incompletely or not at all, while the black pole forms a disc of small cells.

Driesch ('93) observed that abnormally high temperature caused great variations in the cleavage and partial suppression of micromere formation in both Sphaerechinus and Echinus. In a later study ('94) he tested the effect of abnormally high temperature (30° C.) on the gastrulation of Sphaerechinus; exogastrulae were produced. If we suppose that the archenteron grows in the direction of least resistance, we must conclude, as Driesch points out, that the conditions of osmotic equilibrium within the blastula have undergone alteration as the effect of the high temperature.

LITERATURE.

'97 DAVENPORT, C. B. The Rôle of Water in Growth. *Proc. Boston Soc. Nat. Hist.* Vol. xxviii, No. 3. Abstract in *Science*. Vol. v, No. 115.

'93 DRIESCH, HANS. Entwicklungsmechanische Studien: IV. Experimentelle Veränderungen des Typus der Furchung und ihre Folgen; Wirkungen von Wärmezufuhr und von Druck. *Zeitschr. f. wiss. Zool.* 53.

'94 DRIESCH, HANS. Entwicklungsmechanische Studien: VII. Exogastrula und Aventeria. (Ueber die Wirkung von Wärmezufuhr auf die Larvenentwicklung der Echiniden.) *Mitth. aus der Zool. Stat. zu Neapel.* xi.

FREER, PAUL C., AND DUNLAP, F. L. On the Saponification of the Substituted Acetic Esters. *Am. Chemical Journal.* Vol. xiv, No. 5.

'22 GASPARD, B. Einfluss der Temperatur auf die Entwicklung der Schneckeneier (*Helix pomatia*). Magendie's *Journal de Physiologie expérimentale et pathologique.* ii, 295, § 20. Paris.

'96 GURWITSCH, ALEX. Ueber die formative Wirkung der veränderten chemischen Mediums auf die Embryo-Entwicklung. *Archiv für Entwicklungsmech.* iii, pp. 221–260.

'94a HERTWIG, OSKAR. Beiträge zur experimentellen Morphologie und Entwicklungsgeschichte: I. Die Entwicklung des Froscheies unter dem Einfluss schwächerer und stärkerer Kochsalzlösungen. *Archiv für mikr. Anat. u. Entwicklungsgesch.* Bd. xliv, pp. 280–344.

'94b HERTWIG, OSKAR. Ueber den Einfluss äusserer Bedingungen auf die Entwicklung des Froscheies. *Sitzungsber. der königl. preuss. Akad. d. Wiss.* xvii. 3 April, 1894.

'78 MEYER, H. A. Abhängigkeit der Entwicklungszeit des Heringsembryo von der Wasserwärme. *Jahresber. der Commission zur wiss. Untersuchung der deutschen Meere in Kiel.* 4.–6. Jahrg. Berlin. pp. 207–240.

'94 MORGAN, T. H., AND TSUDA, UMÉ. The Orientation of the Frog's Egg. *Quar. Journ. Micr. Sci.* Vol. xxxv, Part 3.

'85 PREYER, W. *Specielle Physiologie des Embryo.* Leipzig. Th. Grieben. Chap. v. *A.* Einfluss der äusseren Temperatur auf den Embryo im Ei. pp. 343–359.

'83 RAUBER. Einfluss der Temperatur, des atmosphärischen Druckes und verschiedene Stoffe auf die Entwicklung thierischer Eier. *Ber. der Naturf. Gesellsch. zu Leipzig.* 8 May, 1883.

'94 SCHULZE, O. Ueber die Einwirkung niederer Temperatur auf die Entwickelung des Frosches. *Anat. Anz.* No. 9.

'86 VINES, SIDNEY F. Physiology of Plants.

THE CELL LINEAGE OF PODARKE OBSCURA.

PRELIMINARY COMMUNICATION.

A. L. TREADWELL.

THE segmentation of Podarke is of especial interest as a representative of the "equal type" of cleavage among annelids. By the first three cleavages the ovum is divided into eight cells of equal size, four above and four below. The next division establishes inequalities in size among the cells, but until the 56-cell stage is reached the quadrants are exactly alike. At the 56-cell stage one quadrant becomes different from the other three, and soon after bilateral divisions begin.

The transition from the 2 to the 4-cell stage is accompanied by a rotation to the left, and the familiar cross furrows appear. The furrow at the top is at right angles to that at the bottom and much shorter. The furrow at the bottom has the same direction that it has in Nereis,[1] Amphitrite,[2] Arenicola,[3] and Crepidula,[4] and retains this direction until it is possible to distinguish the quadrants by means of other landmarks. Later, owing probably to variations in position of the cells forming it, the direction of this furrow may vary. The first two planes of cleavage have the same relation to the median axis of the embryo that they have in Amphitrite and Arenicola.

The second group of micromeres[5] are given off in a left-handed spiral. These cells arise at the same time and are equal in size, there being no large d^2. Simultaneously with the origin of these, the cells $a^{1.1}$, $b^{1.1}$, $c^{1.1}$, $d^{1.1}$ are formed at the upper pole.

In the transition from sixteen to thirty-two cells all the divi-

[1] E. B. Wilson, "Cell Lineage of Nereis," *Journ. of Morph.*, vol. vi.

[2] A. D. Mead, "Early Development of Marine Annelids," *Journ. of Morph.*, vol. xiii.

[3] C. M. Child, *Zoöl. Bull.*, vol. i, no. 2.

[4] E. G. Conklin, "Embryology of Crepidula," *Journ. of Morph.*, vol. xiii.

[5] The term "micromeres" is used here simply for convenience, the division of the "macromeres" being approximately equal during the first four divisions.

sions are dexiotropic. The third group of micromeres appears, and at the same time cells $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, $d^{1.2}$ are formed; $a^{1.1}$, $b^{1.1}$, $c^{1.1}$, $d^{1.1}$ then divide to form the *primary trochoblasts*, and a little later the 32-cell stage is completed by the division of the second group of micromeres.

From thirty-two to sixty-four cells all divisions are laeotropic, and this period may be divided into three stages.

First, from thirty-two to forty cells. This is accomplished by the division of A_3, B_3, C_3, D_3, to form the fourth group of micromeres, and the formation of the apical rosette by the division of $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, and $d^{1.2}$.

The micromeres of this fourth quartette are equal in size, and lie between the cell from which they arose and the left-hand descendant of the second group of micromeres of the corresponding quadrant. At this stage, since the cells given off from the four cells at the top have been smaller than the micromeres of the second and third generations, and since the apical rosette cells are very much smaller than the cells of the fourth generation of micromeres, it follows that the four cells surrounding the rosette are much the largest in the embryo. These cells later divide and form the prominent cross. (See Fig. 4.) The rosette cells elongate and push into the segmentation cavity, retaining their connection with the outside by only a slender stalk. They later come to the surface and divide. (See Fig. 3, where is shown also the relative size of cells at the two poles of the embryo; those at the upper pole are dividing to form the cross.)

Second, forty to fifty-six cells. This is accomplished by a division of cells $a^{1.1.1}$, $b^{1.1.1}$, $c^{1.1.1}$, $d^{1.1.1}$; $a^{1.1.2}$, $b^{1.1.2}$, $c^{1.1.2}$, $d^{1.1.2}$; $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, $d^{1.2}$; and a^3, b^3, c^3, d^3. The first two of these sets of cells are the *primary trochoblasts*. By their division are formed sixteen cells, *all* of which acquire cilia and become the primary prototroch, which here, as in other forms, is composed of four distinct areas of ciliated cells. These later, by the addition of cells from the lower hemisphere, become united into a single band with a dorsal interruption. Before the completion of the prototroch band a strong tuft of cilia is formed at the apical pole.

Third. The 64-cell stage is completed by the division of $a^{2.1}$, $a^{2.2}$, $b^{2.1}$, $b^{2.2}$, $c^{2.1}$, $c^{2.2}$, $d^{2.1}$, $d^{2.2}$; and here arises the first distinction which I have discovered between the quadrants. While in three quadrants the division is such that the upper left-hand cell ($a^{2.2.1}$, etc.) is smaller than the one below it ($a^{2.2.2}$, etc.), in one quadrant the *lower* cell is much the smaller, has a peculiar deeply staining nucleus, and is easily distinguished from the corresponding cells in the other quadrants. (See Figs. 1 and 2, as also Fig. 5, where the size of this cell as compared with the corresponding one in the other quadrants is shown. It should be remembered that the "left-

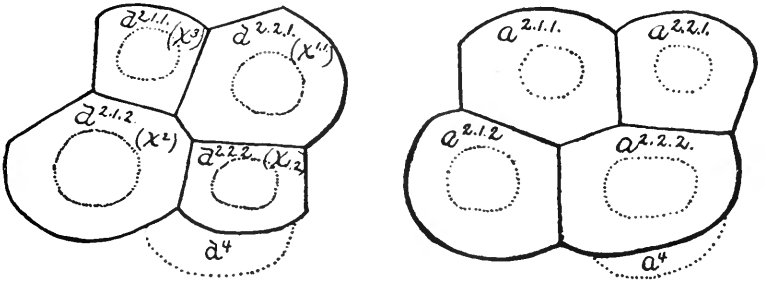


FIG. 1. — Second group of micromeres in D quadrant after their second division (64-cell stage).
FIG. 2. — Second group of micromeres of A quadrant at 64-cell stage. Compare relative sizes of $d^{2.2.2}$ and $a^{2.2.2}$.

hand" cell is the one which is on the left when looked at from the animal pole.) This lies immediately over one of the fourth group of micromeres, and a little to one side of the second cleavage line. For reasons which will appear more fully later on, I believe that this corresponds to the cell $x^{1.2}$, described in Amphitrite, Nereis, Arenicola, Crepidula, and Unio.[1]

The next divisions are those of cells $a^{1.3}$, $b^{1.3}$, $c^{1.3}$, $d^{1.3}$, at the upper pole, leading to the formation of the apical cross. Two of these cells divide equally or nearly so, while the other two divide very unequally. (See Fig. 4.) Here, although the divisions are still of the spiral type, a bilateral arrangement of cells results. The second line of cleavage passes in the direction indicated by the numerals. It is interesting to note that

[1] Lillie, "Embryology of the Unionidae," *Journ. of Morph.*, vol. x.

this division is dexiotropic, the form which it ought to assume under the law of alternating cleavages. A distinction between the anterior and posterior arms can be recognized during several divisions of the cross cells and until meridional divisions of its cells destroy the outline of the cross.

At the vegetative pole the next division is a bilateral one, of one of the fourth group of micromeres, accompanied by a bilateral division of the lower members of the third group which lie on either side of it. (See Fig. 5.) Here $c^{3.2}$ and $d^{3.2}$ have divided, while d^4 is still in process of karyokinesis. Just

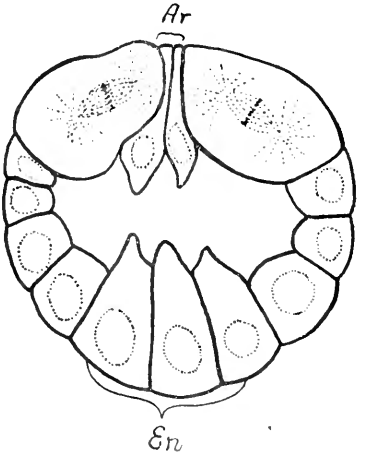


FIG. 3.—Optical section of embryo at the first division after the 64-cell stage. *Ar.*, apical rosette. *En.*, entoderm.

above this cell is the small cell $x^{1.2}$, and a plane passing through these cells coincides with the plane of bilateral symmetry of the cross.

This cell of the fourth quartette is $d^4 = M$; and, while the other three members of the fourth quartette migrate bodily into the segmentation cavity, its products remain at the surface until the blastopore has nearly closed. They then divide, each sending a cell into the segmentation cavity, and form the mesoblast bands.

The inner ends of the entoderm cells A_4, B_4, C_4, D_4 elongate very considerably, and the nuclei migrate to a considerable distance toward their inner end. (See Fig. 3, which shows the

beginning of the process.) Subsequently, however, these nuclei come to the surface and divide, forming the fifth group of micromeres. The eight cells thus formed invaginate, forming with the three members of the fourth quartette an invaginating plate of eleven cells, and the blastopore closes rapidly, closure being effected largely, if not entirely, by the division of the descendants of the third group of micromeres.

For purposes of comparison I have made a few observations on other forms with equal cleavage.

Lepidonotus sp.[1]

In Lepidonotus the bilateral cross and the small cell ($x^{1.2}$) appear exactly as in Podarke. These structures were overlooked by Mead,[2] who was also misled, by the appearance of

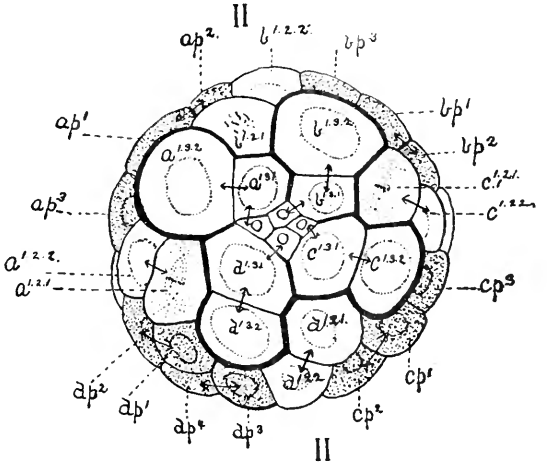


FIG. 4. — Cross when first formed, seen from animal pole. Some of the intermediate cells are dividing. Sixty-eight cells.

a "pseudo-invagination" similar to that of Podarke, into the statement that no fifth group of micromeres are formed. Here, as in Podarke, the nuclei migrate to the surface, and the micro-

[1] The most extended observations previously published on equal cleavage in annelid eggs are those of Mead on Lepidonotus. To Dr. Mead's generosity I am indebted for preparations of some of the later segmentation stages.

[2] *L. c.*, p. 267.

meres of the fifth generation are given off. These differ from other forms in the fact that the resulting micromeres a^5, b^5, c^5, d^5 are larger than the cells at the vegetative pole A_5, B_5, C_5, D_5.

Owing to lack of fresh material I have been unable to follow the history of the cross furrow, but it is interesting to

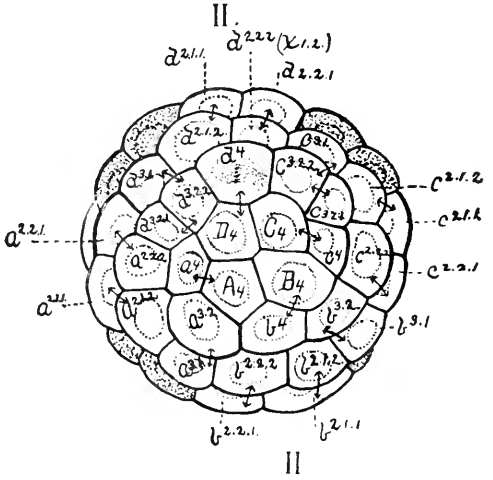


FIG. 5. — Seventy-cell stage from vegetative pole, showing bilateral division of d^4. $c^{3.2}$ and $d^{3.2}$ have already divided. The other three members of the fourth quartette are beginning to invaginate.

note that in all specimens which I have seen it has the same direction with respect to the cell $x^{1.2}$ which it has in Podarke.

I have been unable with the material at hand to discover the formation of the mesoderm.

Sthenolais picta.

I have followed the segmentation of this annelid only as far as the 64-cell stage, but in all respects it agrees exactly with Podarke. The cross furrow takes and retains the typical direction, and the cell $x^{1.2}$ is formed at the same place and time as in the other species.

Hydroides dianthus.

In this annelid the 32-cell stage is formed as in the other cases, but beyond this stage important differences appear. The

cross furrow seems not to retain its original position, and, although the small cell $x^{1.2}$ appears, other divisions of the second group of micromeres occur before any other landmarks are to be seen, and orientation is difficult. A fifth group of micromeres is given off, and the regular invaginating plate of eleven cells appears. One member of the fourth group of micromeres divides bilaterally at the surface, forming what I suppose is the mesoblast. The rosette is formed as in other cases, but the cross remains radially symmetrical until a late stage in the segmentation.

The primary trochoblasts arise as in Podarke, but do not divide again, thus forming two in each quadrant instead of four. Wilson [1] showed that the prototroch of Hydroides is composed of eight cells.

From these observations it is evident that equal cleavage in annelids is not in any way caused by or an expression of a lack of differentiation in the ovum. Although the dorsal cannot be distinguished from the ventral quadrant until the 64-cell stage, it is perfectly possible at the 4-cell stage to determine by means of the cross furrow the median plane of the future embryo. That this cross furrow sometimes varies in direction in later stages is fully explained by the fact that the cells forming it are very small (after the fourth group of micromeres are given off), and more or less of what I have called "pseudo-invagination" occurs. The furrow itself is short as compared with such forms as Nereis or Amphitrite. Under these conditions, as Conklin [4] has shown, the direction of the cross furrow may easily vary because of variations in pressure on the entoderm cells.

Further, the regular alternation from a right to a left-handed cleavage, the regular appearance of certain definite cells at certain definite times, indicate that we have here a differentiation as complete as in any form with unequal cleavage. At the 32-cell stage the embryo is approximately spherical, and is surrounded by a thin, much-wrinkled egg membrane. The four cells at the animal pole are a very little larger than those

[1] E. B. Wilson, *l. c.*, p. 398.

of the vegetative, but *are hard to distinguish from them except for the presence of the polar globules*. The next division, however, leads to the formation of the rosette cells at the top, and the fourth group of micromeres at the bottom, the latter division being nearly an equal one, the former very unequal.[1] It is difficult to explain cases of this kind except on the assumption of a complex differentiation in the protoplasm of the egg.

That mechanical conditions can play but a small part in the regulation of cell divisions is shown especially well by the small cell $x^{1.2}$. This cell arises in exactly the same manner in Amphitrite, Clymenella, Arenicola, Unio, and Crepidula (Conklin's $2d^{2.2}$) of the unequal, and in Lepidonotus, Sthenolais, Podarke, and Hydroides of the equal type. Comparison of the figures of the first forms with those of the second will show that mechanical conditions must be very different in the two cases. On the one hand, we have large mesoderm and entoderm cells which must exert considerable pressure on the cells surrounding them; on the other, the largest cells in the embryo are at the animal pole. These facts point to some definite complex organization of the egg protoplasm.

The problem to be settled by a study of equal cleavage has been stated by Mead thus:[2] "Whether one of the two cells in equal cleavage is homologous with the larger cell in unequal cleavage." I believe that one of these cells in the one case is homologous with one in the other, and that the second of Mead's alternatives is correct, that the "peculiar destiny of the cell" is "the cause of its larger size," and I would suggest that the cell *D* in the unequal type is larger, not simply because it contains somatic and mesodermic material, as Wilson supposed, but because it contains *an extra supply* of this material. Sufficient data for wide generalizations are not at hand, but such as we have bear out this supposition.

The trochophore of Podarke is small, with very thin walls and a feeble development of mesodermal tissue. It grows very slowly, so that scarcely any change except a slight increase in length is perceptible from twenty-four to seventy-two hours. Dr. Mead informs me that the same is true of the trochophore

[1] *Cf.* Mead, *l. c.*, p. 293. [2] *L. c.*, p. 278.

of Lepidonotus. Amphitrite, on the other hand, rapidly develops strong parapodia with numerous setae, and by sixty hours has four trunk segments. Compare also the four-day trochophore of Eupomatus [1] (Hatschek's Fig. 50, which strongly resembles the Podarke trochophore) with the sixty-hour trochophore of Nereis (Wilson's Fig. 91). The former is thin-walled with a large cavity and no trace of metameric segments or of parapodia. The latter has three pairs of segments, with large parapodia, setae, and cirri. If the law that the size of a cell bears some definite relation to the size and time of appearance of the organ to which it gives rise [2] applies in other cases, may it not apply here as well, and may we not suppose the extra amount of material stored in cell *D* of Nereis, Amphitrite, etc., is in some way related to the need for an extra amount of somatic and mesoblastic material in the young larva?

I think that the supposition is a reasonable one, and would state my conclusions thus. The large size of the posterior macromere *D* in forms with unequal cleavage is due to the fact that the young larvae of these forms require for their development an excessive amount of the characteristic products of this cell, — mesoblastic and somatic tissue, — and hence arises an accumulation of this material in this particular cell. This material differs, not in quality, but in quantity from that in the corresponding cell in equal cleavage (hence there is as truly a "precocious segregation" in the one case as in the other), and the two cells are to be regarded as absolutely homologous.

MARINE BIOLOGICAL LABORATORY,
WOODS HOLL, MASS.,
Aug. 23, 1897.

[1] Hatschek, "Entwick. d. Trochophore von Eupomatus." Wien, 1885.
[2] *Cf.* Lillie, *l. c.*, and Conklin, *l. c.*

NOTES UPON CORDYLOPHORA LACUSTRIS

CHAS. W. HARGITT.

VARIABILITY among living things, whether in habits, structure, or development, is now so fully recognized as to need no special emphasis as an important law of nature. The list of facts is not, however, so complete as to render others unimportant; and it is with this point in view that the following notes have been thought worthy of record.

Cordylophora as a genus of hydroids has long been of more than usual interest to the biologist as affording among its phylum a rather striking illustration of remarkable range of environmental adaptation. Its ability to range from a distinctively marine habitat to that of fresh water has long been known, and is well expressed in the specific name applied to it. Allman was, I think, the first to call particular attention to this peculiarity and to make some experiments and repeated observations of very interesting nature in connection with it.[1] It is with a view to confirming and extending these observations that attention is directed to them in this connection.

Through the kindness of Mr. H. W. Britcher, of Johns Hopkins University, I received in December, 1895, a colony of these hydroids obtained from near Baltimore, Md. They had been collected by Mr. Britcher some time earlier and were brought to Syracuse in about a pint of brackish water, attached to a bit of slag upon which they grew, and upon which were also growing several specimens of acorn barnacles, Balanus, and bits of a filamentous marine alga. They remained in my laboratory for several weeks, where they were inspected by students and visitors. Twice during this time they were frozen almost solid and, as I supposed, killed by the ordeal. They were therefore set aside and for several weeks unobserved. A subsequent examination, however, showed a few

[1] Allman, Gymnoblastic Hydroids, p. 253.

living specimens, though the colony had evidently suffered as a whole. No particular attention was given them, and the later growth of algae had seemed to entirely stifle the animal life of the jar. A portion of the water was poured off and the jar replenished from the laboratory tap, which is supplied from the city water system. Once more the jar was set aside and unnoticed for at least a fortnight, when, to my surprise, one day the barnacles were seen to be living and active. This observation led to the introduction of specimens of Protozoa and Ostracoda from another aquarium to serve as possible food supply. No further attention was given to the matter till incidentally late in May, 1896, when from a cursory examination no life was apparent except that of the Ostracoda, which had evidently multiplied to considerable extent. The water was once more poured off and once more renewed from the tap. Observations made some time later revealed the presence of several colonies of hydroids in apparently flourishing condition, and the barnacles were also living and active.

While the observations as a whole are interesting, those aspects pertaining to the barnacles were specially so. While these animals can endure long periods of removal from the water, as removal above tide-water, etc., there is no record, so far as I am aware, that they can endure changes so radical in their nature as those above indicated. That the water was at first saline to considerable extent was evident enough in the fact of their presence in it. That they could endure a change to practically fresh water is likewise evident. It is true that the changes were not sudden ; and this would indicate the no less interesting fact of the adaptability of the organism to changing conditions of environment.

Allman records that in his experiments he found that the hydroids when changed from the slightly brackish water of the Indian docks to that of fresh showed unmistakable signs of decline, many of the hydranths falling from the stem and the colonies as a whole showing decadence. On the additions of slight portions of sea water they soon recovered and grew freely. I have had similar results from the additions of small quantities of salt to the water of the aquarium.

While no chemical analysis was made of the quantitive composition of the water at the beginning and conclusion of the observations, it is, however, evident that it must have been almost completely changed in the several operations through which it passed.

Lankester[1] has recorded the fact that Cordylophora may be kept for some time in cans of water if kept in a dark place. My observations show that this precaution is not essential, since they were frequently exposed to direct sunlight and were constantly open to strongly diffused light in a north window. The following extract from a letter of Mr. Britcher will show that his own observations on this point are quite in accord with my own: "From December 15 to January 15 they were kept in the cellar, and when taken out only a few individuals seemed to be living. Since this time they have been on my window, where they get direct sunlight from 2 to 4 P.M. The water has frozen several times, once apparently solid. . . . To-day, March 5, there are at the lowest estimate over a hundred individuals in the colony."

At the present writing, October 20, both hydroids and barnacles are still living in less than a pint of water containing only a trace of salts in solution, the hydroids feeding freely upon the Ostracoda, entangling them in the long and graceful folds of their tentacles and engulfing them entire. They seem also to be reproducing both by budding and from sexual gonophores in perfectly normal fashion. While the barnacles are alive and sweeping their tentacles as usual, the movement is apparently feeble and would indicate a low stage of vitality.[2]

[1] *Quar. Journ. Mic. Sci*, vol. xvi, p. 26.

[2] Since the foregoing "Notes" were in type the vessel in which the organisms described were originally placed has been reëxamined with some care and not a little interest. Although left without attention of any sort during the entire year, except for the precaution of a close cover to prevent access of dust or loss from evaporation, colonies of hydroids are still living and thriving apparently as normal as when first examined. It should be said, however, that those living at present are not the identical colonies, though of course direct offspring of them. No food of any sort has been provided from without, though the examination revealed the presence of several species of Protozoa, Rotifera, Vermes, and also several species of Algae, Diatoms, etc.

The barnacles, on the other hand, have apparently perished. This is, however, not strange. The wonder is that under the extremely artificial conditions any life should have persisted during so long a period with no precautions to prevent deterioration. Such, however, are the facts, open attention to which has been directed at several times within the past few weeks. If a similar experiment has been made I should be glad to have my attention directed to it.

SYRACUSE UNIVERSITY,
October 10, 1897.

HIRUDINEEN STUDIEN.

PRELIMINARY NOTICE.

ARNOLD GRAF, PH.D.,

ASSOCIATE IN BIOLOGY, STATE OF NEW YORK PATHOLOGICAL INSTITUTE.

THE complete paper which will be published under the above title was finished in April, 1896. Many difficulties have arisen with regard to its appearance in print, and when it will be given to the public is as yet entirely uncertain.

The fact that certain of the problems which I tried to solve are being worked out by at least one other author compels me to communicate the more important of my results in a condensed preliminary form.

The primary object of my paper was to investigate the anatomy of the excretory organs of Nephelis and Clepsine. During the progress of the work, however, it became evident that without an insight into the finest structure of the nephridial cells no inferences could be drawn as to the physiology of excretion.

On the other hand, it was seen that without a fair knowledge of the general organization of these animals no deeper anatomical understanding of the organs in question could be gained, and this conviction induced me to devote some time to the study of the other organs. During this study it was found that the lymphatic cells stand in intimate relation to excretion, and considerable time was given to the investigation of these cells.

The whole paper was finally divided into:

1. A short account of the general organization of Nephelis and Clepsine.
2. The anatomy of the excretory organs.
3. The cytology of the excretory organs.
4. The physiology of excretion.
5. The origin of the pigment.
6. The cause of the color pattern in the skin.

The subjects of the last two items have been shortly dealt with in a preliminary account which appeared in the *Zool. Anz.*, No. 468, February, 1895, "Ueber den Ursprung des Pigments und der Zeichnung bei den Hirudineen."

The physiology of excretion has been made the subject of a lecture at the Marine Biological Laboratory at Wood's Holl, Mass., in August, 1896, which will be published in the volume of lectures for 1896 and 1897.

The few new points in No. 1 I shall not record here, and it therefore only remains to me to outline my results on the anatomy and cytology of the nephridium.

I. *Anatomy of the Nephridium of Nephelis and Clepsine.*

The following terms have been proposed for the different parts of the nephridium of these forms:

1. Portio afferens: the funnel apparatus.

2. Portio afferens-glandulosa: the part of the nephridial gland which both receives excretory products and which at the same time by its own chemical activity produces oxydized end-products.

3. Portio glandulosa-efferens: the part of the nephridium which has, besides the chemical excretory function, the task of conveying excretory end-products to the outside.

4. Portio efferens: the terminal vesicle and the terminal canal, opening at the surface of the animal to the exterior.

The portio afferens. — The funnel apparatus consists of two distinct parts: the crown and a vesicle.

The funnel crown is formed in Nephelis by from five to eight bilobed ciliated cells grouped around a central lumen. The cells are all strongly curved to the outside and downwards, and the funnel crown may in its shape be best compared with the flower of a tiger-lily (Lilium tigrinum or Lilium martagon). The crown cells are covered with cilia on the convex (upper and inner) surface, and these cilia produce by their motion a stream running centripetally, by which little granules floating in the surrounding liquid are conveyed into the central opening.

In Clepsine the funnel consists of three cells, two crown cells and one peduncle cell. The crown cells are differently shaped in different species, but they all more or less resemble the crown cells of Nephelis. The peduncle cell is one long cylindrical cell with an intracellular lumen, which lumen is lined with long cilia.

The crown cells are situated opposite each other on the outer end of the peduncle cell, and the whole funnel crown therefore resembles the letter T.

I have been fortunate enough to determine the mode of growth of the funnel cells by dissecting out funnels of very young and of very old specimens.

The vesicle, which is the second important part of the funnel apparatus, has the same appearance in all the leeches. I propose for it the term "receptaculum excretorium." It is a hollow vesicle, the walls of which are composed of flattened connective-tissue cells, and it is only open toward the central opening of the funnel crown, and closed *in all other places*.

The connection of this vesicle with the funnel crown cells has been generally misunderstood.

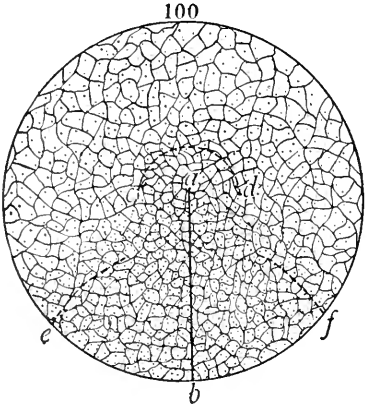
The connective-tissue wall of the receptaculum is continuous with a connective-tissue membrane covering the entire outer and under surface of the crown cells in Nephelis and enveloping the whole peduncle cell in Clepsine. Thus the ciliated funnel crown is entirely wedged in with its unciliated surface into the receptaculum, as the petals of some flowers are imbedded in the calyx leaves.

In very young funnels of Clepsine I have found that both the peduncle cell and the receptaculum are extremely minute, the receptaculum being hardly demonstrable, and thus the conclusion was easily drawn that this receptaculum is only a by-product in the process of growth of the funnel proper, growing with the increase of excretion (see Physiology of Excretion). That the receptaculum is a hollow vesicle filled with débris was proved by pricking it with a needle, when the contents were seen flowing out, while the walls collapsed.

The funnel of Nephelis lies in the first ampulla of the segment, whereas in Clepsine the position of the funnels is very

variable. In some species it lies in the ventral lacuna near the ganglionic chain; in others it is situated in a lateral pouch of this ventral lacuna; in others it still lies ventrally in the so-called intermediate lacuna (Zwischen lacune) of Oka; and, finally, it may assume a dorsal position in a coelomic section near the lateral lacuna.

In Clepsine Hollensis the funnels of the anterior segments lie near the ventral lacuna, whereas in the posterior segments



they lie dorsally, very near the lateral lacuna; a fact which would imply that the more primitive position is the ventral one.

The other part of the nephridium of both genera consists of a chain of cells possessing a different structure in different parts.

The portio afferens-glandulosa, the innermost cells of which stand in close contact with the receptaculum (not in open communication, however), is characterized by an intricate network of vacuoles and canals filling the cells of that portion. The portio glandulosa-efferens is pierced by a single central canal only. Both portions are covered with a connective-tissue membrane.

The whole course of the nephridium with all its intricate windings and loops was outlined with the help of the camera lucida from a very young specimen of Clepsine Hollensis lightly

pressed under a cover glass while living, and it was found that the shape of the nephridium adapts itself to the shape of the spaces left free by the surrounding tissues. It is impossible and idle to establish morphological types of nephridia for the different genera of leeches.

The vesicula terminalis is lined by an epithelium-bearing cilia on the inner surface. The canalis terminalis is to be regarded as a simple invagination of the epidermis.

The anatomical investigation was almost exclusively prosecuted on living material, partly on very young uninjured animals, partly by the study of rapidly made dissections and teased preparations. This was of paramount importance in order to gain perfectly trustworthy results and to eliminate the subjective interpretation of reconstructions from sections. This anatomical investigation was finished in all its details in the fall of 1894, and all the drawings pertaining to this part of the work were executed in Wood's Holl during the summer of 1894.

In the fall and winter of 1894 the investigations concerning the pigment formation and origin of markings were finished, and in the spring and summer of 1895 the greatest part of the cytology of the nephridium was worked out.

II. *The Cytology of the Excretory Organs.*

New points were gained with regard to the finest structure of the ciliated cell by the study of the ciliated crown cells of the funnel.

The cilia were found to consist of three parts, a basal piece staining deeply with haematoxylin, a middle piece staining very faintly with the same agent, and the flagellum or cilium proper staining with the acid anilins, *e.g.*, Bordeaux red. The basal piece is rod-shaped, and stands not quite vertically to the ciliated surface, but a little inclined. The middle piece is more round, and it seems as if the middle pieces of one row of cilia touched each other. The flagellum is thin, very long, and elastic, and is regarded by the author as a passive material, a metamorphosed protoplasm, like a secretion.

The cause of the ciliary motion is to be sought in the contraction of the basal piece, the middle piece playing a nervous rôle.[1]

This structure seems to be of general occurrence, as the writer found it also, during the last winter, in the ciliated intestine cells of the leeches in all its details.

The cytoplasm of the ciliated funnel cell is specifically modified, inasmuch as the cytoplasmic threads are arranged parallel to each other, each cilium standing in connection with one of these threads.

It was observed at first that near the edge of the ciliated cell, where the basal pieces of the cilia stand in communication with the cytoplasmic threads, a great number of little granules are assembled which make this connection obscure, and it was at first inferred that those granules all consisted of food matter destined for the regeneration of the basal pieces. I have, however, after the study of the cells of the intestine in these animals, altered my views in that respect. Only a portion of these granules is food material. The basal piece of the cilium is connected with a fine, short thread projecting into the cell and ending inwardly in a thick granule. (These granules have been discovered and described by me as peripheral organs in other cells.) Only this granule is connected with the cytoplasmic thread.

The function of these peripheral organs is a problem of very great interest, but I must leave a discussion of this to a later time, when I have completed my observations.

The cytoplasmic threads of the ciliated cell are all parallel and show not the slightest connection in their arrangement with the nucleus.

The nucleus is large, shows a very clear, distinct linin network, and the chromatin is suspended in that network in the form of little granules.

The cells of the portio afferens-glandulosa. — The row of cells forming that part of the nephridium which I call portio afferens-glandulosa presents under the microscope very various structures, according to the place from which they are taken.

[1] See Physiology of Excretion.

The cells nearest to the receptaculum are very large, possess a large nucleus with loose linin network, very large nucleoli, and loosely scattered chromatin granules. The membrane of the nucleus is very thin. The shape of the latter is very irregular, numerous processes projecting all over the surface.

The cytoplasm appears as a beautiful clear network with rather wide meshes. This network is not, as Bolsius states, radially arranged, but it is equally well developed in every direction. The microsomes cluster around the cytoplasmic threads.

These cells are filled with two kinds of vacuoles, some large ones near the periphery and great masses of exceedingly small ones near the center of the cell.

In the cells farther away from the receptaculum the peripheral vacuoles have partly fused together, forming an irregular network of canals, and the vacuoles of the central masses also fuse more and more together and ultimately form one single central canal in the cell.

As soon as this central canal is definitively formed a new group of structural elements becomes conspicuous in the cell, namely, the peripheral organs.

They appear at first only in the shape of microsomes, a trifle larger than the other microsomes of the cell and more regularly arranged along the inner edge of the cell surface. The farther away we go from the receptaculum the larger these peripheral microsomes grow and the more regularly they are arranged, until they become finally eight to ten times as large as the other microsomes and are each of them attached by means of a thick, strongly staining cytoplasmic thread to the surface of the cell. In the meantime the central canal becomes a wide tube (in some cases bifurcating in places), and the network of peripheral canals becomes united with the central canal, forming elegantly ramifying side branches of the latter.

The cells of the portio glandulosa-efferens next to these are pierced only by the central canal, which shows no more bifurcations and possesses no more side branches.

The cells are smaller than those of the portio afferens-glandulosa, as are also the nuclei, which are perfectly smooth and

without processes. The nuclear membrane is thicker, the chromatin granules more crowded.

The central canal is surrounded by a cuticular layer in which the intracellular musculature, consisting of parallel rings studded with granules and united by cross anastomoses, is developed.[1]

The peripheral organs are best developed in the cells of this part of the nephridium.

There is nothing new to be said with regard to the structure in the terminal vesicle and the terminal canal.

The main point of the cytological part of the paper is the demonstration of true organs in the cell which develop gradually under a gradually increasing stimulus.[2]

With regard to the rest of the paper, I simply mention that it chiefly deals with the distribution of the pigment and with the cytology and physiology of the elements connected with that process.

[1] See Physiology of Excretion. [2] *Ibid.*

Volume I. *February, 1898.* *Number 5.*

ZOÖLOGICAL BULLETIN.

A NEW GENUS OF DOLICHOPODIDAE FROM FLORIDA.

WILLIAM MORTON WHEELER.

Drepanomyia,[1] *gen. nov.*

LARGE species, dull metallic green, overlaid with white dust. Proboscis swollen, projecting; palpi large; face very broad in the male, not reaching to the lower corner of the eyes, its lower portion somewhat swollen. Eyes covered with very distinct white pubescence. First antennal joint hairy above, second subglobular, third rather long with a ventral projection and apically inserted arista. The latter is two-jointed, short, thick, and covered with scarcely perceptible pubescence. Thoracic dorsum evenly arched, without acrostichal and dorsal bristles; prescutellar region convex. Along the edges of the thoracic dorsum, between the humerus and the insertion of the wing, there are three macrochaetae. Scutellum with six bristles along its posterior edge, the innermost pair longest, the outermost shortest. Abdomen of male with six visible segments and a rudiment of a seventh on the left side overlapping the swollen hood-shaped base of the partially imbedded hypopygium. The venter of the fourth segment is somewhat dilated posteriorly for the reception of the hypopygial appendages. Legs rather bristly; tarsi with somewhat dilated pulvilli in the male. Fore femora thickened towards their bases. First tarsal joint of the hind legs nearly as long as the four succeeding joints taken together, without bristles on its upper surface.

[1] From δρέπανον, a sickle, and μυῖα, fly.

Wings rather long, with nearly parallel costal and posterior margins. Third and fourth veins somewhat lyrate towards their apices; posterior cross vein oblique, longer than its distance from the posterior margin. Tegulae with very short cilia.

The characters of this genus are strongly marked. The neuration resembles that of *Hydrophorus* and allied genera. The antennae are very aberrant, and, especially in the first species to be described, remind one of the antennae of *Tabanus*. In this genus, however, the projection on the third joint is dorsal, and not ventral as it is in *Drepanomyia*. Other very marked peculiarities of the new genus are the broad face in the male, the large palpi, and the absence of the rows of thoracic macrochaetae which are so generally present in *Dolichopodidae*.

Drepanomyia pruinosa, n. sp.

Male. — Length of body, 6.5 mm. Length of wing, 5 mm. Dull metallic green, thickly coated with glistening white dust. Palpi yellow, with short black bristles. Antennae black, ventral surfaces of the first and second joints yellow; basal joint twice as long as the second; third joint large, flattened, its ventral edge with a very pronounced projection, half as long as the dorsal projection on which the arista is inserted. Arista short, thick, two-jointed, pubescent, bent downwards. Face and front broad, thickly covered with white dust; vertex and occiput more metallic green. Cilia of the superior orbit not very numerous, short, black; those on the inferior orbit longer and more abundant, yellowish white. Thoracic dorsum opaque, with two conspicuous accumulations of white dust on either side, one in the humeral, the other in the prealar depression. Middle of the thorax traversed longitudinally by two darker bands which fade out posteriorly. Pleurae uniformly and thickly covered with pale dust. Abdomen with a moderate layer of dust and with short black hairs on all the segments like those which cover the thoracic dorsum. The swollen and projecting base of the hypopygium is without hairs, but covered with thick yellowish dust. The partially projecting inner

appendages yellow. All the tibiae and tarsal joints yellow with black apices; pulvilli somewhat dilated, yellow. Coxae concolorous with the pleurae, the anterior pairs beset with long yellow hairs on their front faces. Trochanters yellow. Fore femora dull metallic green, dusted with white, with yellow bases and apices; middle femora yellow with a metallic green, white-dusted streak on the upper and lower face; hind femora with their basal halves and extreme apices yellow and their

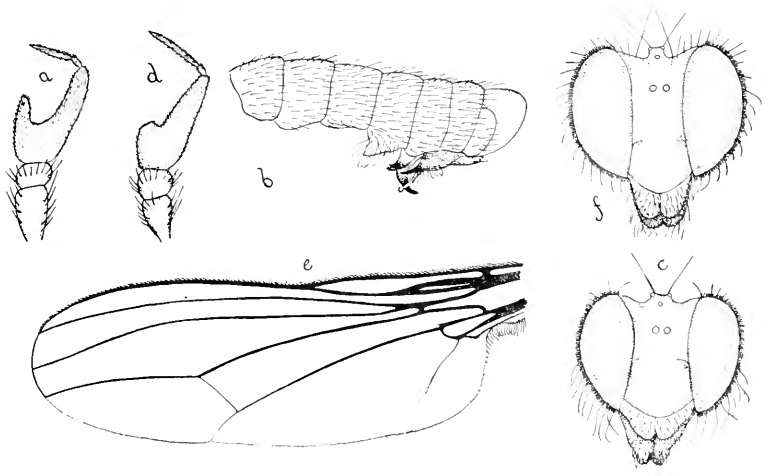


FIG. 1. — *a*, antenna; *b* abdomen; *c*, face of *Drepanomyia pruinosa* ♂; *d*, antenna; *e*, wing; *f*, face of *D. Johnsonii* ♂.

distal halves of the same color as the pleurae. Bristles on all the joints of the legs rather short, black. Wings grayish hyaline with yellow veins. Halteres and tegulae yellow, the latter with extremely short white cilia.

One specimen from St. Augustine, Fla., taken May 21, 1894, and received from Mr. Chas. W. Johnson.

Drepanomyia Johnsonii, n. sp.

Male. — Length of body, 7 mm. Length of wing, 6 mm. Metallic blue-green with a thin covering of white dust. Palpi yellow, with long black hairs. Face somewhat narrower than in the preceding species and with its green ground color dis-

tinctly showing through the dust. Antennae black, third joint narrower than in *D. pruinosa*, ventral projection short, truncated; arista somewhat longer than that of the preceding species. Cilia of the superior orbit more, those of the inferior orbit less, conspicuous. Thoracic dorsum with two approximated median dark lines, and accumulations of white dust in the humeral and prealar depressions. Abdomen tapering to the hypopygium, which is distinctly smaller and more imbedded than in the preceding species. Of the appendages only the small lamellae, beset with yellow hairs, are visible. The rounded base of the hypopygium lacks the black hairs which cover the abdominal segments. The dark metallic green color of the pleurae and coxae is overlaid with a thick and uniform layer of pale dust. There are a few stout yellow bristles above the insertions of the fore coxae. The fore and middle coxae have long yellow hairs on their anterior faces; the middle coxae have, besides, some black bristles on their lateral surfaces. Trochanters yellow. Femora metallic green, dusted with white, and with yellow apices. Tibiae yellow, growing black towards their apices, which are somewhat incrassated on the hind pair of legs. Tarsal joints black, with yellow bases. Pulvilli yellow. Wings hyaline, veins brown, becoming yellow towards their bases. Halteres and tegulae yellow, the latter with somewhat longer pale cilia than in *D. pruinosa*.

A single specimen bearing the same date and locality as the preceding species and also received from Mr. Chas. W. Johnson.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO, October 15, 1897.

THE OLFACTORY LOBES, FORE-BRAIN, AND HABENULAR TRACTS OF ACIPENSER.

A SUMMARY OF WORK ON THEIR MINUTE STRUCTURE.[1]

J. B. JOHNSTON,

INSTRUCTOR IN ZOÖLOGY, UNIVERSITY OF MICHIGAN.

THE sturgeon of the Great Lakes, *Acipenser rubicundus*, Le Sueur, grows to a maximum length of about two meters. In my investigation I have used partly fish 25 to 40 cm. in length, and partly the smallest of the fish taken at the fisheries, 1 to 1½ meters in length. The greater part of the work has been done by the method of Golgi, although I have used the ordinary histological methods, and also methylene blue and acid fuchsin.

The form and relations of the fore-brain and olfactory lobes of Acipenser have been described and figured by Goronowitsch ('88). The figures accompanying the present paper illustrate the gross anatomy of these regions sufficiently well for an understanding of their minute structure. All the figures are from the brain of fishes 25 to 40 cm. in length.

A. *The Olfactory Lobe.*

In either transverse or longitudinal sections of the olfactory lobe three zones are easily distinguished (Fig. 1). These are, from without inward: the zone of olfactory fibers (*o.f.z.*), the zone of olfactory glomeruli (*gl.z.*), and the granular zone or zone of granule cells (*gr.z.*). The bundles of olfactory fibers spread out over the surface of the gray matter in such a way as to form a cap whose thickness is greatest at the anterior end and becomes *nil* near the junction of the olfactory lobe with the fore-brain. This zone is made up of intercrossed bundles of olfactory fibers with a small amount of connective tissue and a few pigment cells between them. The glomerular zone is char-

[1] From the Zoölogical Laboratory of the University of Michigan, Jacob Reighard, Director.

acterized, in preparations stained with haematoxylin, by the compact masses of branching dendrites and nerve fibers known as olfactory glomeruli. This zone constitutes about one-third of the thickness of the wall of the lobe. The granular zone is made up of numerous nerve cells and fibers. There is no distinct white zone. The axis cylinders of the cells of the lobe, as will be shown later, take the shortest course toward the fore-brain. In consequence, the granular zone is a mixed white and gray zone. It is readily distinguished from the glomerular zone, in ordinary preparations, by the absence of the glomeruli; but the line of division between the two zones is not sharply defined. The central cavity of the lobe is large and communicates widely with the cavity of the fore-brain (Fig. 1, *B*).

a. ZONE OF OLFACTORY FIBERS. — The fibers coming from the sense cells of the olfactory epithelium are gathered in bundles from 80 to 400 μ in thickness (Fig. 1, *o.f.z.*). The individual fibers differ considerably in thickness. They are very direct in their course and present definite varicosities at irregular intervals throughout their entire length. There is no evidence that the appearance of varicosities is due to imperfect impregnation, as suggested by Van Gehuchten and Martin (**91**). (I learn from Miss Langdon that in her forthcoming paper on *Nereis virens* she will present evidence to show that such varicosities are artifacts.) The bundles break up into smaller bundles as they enter the gray matter.

b. ZONE OF OLFACTORY GLOMERULI. — The olfactory fibers approach their termination in the glomerular zone either in small bundles or singly. Occasionally the fibers divide shortly after entering this zone and end in two or more glomeruli, but the greater number divide only in the glomeruli in which they terminate. I shall consider the glomeruli after the various elements which may contribute to their formation have been described.

I have found in the glomerular zone three distinct forms of cells: mitral cells, stellate cells, and cells with short axis cylinders.

(1) There are present *mitral cells* of two varieties. The first (Fig. 1, *g*) are large, measuring from 16 to 48 μ in their short

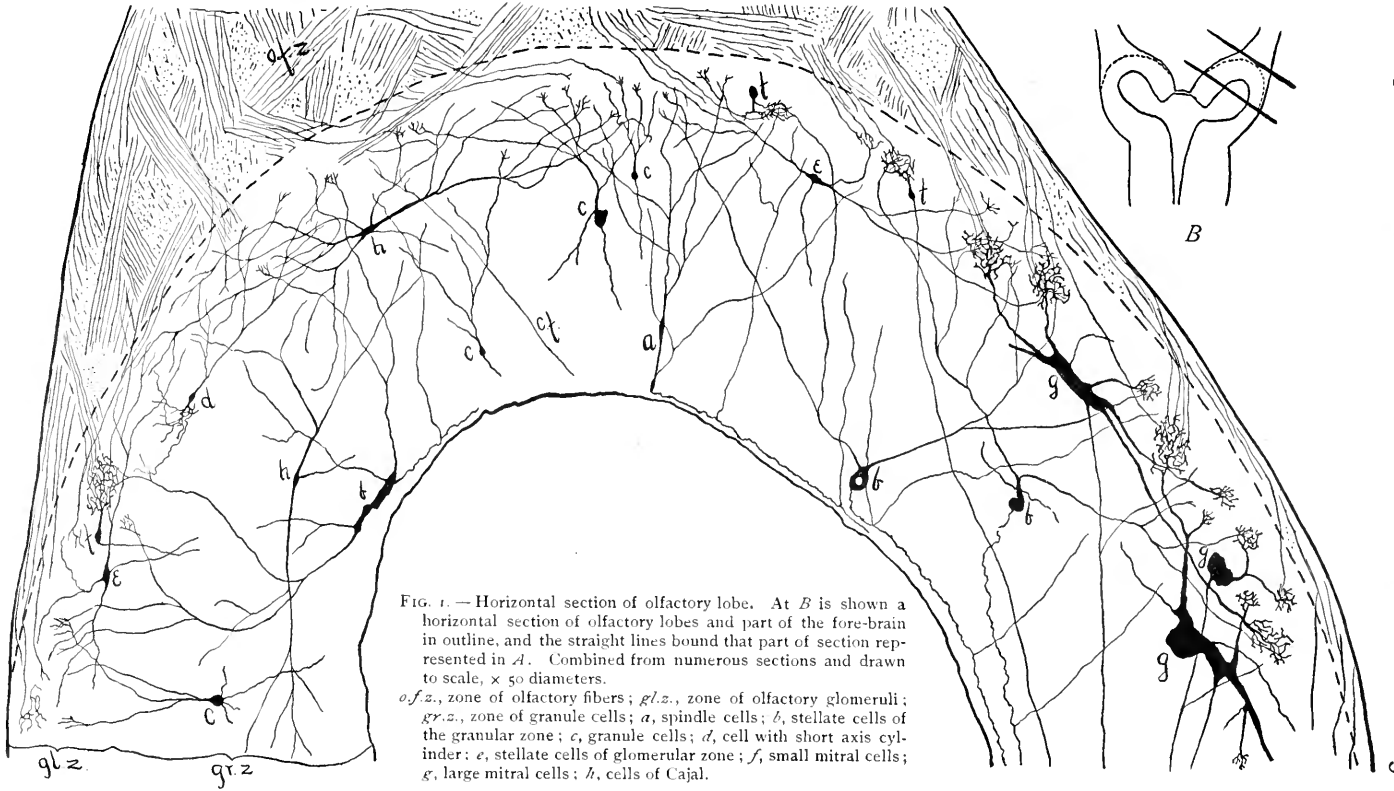


FIG. 1. — Horizontal section of olfactory lobe. At *B* is shown a horizontal section of olfactory lobes and part of the fore-brain in outline, and the straight lines bound that part of section represented in *A*. Combined from numerous sections and drawn to scale, × 50 diameters.

o.f.z., zone of olfactory fibers; *gl.z.*, zone of olfactory glomeruli; *gr.z.*, zone of granule cells; *a*, spindle cells; *b*, stellate cells of the granular zone; *c*, granule cells; *d*, cell with short axis cylinder; *e*, stellate cells of glomerular zone; *f*, small mitral cells; *g*, large mitral cells; *h*, cells of Cajal.

diameter and from 32 to 240 μ in their long diameter, possess from two to five thick dendrites, each of which supplies one or more olfactory glomeruli, and frequently have one or more slender dendrites which end freely in the glomerular zone without forming glomeruli. The axis cylinders are moderately thick fibers which take a direct course toward the fore-brain, and give off collaterals which rise toward the glomerular zone. The second variety of mitral cells (Fig. 1, *f*) are smaller, measuring 12 to 16 by 16 to 32 μ, have each a single thick dendrite which immediately breaks up into a glomerulus or into two or three imperfectly separated glomeruli, and have no non-glomerular processes. Their axis cylinders are usually directed centrally.

(2) *Stellate cells.* I have found numerous cells (Fig. 1, *e*) measuring 12 to 16 by 12 to 32 μ which have from two to five dendrites usually disposed parallel to the surface of the lobe, so that the cells have a stellate appearance in surface view. The dendrites are richly branched and long, their greatest expansion sometimes exceeding 1 mm. The branches of the dendrites end in olfactory glomeruli. The axis cylinders, which I have found in only a few cases, are directed either centrally or backward toward the fore-brain.

(3) The *cells with short axis cylinders* (Fig. 1, *d*) measure about 16 by 20 μ. From one end of the cell arises a thick dendrite whose branches end in glomeruli. A smaller process, which I take to be the axis cylinder, arises from the end of the cell opposite the dendrite and breaks up into numerous slender, smooth fibers which are lost in the glomerular zone. These cells are very few in number and very distinct in their character.

c. ZONE OF GRANULE CELLS. — Besides the granule cells which give this zone its name, I have found in it three other distinct forms of cells, which I shall call stellate cells, spindle cells, and cells of Cajal.

(1) The *stellate cells* (Fig. 1, *b*) measure 16 to 32 by 24 to 128 μ. They possess from three to five widely diverging dendrites whose branches end in the glomerular zone, where they enter into the formation of olfactory glomeruli. Their axis

cylinders are thick, strongly varicose fibers which run toward the central cavity of the lobe, and then along it close over or among the ependyma cells toward the fore-brain. From their horizontal portion the axis cylinders give off collaterals which branch once or twice in the outer part of the granular zone. They may enter the glomerular zone.

(2) The *spindle cells* (Fig. 1, *a*) measure 8 to 16 by 24 to 40 μ. The spindle-shaped cell body stands in a radial position in the internal one-third of the granular zone. From its peripheral end arises a dendrite whose branches end in olfactory glomeruli in the glomerular zone. From the central end of the cell a thick process runs toward the cavity and usually ends with an enlargement close to or among the bodies of the ependyma cells. From this enlargement an axis cylinder similar to those of the stellate cells runs backward to the fore-brain. Collaterals rise from it toward the glomerular zone. From the central process, and especially from its enlarged end, arise collaterals which branch and enter the glomerular zone. Sometimes the central process is without an enlargement, and it is perhaps to be regarded as part of the axis cylinder.

(3) The *granule cells* (Fig. 1, *c*) are numerous rounded or pyramidal cells measuring 8 to 32 μ in their greatest diameter. They occur at regular intervals throughout this zone. The character of these cells in other vertebrates has been much in dispute. Cajal in his earlier work ('90) considered them as certainly nervous, the peripheral process being the axis cylinder. Van Gehuchten and Martin ('91) are in doubt as to their character, having found no axis cylinder. Cajal later ('95) compares them with the spongioblasts of the retina, and considers them as nerve cells without axis cylinders. Koelliker ('96, pp. 709–713), on the other hand, regards them as certainly neuroglia. In Acipenser I have found these cells presenting characters showing them to be nerve cells conveying impulses from the olfactory fibers to the fore-brain. A description of them follows. Their peripheral processes rise to the glomerular zone and end in olfactory glomeruli; either forming, with olfactory fibers, glomeruli into which no other central elements enter, or entering glomeruli formed chiefly by other cells

described above. From the central end of the cell arises a slender, uniform, slightly varicose axis cylinder which runs either centrally or backward — that is, in the direction of all other axis cylinders. I have also found in some cases several short, slender dendrites arising from the central end of the cell about the base of the axis cylinder.

(4) *Cells of Cajal.* I have found at all levels of the granular zone very conspicuous elements (Fig. 1, *h*), measuring 8 to 20 by 24 to 40 μ, which in my earlier preparations seemed to correspond closely with certain cells with numerous axis cylinders described by S. Ramon y Cajal ('91) in the cortex of the rabbit, and afterward named by Retzius ('93) cells of Cajal. Later preparations brought to view in many of these cells characteristic end-branchings of some of the processes in olfactory glomeruli, and a single distinct axis cylinder arising from the cell body or from one of the thick processes and running centrally and backward toward the fore-brain. These cells, therefore, like all others of the olfactory lobe except those with short axis cylinders, receive impulses from olfactory fibers and transmit those impulses to the fore-brain. It seems probable, however, that some of the dendrites do not end in glomeruli, and that such dendrites may receive impulses from many collaterals and so serve as important parts of the collateral paths. Since coming to the above conclusions there has come to my notice the recent paper of Veratti ('97) criticising Cajal's interpretation of the cells in the rabbit's cortex and announcing that each cell has a single true axis cylinder. My account agrees with that of Veratti, and I have therefore called the cells here described cells of Cajal.

In addition to the elements described above, I have found in a very few cases fibers ending freely in the glomerular zone (Fig. 1, *c.f.*) which probably correspond to the centripetal fibers of Golgi ('75) and S. Ramon y Cajal ('90).

The olfactory glomeruli do not differ in general appearance from those described by S. Ramon y Cajal, Van Gehuchten and Martin, Koelliker, Retzius, Loewenthal, P. Ramon y Cajal, and others in mammals, birds, reptiles, and amphibia. In Acipenser the glomeruli vary in diameter from 16 to 240 μ, and

are much more complex than in mammals. They may receive from two or three to a large number of olfactory fibers, and the size of the glomerulus is probably determined by the number of olfactory fibers entering it. Each glomerulus may be supplied by a single large or small bundle of fibers, or by several single fibers or small bundles of fibers coming, it may be, from widely separated bundles of the olfactory nerve. Each olfactory fiber usually has from two to five end-twigs produced by dichotomous divisions, but frequently the branching is irregular, and it may be very complex. I have found no indication of anastomosis or the formation of a network in these endings. To the central portion of the glomerulus dendrites may be contributed by any one or several of the eight forms of cells described above. The largest part of a typical glomerulus is formed by the end-branching of the mitral cell dendrites, but almost all the glomeruli in Acipenser are formed in part by the dendrites of other cells. The whole makes up a very complicated mass of interwoven nerve twigs among which ramify the processes of glia cells. The essential condition for the transference of nerve impulses in the glomerulus is the contact of the tips of the olfactory fiber end-branches with the dendrites. There is in Acipenser no evidence of continuity of nerve substance between olfactory fibers and dendrites. Besides these typical glomeruli, there are many small ones whose central portion is formed wholly by the dendrites of cells other than the mitral cells.

I have thus recognized eight forms of nerve cells in the olfactory lobe. On the whole, these cell forms are distinct and present well-marked characters. However, there are to be found occasional intermediate forms: between the cells *a* and *c*, *b* and *c*, *b* and *e*, *c* and *f* (Fig. 1). Only the large and small mitral cells (and the granule cells?) can with certainty be compared with cell forms heretofore described in other vertebrates. The olfactory lobe of other fishes (especially Cyclostomes and Elasmobranchs) and of Amphibia needs to be investigated by the Golgi method and the lobe of higher vertebrates reëxamined with reference to the occurrence of the other cell forms. The only work done by modern methods on the olfactory lobe of

fishes hitherto is that of Van Gehuchten ('94) on the trout and that of Sauerbeck ('96) on Elasmobranchs. Van Gehuchten gives a single small figure of the end-branching of several olfactory fibers, and Sauerbeck states that he has had a few olfactory glomeruli and mitral cells impregnated, but gives no figure. In the frog I have found stellate cells similar to those in the granular zone of Acipenser, their axis cylinders directed toward the fore-brain. Similar cells, but devoid of axis cylinders, are shown in a figure copied by Edinger ('96b, p. 142) from a paper by P. Ramon y Cajal ('94) which I have not seen. The question of the interpretation of the various forms of nerve cells in the olfactory lobe of vertebrates I am not yet prepared to discuss.

S. Ramon y Cajal ('96a) makes use of the mitral and granule cells of the olfactory lobe to illustrate his principle that a nerve cell gains a higher morphological development by the growth of new processes, which by their position and direction set up connections with a greater number of cells. Interpreted by this statement, the large mitral cells of Acipenser present as high a stage of differentiation as those of mammals, or higher. The dendrites of these cells supply several, usually large glomeruli, into which a very large number of olfactory fibers enter. Thus the number of cells with which they come into relation is greater than in mammals. I have now shown also that non-glomerular protoplasmic processes which were hitherto unknown in lower vertebrates are present in Acipenser. With regard to the granule cells, if centrally directed axis cylinders should be found in higher vertebrates, all previous interpretations of them will fall to the ground. Here, again, the presence of basal processes goes to show that the morphological distinction between nerve cells in fishes and mammals is not so great as previous work has led us to suppose.

B. *The Fore-Brain.*

a. GENERAL DESCRIPTION OF NUCLEI AND FIBER TRACTS. — In sections stained with methylene blue and acid fuchsin or with haematoxylin there are to be seen several distinct collections of

cells whose size and position I shall briefly indicate (Figs. 2–4). First is the large dorsal and median portion bulging into the central cavity and constituting about one-half of the entire fore-brain. It is the corpus striatum. At the anterior end, near the border of the olfactory lobe, are two compact groups of cells, one ventral and median and one lateral, which together make up the lobus postolfactorius. I shall refer to these nuclei as the ventral and lateral nuclei postolfactorii (Fig. 2). Over the greater part of the lateral surface of the fore-brain are scattered small cells whose character I have not yet certainly determined. I shall describe this area under the name of the cortex (Fig. 3). The cells immediately surrounding the ventral portion of the cavity of the fore-brain behind the anterior commissure together with those surrounding the recessus praeopticus form a distinct nucleus which corresponds with the nucleus thaeniae of Edinger ('96a).

Three main fiber tracts are to be mentioned here (Figs. 2–4). The large number of fibers entering the fore-brain from the olfactory lobe constitute a tractus olfactorius, although so short as not to appear in the gross anatomy of the brain. More conspicuous is the large bundle of fibers traversing the ventro-lateral portion of each half of the fore-brain and entering the 'tween-brain, the tractus strio-thalamicus. A smaller bundle connects the fore-brain with the Ganglion habenulae, the tractus olfacto-habenularis. In addition to these longitudinal tracts is the large anterior commissure connecting the two halves of the fore-brain at about the middle of their length.

b. Histology of the Several Nuclei. — (1) The *corpus striatum*. The striatum contains two distinct types of cells imperfectly separated into two nuclei. The internal (dorso-median) nucleus (Figs. 2, 3, *Epistriatum*) is composed of pyramidal cells measuring 12 to 26 by 16 to 40 μ arranged in about ten to twelve compact layers parallel with the internal surface of the striatum. The apices of the cells are directed latero-ventrad. Each cell has several basal processes, and from the apex arise from one to four dendrites which are marked by characteristic spiny projections described by Van Gehuchten ('94)

for the cells of the fore-brain of the trout. The axis cylinders arise from the side or apex of the cell body or from the first portion of one of the basal or apical dendrites. Each fiber is of medium thickness and quite smooth. It runs latero-ventrad, grows distinctly more slender as it is traced further from the

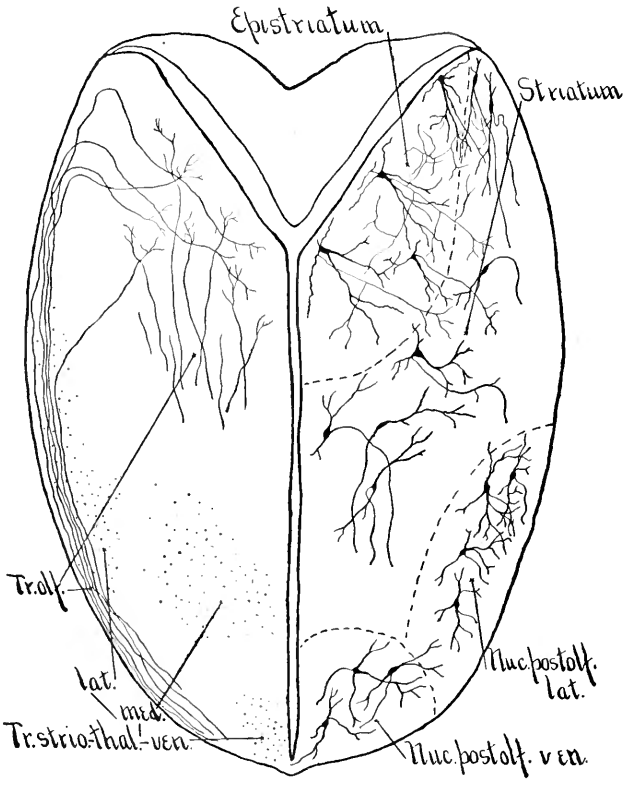


FIG. 2. — Diagrammatic section of the fore-brain in the plane indicated by the dotted line in Fig. 4, *a*. Outline, × 25 diameters; cells, × 50 diameters.

cell, gives off numerous collaterals which ramify widely in the striatum, and ends with terminal branches in the external portion of the striatum. All my preparations contain many cells of this sort.

The external nucleus (Figs. 2, 3, *Striatum*) is composed of irregular cells with two or more dendrites, which are not arranged in layers and are not so compactly grouped as are those of the internal nucleus. The cells measure 12 to 24 by

12 to 48 μ. The dendrites present the same appearance as those of the pyramidal cells. The axis cylinders arise from the cell bodies or from the basal part of the dendrites, are medium-sized, smooth fibers of uniform diameter, give off few and short or no collaterals, and enter the tractus strio-thalamici. Many cells of this description are scattered among the pyramidal cells of the internal nucleus and some of them have a pyramidal form, so that it is sometimes difficult to distinguish between

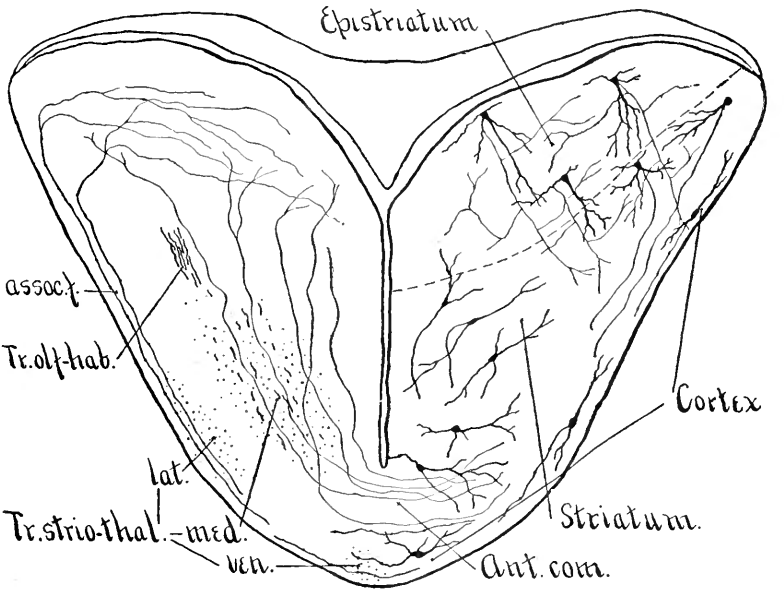


FIG. 3. — Diagrammatic frontal section of the fore-brain through the anterior commissure. Scale as in Fig. 2.

the two types of cells. In a single series in which the axis cylinders are very well impregnated, I have in a few cases found the two kinds of cells side by side in the same section. It should be added that the cells in the ventral part of the striatum are smaller, have usually only two dendrites, send their axis cylinders into the median portion of the tractus strio-thalamici, and are readily distinguished from the pyramidal cells of the internal nucleus.

Goronowitsch ('88) describes the appearance of these two nuclei in picrocarmine preparations of the brain of *Acipenser ruthenus*. Van Gehuchten ('94) states that in the trout all the

cells impregnated in his preparations are multipolar with axis cylinders entering the tractus strio-thalamici (basal bundles of Edinger's earlier work). He does not mention or figure collaterals. Either the pyramidal cells with short axis cylinders described above for the internal nucleus were not impregnated in his preparations, or from seeing the axis cylinders directed toward the basal bundle he has concluded that they join it. Whether the cells with short axis cylinders described above are the same as those referred to by Van Gehuchten ('94) as having been described by Bellonci in teleosts ('79 ?) I cannot tell, as I have not had access to Bellonci's paper. In a single case I have found a small cell in the intermediate portion of the striatum with two dendrites and with short axis cylinder disposed longitudinally.

The fibers having their endings in the corpus striatum come from two or three sources: the olfactory lobe, the thalamus, and probably the cortical area. The fibers from the olfactory lobe which pass into the corpus striatum are best seen in such a section as that represented in Fig. 2 (*tr. olf.*). These fibers end, so far as I have seen, only in the internal nucleus of the corpus striatum. The fibers entering the corpus striatum from the thalamus (Fig. 4, *D*) are the ascending or sensory fibers described by Van Gehuchten ('94) in the Tractus striothalamici (basal bundles) of the trout. Probably the greater number of these fibers cross in the anterior commissure (Fig. 3, *ant. com.*), but some seem to find endings on the same side. They are fine or medium-sized varicose fibers which end by fine ramifications in the internal nucleus of the corpus striatum. I have not found their endings in the external nucleus. In addition to the fibers last mentioned, the anterior commissure contains a small number of thick, strongly varicose fibers which course around the lateral surface of the fore-brain and enter the corpus striatum at its dorso-lateral angle (Fig. 3, *assoc. f.*). Either before or after entering the corpus striatum these fibers break up into several very fine branches which run for a long distance in the internal nucleus parallel with its layers of cells. These fibers will be described below as the probable axis cylinders of the cells of the cortex.

The two nuclei of the corpus striatum described above are not sharply differentiated. Since the fibers from the tractus olfactorius are found to end in the internal nucleus and have not been seen to end elsewhere in the striatum, this nucleus is probably homologous with the epistriatum as described by Edinger ('96a, b) in reptiles, amphibia, and teleosts. The cells of the external nucleus give rise to descending fibers of the tractus strio-thalamici, and are therefore to be considered as the motor component of the corpus striatum. In the intermediate portion there is a mingling of cells of the two kinds, and hence a region of mixed functions. It seems probable that the epistriatum consists properly of the cells with short axis cylinders, here not fully separated from the striatum, and that they receive all sensory and associational impulses (*e.g.*, from the three sources indicated above), and in turn stimulate the motor cells which constitute the striatum proper.

(3) *Nuclei postolfactorii.* The nucleus postolfactorius ventralis, occupying the ventro-median angle of the fore-brain at its extreme anterior end, is made up of multipolar and bipolar cells. The multipolar cells measure 12 to 16 by 16 to 26 μ. They have irregularly spreading dendrites. From the cell body arises a medium-sized smooth axis cylinder which is directed backward along the ventral surface of the fore-brain, near the median line. The bipolar cells have bodies measuring 16 to 17 by 16 to 32 μ. From each end of the cell arises a thick dendrite directed parallel with the long axis of the brain. The dendrites give off several small branches at right angles. The posteriorly directed dendrite gradually becomes transformed into a thick, varicose axis cylinder, which I have traced more than half the length of the fore-brain. The axis cylinders of both multipolar and bipolar cells traverse the length of the fore-brain, forming a small bundle at each side of the mid-ventral line, pass among the cells surrounding the recessus praeopticus, and pierce the large bundles of the optic nerve. I have not yet certainly determined their place of ending (Fig. 4, *B*). The fibers which enter and end in this nucleus come from the olfactory lobe. Most or all of them are fibers which run close over the central cavity; hence, probably, the axis cylin-

ders of the stellate and spindle cells of the granular zone. The terminal branches are thickened and varicose.

The nucleus postolfactorius lateralis is much larger than the ventralis. It is made up of multipolar cells similar to those of the ventralis, measuring 16 to 20 by 20 to 24 μ. Their axis cylinders, together with fibers from the anterior end of the striatum, form a bundle of fibers which runs ventro-posteriorly over the lateral surface of the fore-brain (Fig. 4, *C*). At the optic thalamus the bundle turns mesad, plunges through some of the more mesial optic bundles, and continues into the 'tween-brain with the tractus strio-thalamicus medius, on the lateral surface of which it forms a distinct bundle. Its destination I have not yet worked out. The fibers which end in the lateral nucleus postolfactorius come from the olfactory lobe, but from what cells of the lobe I cannot at present say. They seem to be those axis cylinders which take the most direct course to the fore-brain and do not run close over the central cavity; hence, some of the axis cylinders of the granules, the cells of Cajal, the mitral cells, and the stellate cells of the glomerular zone.

There seems to be a third nucleus occupying the antero-dorsal angle of the fore-brain having relations similar to those of the lateral nucleus. Since its cells have been impregnated in only a single series, I cannot describe it further. It may be a nucleus postolfactorius dorsalis, or it may belong to the epistriatum.

In addition to the fibers which run from the nuclei postolfactorii to the ventral portion of the 'tween-brain, there are several small bundles of fibers which connect these nuclei with the ganglia habenulae. Tracing the fibers forward from the ganglion of habenula, the bundle breaks up in the posterior dorsal portion of the fore-brain into several small bundles, which diverge like the ribs of a fan and penetrate to the ventral part of the fore-brain *throughout its entire length*. Here I have been unable to find either terminal arborizations or cells of origin, owing in some cases to imperfect impregnation and in others to the great number of fibers of other tracts, making it impossible to trace these through successive sections. How-

ever, in the ganglia habenulae the fibers in question cross to the opposite side through the commissura habenularis and seem to end by free branching among the dendrites of the cells which give rise to the bundles of Meynert. This renders it probable that these fibers constitute the tractus olfacto-habenularis (Fig. 3, *Tr. olf.-hab.;* Fig. 4, *E*).

I will here insert a note on the course of the bundles of Meynert as throwing light on the function of the olfactory nuclei as well as on that of the ganglia habenulae. For these conspicuous bundles traversing the internal faces of the walls of the 'tween-brain I have not adopted the name proposed by Edinger,—tractus habenulo-peduncularis,—for the reason that in Acipenser their fibers do not end in the corpus interpedunculare as described by Mayser ('82), Van Gehuchten ('94), Edinger ('96b), and S. Ramon y Cajal ('96b) for other forms. In the corpus interpedunculare a majority of the fibers of the bundles of Meynert cross to the opposite side, and both the crossed and uncrossed fibers pass on back toward the medulla. I have not traced them to their endings. I give a figure (Fig. 5) of the course of the fibers through the corpus interpedunculare.

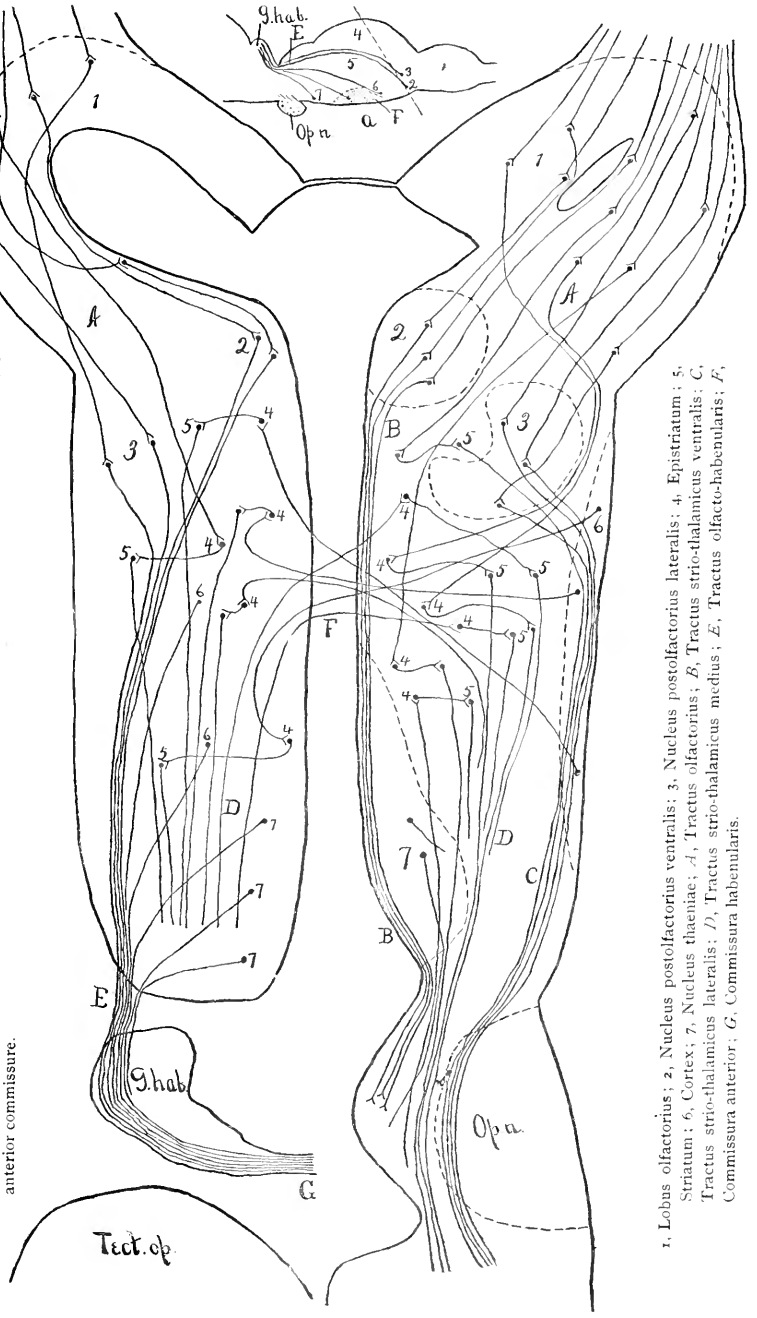
(4) *The cortex.* Among the fibers from the lateral nucleus postolfactorius and striatum on the lateral and ventral surfaces of the fore-brain occur cells measuring 12 to 18 by 16 to 40 μ with two or more dendrites usually disposed parallel with the external surface (Fig. 3). In the anterior commissure are found a small number of thick fibers with very definite, round varicosities at regular intervals (Fig. 3, *assoc. f.*). In a few cases I have found the axis cylinders arising from the superficial cells just mentioned directed toward the anterior commissure and having the characters of these thick fibers. Tracing the fibers, they are found to end in the epistriatum in the manner described above. Some of the cells of this region have very slender, smooth axis cylinders directed toward the anterior commissure. I have been unable to trace them to their destination. The fibers which end among these cells are collaterals from the axis cylinders of striatum cells, occasional short axis cylinders from the epistriatum, and probably fibers from the tractus olfactorius.

It seems probable that at least some of the cells here described are associational cells serving to coördinate the motor impulses descending from the two halves of the fore-brain, while other cells may belong to the olfactory area and give rise to a part of the tractus olfacto-habenularis. The disposition of the bundles of the tractus olfacto-habenularis, to be noted below, suggests homology of the cells just described with the cortex lateralis of reptiles, to which they correspond in position. In the choroid roof of the fore-brain in Acipenser I have had one or two nerve cells and fibers impregnated in a few preparations. These cells correspond in position with the cortex dorsalis of higher forms.

(5) *Nucleus thaeniae.* The cells surrounding the recessus praeopticus and the ventral portion of the central cavity of the fore-brain as far forward as the anterior commissure constitute a nucleus corresponding to the nucleus occipito-basalis of Herrick ('91) and the nucleus thaeniae of Edinger ('96a). Their dendrites spread widely toward the lateral surface of the fore-brain. At least some of their axis cylinders run backward through the optic chiasma; I have been unable thus far to trace them to their endings. There is a decussation of some of the fibers beneath the recessus praeopticus. It may be that this is an important olfactory nucleus, but I have been unable to trace any fibers to it from the olfactory tract. It would be impossible, however, to trace such fibers if they were present in my preparations, owing to the enormous number of other fibers among which they must run. There is some evidence that this is an olfactory nucleus in the fact that some bundles of the tractus olfacto-habenularis arise from this region, probably from some of the cells of the nucleus thaeniae here described.

c. THE FIBER TRACTS. — Under this head I collect the statements scattered through the preceding pages and make some additions to them.

(1) The tractus olfactorius is for the most part very diffuse. The great majority of its fibers enter the fore-brain singly and are at once mingled with the fibers of other tracts. Only a small part of its fibers are gathered into a compact bundle.



Scheme of nuclei and fiber tracts in fore-brain and olfactory lobes. A diagrammatic horizontal section is represented. The plane of the left half of the section is dorsal to that of the right half. At *a* is shown in a sagittal section laterad of the median plane, the course of the bundles of the Tractus olfacto-habenularis with reference to the anterior commissure.

1, Lobus olfactorius; 2, Nucleus postolfactorius ventralis; 3, Nucleus postolfactorius lateralis; 4, Epistriatum; 5, Striatum; 6, Cortex; 7, Nucleus thaeniae; *A*, Tractus olfactorius; *B*, Tractus strio-thalamicus ventralis; *C*, Tractus strio-thalamicus lateralis; *D*, Tractus strio-thalamicus medius; *E*, Tractus olfacto-habenularis; *F*, Commissura anterior; *G*, Commissura habenularis.

FIG. 4

This bundle is formed in the ventral part of the olfactory lobe, enters the fore-brain through the nucleus postolfactorius ventralis, courses round the lateral surface of the nucleus postolfactorius lateralis, and enters the epistriatum at its dorso-lateral angle (Fig. 2). I have traced olfactory tract fibers into the epistriatum and into the two nuclei postolfactorii. I have been unable to trace any fibers between the anterior commissure and the olfactory lobe. Although we might expect to find olfactory tract fibers ending in the nucleus thaeniae and in the area described as cortex, I have been unable to trace them to either with certainty.

(2) The tractus strio-thalamicus, considered as including all fibers connecting the fore-brain with the ventral portion of the 'tween-brain, is divided into three portions: the median, lateral, and ventral bundles. The median bundle is much the largest, and occupies the position indicated in Figs. 2, 3 (*Tr. strio-thal. med.*), and 4 (*D*). It contains the descending axis cylinders of the cells of the striatum and the ascending fibers from the thalamus to the epistriatum. The lateral bundle (Figs. 2 and 3, *Tr. strio-thal. lat.*, and Fig. 4, *C*) contains fibers arising from the nucleus postolfactorius lateralis and from the anterior part of the striatum. Its course has been described. The ventral bundle (Figs. 2 and 3, *Tr. strio-thal. ven.*, and Fig. 4, *B*) consists only of fibers from the nucleus postolfactorius ventralis whose course has been described above.

(3) The tractus olfacto-habenularis (Fig. 4, *E*) arises from cells of the nuclei postolfactorii and probably from cells of the cortical area and of the nucleus thaeniae, and passes to the ganglion habenulae where most or all the fibers cross to the opposite side to end among the dendrites of the cells giving origin to the bundles of Meynert. That the bundles here described are equivalent to more than Edinger's tractus olfacto-habenularis is shown by their relation to the anterior commissure. The tract to which Edinger gives this name arises in the ventrally situated nucleus thaeniae and area olfactoria and runs behind and below the anterior commissure (see Edinger, '96a, p. 343, Fig. 5, or '96b, p. 148, Fig. 100), while the larger part of the fibers here included under that name arise in the

nuclei postolfactorii (and cortex?) and run in front of and above the anterior commissure. It is probable that this portion includes Edinger's tractus cortico-habenularis. The bundles in Acipenser from the nuclei postolfactorii do not seem to be represented in Edinger's scheme.

(4) The bundles of Meynert arise from cells in the ganglia

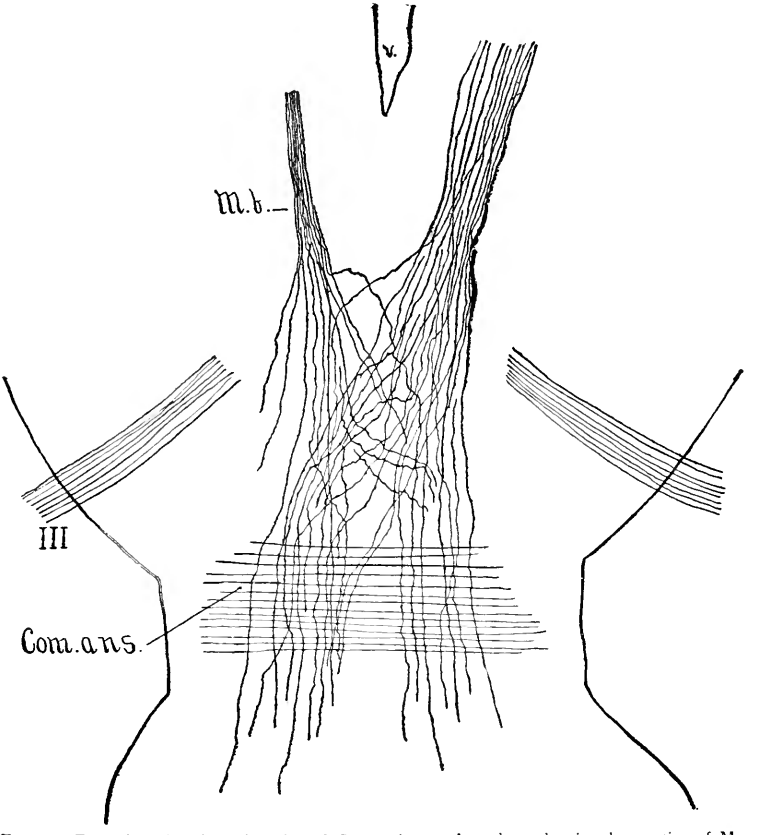


FIG. 5. — Frontal section through region of Corpus interpedunculare, showing decussation of Meynert's bundles. *M.b.*, Meynert's bundles; *III*, Nervus oculomotorius; *Com. ans.*, Commissura ansiformis; *v.*, cavity of mid-brain. Camera drawing.

habenulae as described by Van Gehuchten ('94) in the trout, traverse the walls of the 'tween-brain to the region of the corpus interpedunculare in the base of the mid-brain, thence, after decussation of most of the fibers, pass back toward the medulla. Other habenular tracts will be described in my final paper.

(5) The anterior commissure is made up chiefly of ascending fibers which cross to end in the epistriatum of the opposite side. In addition to these are the somewhat doubtful associational fibers of the cortical area, crossing likewise to terminate in the epistriatum.

Summary of Results.

A. The olfactory lobe:

(1) In addition to mitral cells of two sorts, six other forms of cells, concerned in receiving and transmitting olfactory impulses, are found in the olfactory lobe.

(2) The granule cells are provided with axis cylinders and glomerular dendrites, and are therefore nerve cells.

(3) The olfactory lobe contains cells which are morphologically identical with the cells of Cajal.

(4) The glomerular zone of the olfactory lobe contains cells with short axis cylinders (associational cells).

(5) The large mitral cells are provided with non-glomerular dendrites.

B. The fore-brain:

(6) There is in the dorso-median region of the fore-brain a large incompletely differentiated nucleus of cells with short axis cylinders, constituting an imperfect epistriatum.

(7) A group of cells is found on the lateral surface of the fore-brain which agrees in position and apparently also in connections with the cortex lateralis of *Reptilia*.

(8) The cortical region of the fore-brain is connected with the ganglion habenulae by a tractus cortico-habenularis. A tractus olfacto-habenularis is also present.

C. The habenular tracts:

(9) Meynert's bundles do not end in the corpus interpedunculare, but undergo partial decussation there and pass on toward the medulla.

ZOÖLOGICAL LABORATORY, UNIVERSITY OF MICHIGAN,
ANN ARBOR, MICHIGAN, September 24, 1897.

BIBLIOGRAPHY.

'79. [1] Bellonci. Ricerche intorno all' intima tessitura del cervello dei Teleostei. *Atti R. Acad. dei Lincei.* 3 Ser., Vol. iii. 1879.

'94. [1] Cajal, P. Ramon y. Invest. micr. en el encéfalo de los batraceos y reptiles. Sargossa, 1894.

'90. [1] Cajal, S. Ramon y. Origen y Terminacion de las fibras nerviosas olfatorias. *Gaz. sanit. municipal.* Barcelona, Dec. 10, 1890.

'91. Cajal, S. Ramon y. Sur la structure de l'écorce cérébrale de quelques mammifères. *La Cellule.* T. vii. 1891.

'95. Cajal, S. Ramon y. Les nouvelles idées sur la structure du système nerveux chez l'homme et chez les vertébrés. Paris, Reinwald, 1895.

'96a. Cajal, S. Ramon y. Allgemeine Betrachtungen über die Morphologie der Nervenzelle. *Arch. f. Anat. u. Phys. Anat. Abth.* 1896. Hefte 3–4.

'96b. Cajal, S. Ramon y. Beiträge zum Studium der Medulla oblongata u. s. w. Leipzig, Barth, 1896.

'88 and '92. Edinger. Untersuchungen über die vergleichende Anatomie des Gehirns. I. Das Vorderhirn. Frankfurt a. M. 1888. Neuabd. 1892.

'96a. Edinger. Untersuchungen u. s. w. 3. Neue Studien über das Vorderhirn der Reptilien. Frankfurt a. M., Diesterweg, 1896.

'96b. Edinger. Vorlesungen über den Bau der nervösen Centralorgane. 5. Auflage. Leipzig, Vogel, 1896.

'75. [1] Golgi. Sulla fina struttura dei bulbi olfattorii. Reggio Emilia, 1875.

'88. Goronowitsch. Das Gehirn und die Cranialnerven von *Acipenser ruthenus. Morph. Jahrb.* Bd. 13. 1888.

'91. Herrick. Topography and Histology of the Brain of Certain Reptiles. *J. Comp. Neur.* Vol. i. 1891.

'96. Koelliker. Gewebelehre. 6. Auflage. Bd. ii. Leipzig, Engelmann, 1896.

'82. Mayser. Vergleichende anatomische Studien über das Gehirn der Knochenfische mit besonderer Berücksichtigung der Cyprinoiden. *Zeit. f. wiss. Zool.* Bd. xxxvi. 1892.

'93. Retzius. Die Cajal'sche Zellen der Grosshirnrinde beim Menschen und den Säugethieren. *Biol. Untersuch.* Bd. v. 1893.

'91. Van Gehuchten et Martin. Le bulbe olfactif chez quelques mammifères. *La Cellule.* T. vii. 1891.

'94. Van Gehuchten. Contribution a l'étude du système nerveux des téléostéens. *La Cellule.* T. x. 1894.

'97. Veratti. Ueber einige Structureigenthümlichkeiten der Hirnrinde bei den Säugethieren. *Anat. Anz.* Bd. xiii. 1897.

[1] Papers marked thus I know only from abstracts.

A METHOD OF REMOVING CUTICULA FROM MARINE ANNELIDS.

THE following simple method has been found successful for the removal of cuticula from marine annelids:

The worms are first narcotized in sea water to which from 5 to 10% commercial alcohol has been added. They are then placed in a 10% solution of sodium chloride in distilled water for from 24 to 48 hours. The time of immersion in the salt solution varies with the different species. The worm is now removed to fresh water and an incision with the points of scissors is made along the dorsal surface of the animal. A little shaking with forceps aided with scalpel is sufficient to remove the cuticula entire, although it is usually more convenient to first cut the worm into pieces. The pieces of cuticula thus obtained are floated upon slides and the water is allowed to evaporate. In this way permanent mounts are obtained. Such mounts are especially valuable for studying the distribution of sense organs, gland pores, setae, etc. If the worm has been left in the salt solution a sufficient time all the epidermis cells will be left behind and a mount of perfectly clean cuticula will be obtained. Care must be taken to wash thoroughly in fresh water before mounting upon slides; otherwise salt crystals will form and injure the preparations.

MARGARET LEWIS.

A SIMPLE METHOD OF DILUTING ALCOHOL TO ANY DESIRED PER CENT.

Of alcohol of known per cent take the number of c.c. represented by the figures in the required per cent, add enough water to bring the whole up to the number of c.c. represented by the figures in the known per cent. The mixture will be of the required per cent. To illustrate:

> To make 50% alcohol from 95% alcohol, add to 50 c.c. of 95% alcohol enough water to make 95 c.c. The result will be 95 c.c. of 50% alcohol.
>
> To make 40% alcohol from 70% alcohol, add to 40 c.c. of 70% alcohol enough water to make 70 c.c. The result will be 70 c.c. of 40% alcohol.

If one of these illustrations is firmly fixed in the mind, one can, working by analogy, make up very quickly alcohol of any desired per cent without laborious calculation or reference to tables. Larger or smaller quantities than those indicated in the illustrations can be made by using different units of measurement or by multiplying or dividing those quantities by the proper number.

Wm. A. Redenbaugh.

Dartmouth College,
Hanover, N. H.

THE VARIATIONS AND MUTATIONS OF THE INTRODUCED LITTORINA.

A THIRD CONTRIBUTION TO THE STUDY OF VARIATION.

HERMON C. BUMPUS.

THE observations recorded in this communication were made for the purpose of ascertaining additional facts relative to the variability of "introduced species," and with the design of eliciting further evidence in corroboration of certain conclusions which had been reached after an examination of a large number of eggs of the English sparrow (Bumpus, '98).

The periwinkle, *Littorina littorea* Linné, is "extremely common" among stones and on the rocks of the British shores, and is reported from Greenland, the White Sea, and the European coast as far south as Lisbon (Jeffreys, '65). Along the New England shore it is by far the most abundant mollusc at the present time. Stones, piles, and seaweed are everywhere dotted with the dark-colored shells which often form a distinct band near high-water mark.

When the tide is low, the snails often lose their hold and roll from the slanting rocks into hollows, where they may be scooped up by the handful. At Seaconnet no less than 2500 shells were taken from a small depression not more than a foot square.

The history of the introduction and distribution is as follows :

In 1841 Gould published his *Report on the Invertebrate Animals of Massachusetts*, but no mention was made of the present species.

In 1855 Morse received specimens from Bathurst, on the Bay of Chaleur, an inlet of the Gulf of St. Lawrence (Morse, 1880).

In 1870 Binney revised Gould's *Report*, gave a description of the species, and mentioned the shell as reported from Halifax

by Willis. He evidently had no idea that the Massachusetts shore was soon to be invaded. In the same year Mr. Charles B. Fuller, curator of the Portland Society of Natural History, found a few specimens in Maine at Portland and at Kennebunk (Morse, '80).

In 1871 the species was found at Hampton Beach, New Hampshire (Gray, '79).

In 1872 Professor Morse found it at Salem (a single specimen), and Verrill ('80) found it "very rare" at Provincetown, Mass.

In 1875 two specimens were taken at Woods Holl, Mass. (Verrill, '80).

In 1880 Prof. S. I. Smith found the first specimen at New Haven.

At all of these localities the shell became abundant in a very few years after its first appearance.

Thus the history of the introduction, the rapid dispersion and the remarkable increase of the periwinkle are not essentially different from that of the introduction, dispersion, and increase of the sparrow.

It is, of course, well known that *Littorina littorea* is, on its native shore, like many gasteropods, subject to variation (Jef-

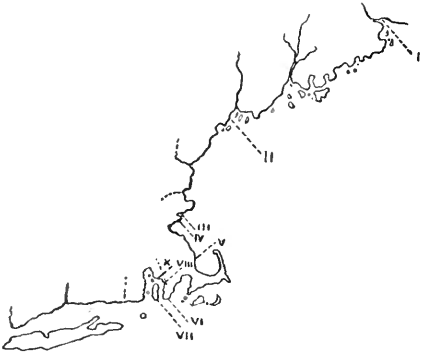


FIG. 1. — Coast line of New England, indicating the localities from which collections of *Littorina littorea* have been made. I, St. Croix River; II, Casco Bay; III, Beverly; IV, Nahant; V, Plymouth; VI, Seaconnet; VII, Newport; VIII, Bristol; IX, Bristol; X, Warren River.

freys, '65). Jeffreys gives the diagnostic characters of four "varieties," though these are not characterized by definite

CHART V. 1,000 LITTORINA LITTOREA. CASCO BAY, ME.

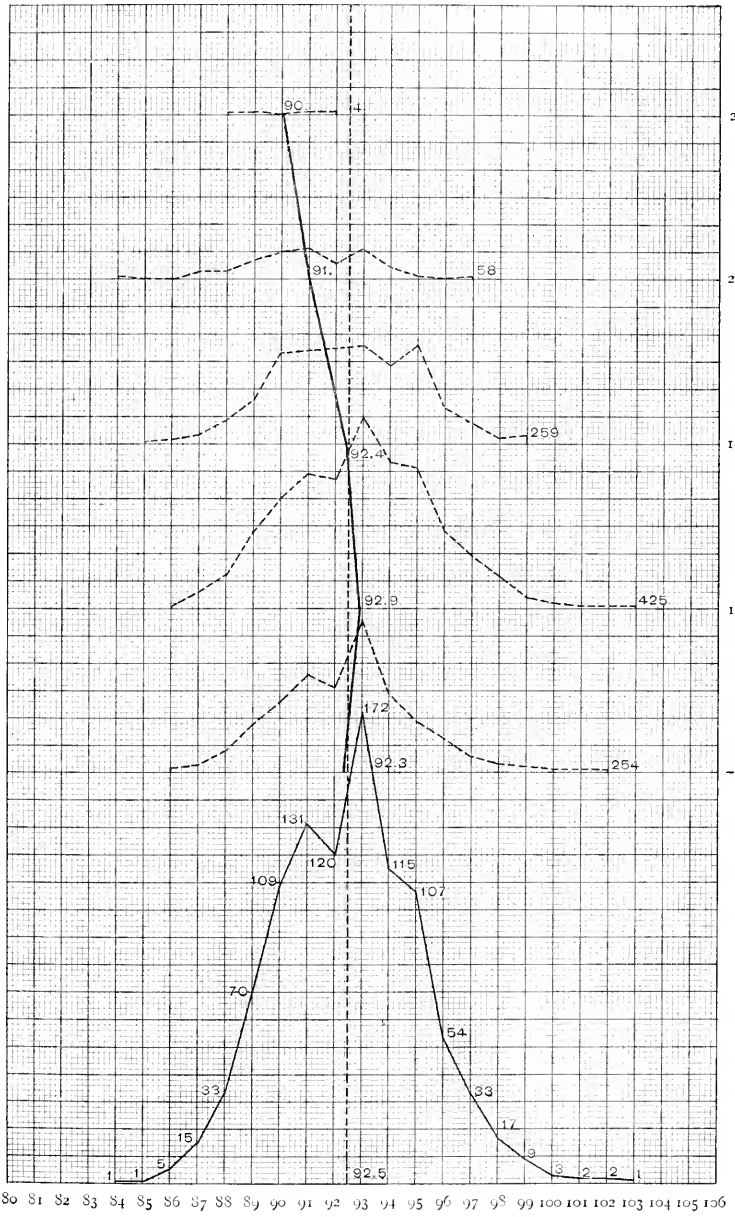


CHART VI. 1,000 LITTORINA LITTOREA. BEVERLY, MASS.

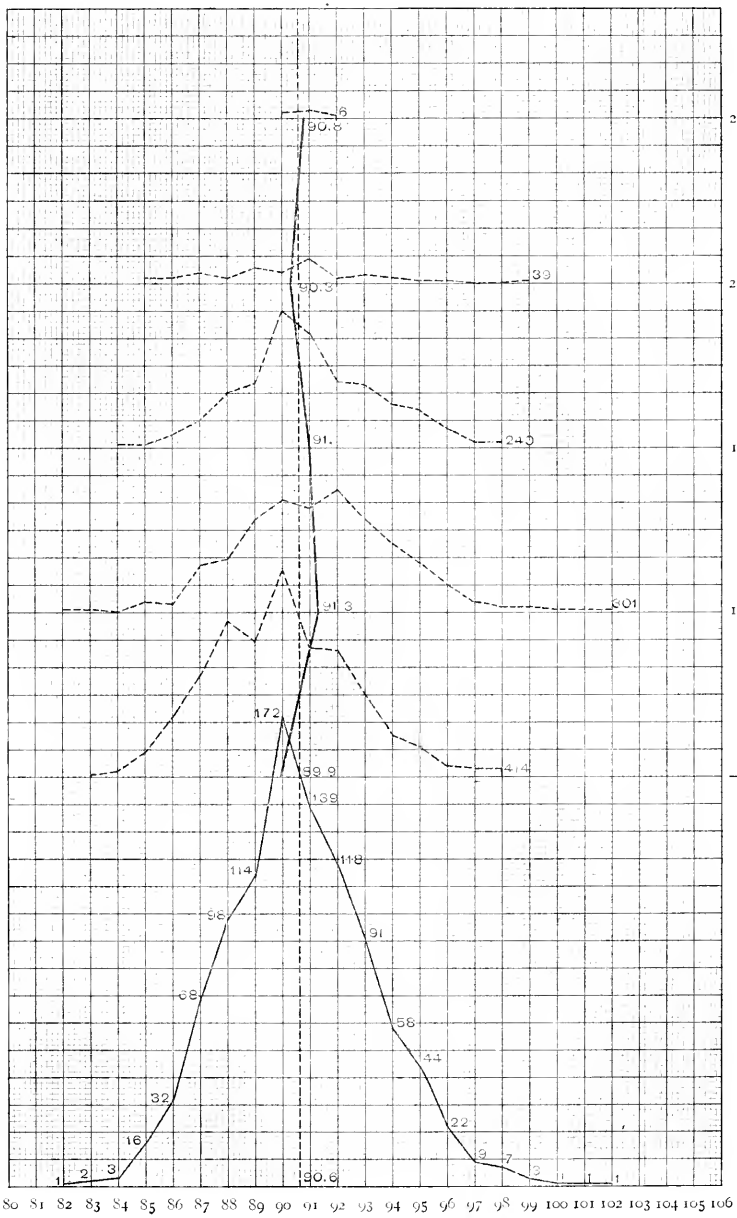


CHART VII. 1,000 LITTORINA LITTOREA. NAHANT, MASS.

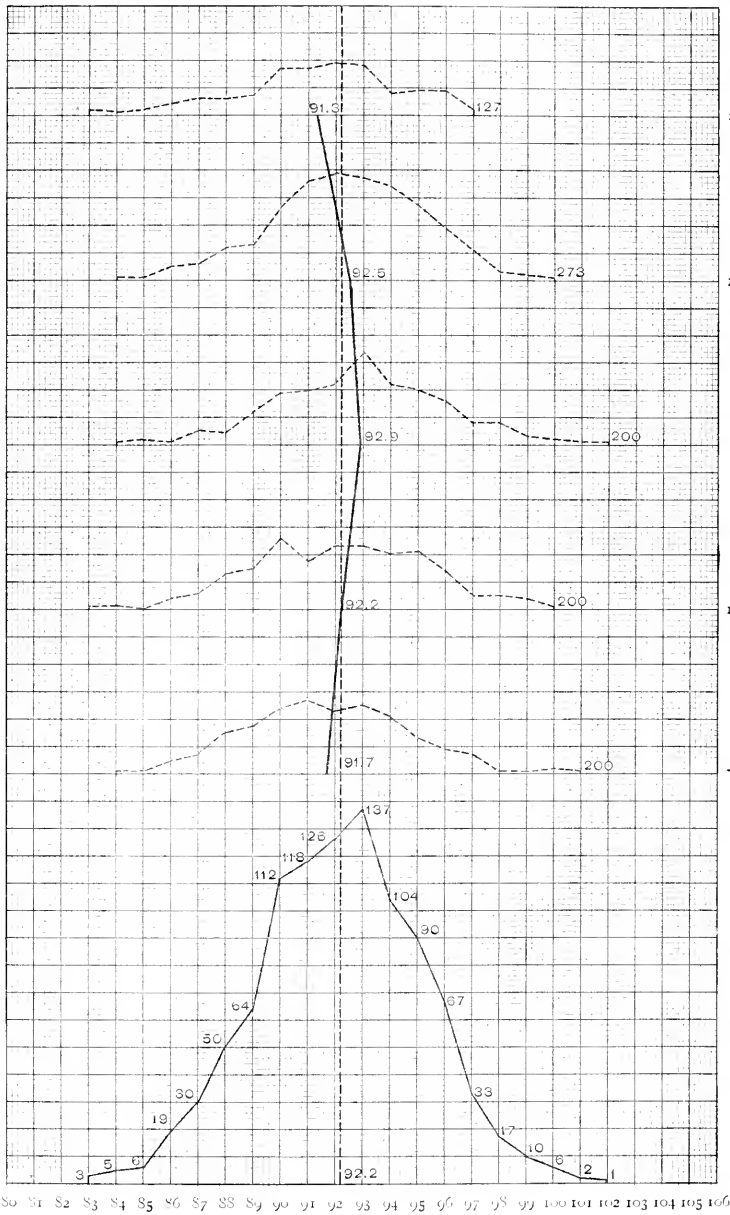


CHART VIII. 1,000 LITTORINA LITTOREA. PLYMOUTH, MASS.

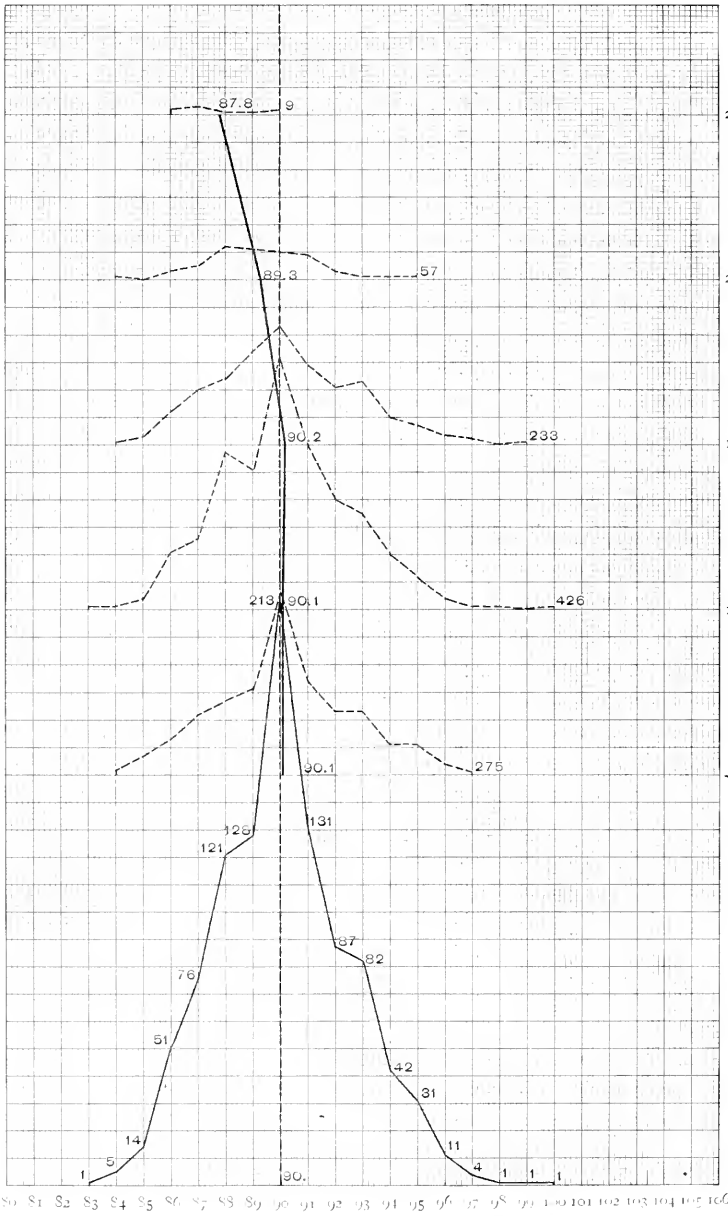


CHART IX. 1,000 LITTORINA LITTOREA. SEACONNET, R. I.

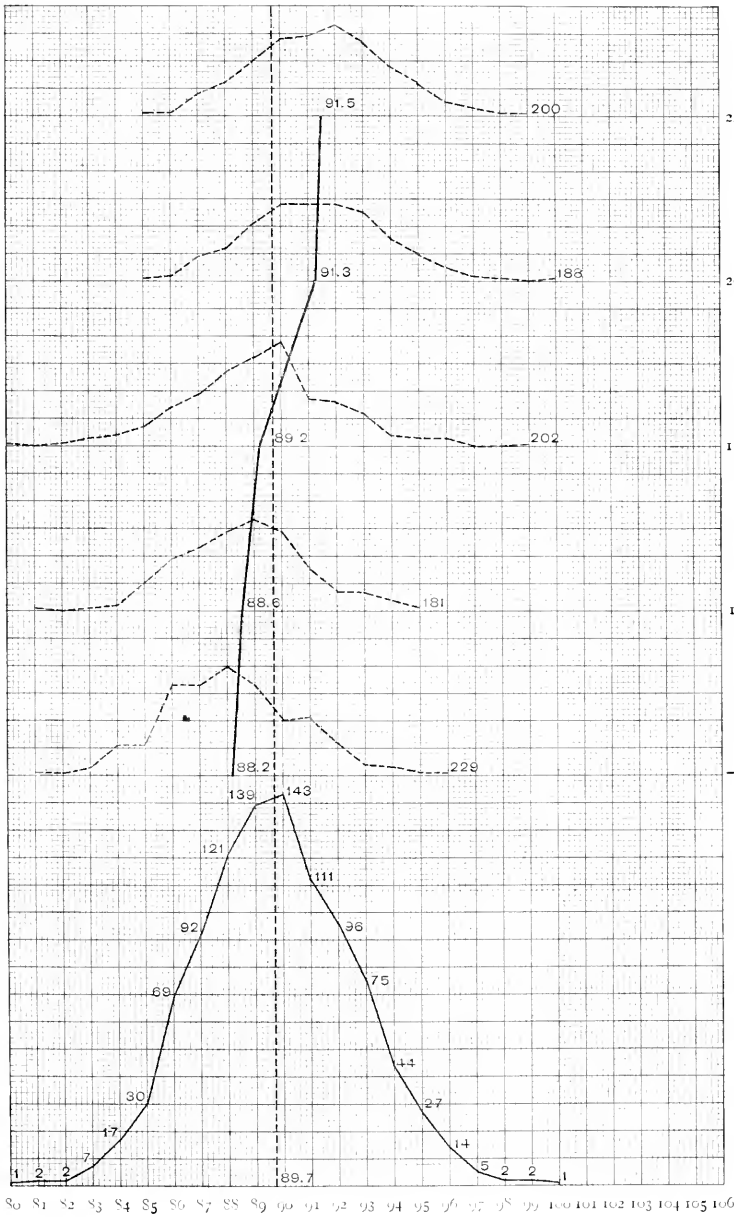


CHART X. 1,000 LITTORINA LITTOREA. NEWPORT, R. I.

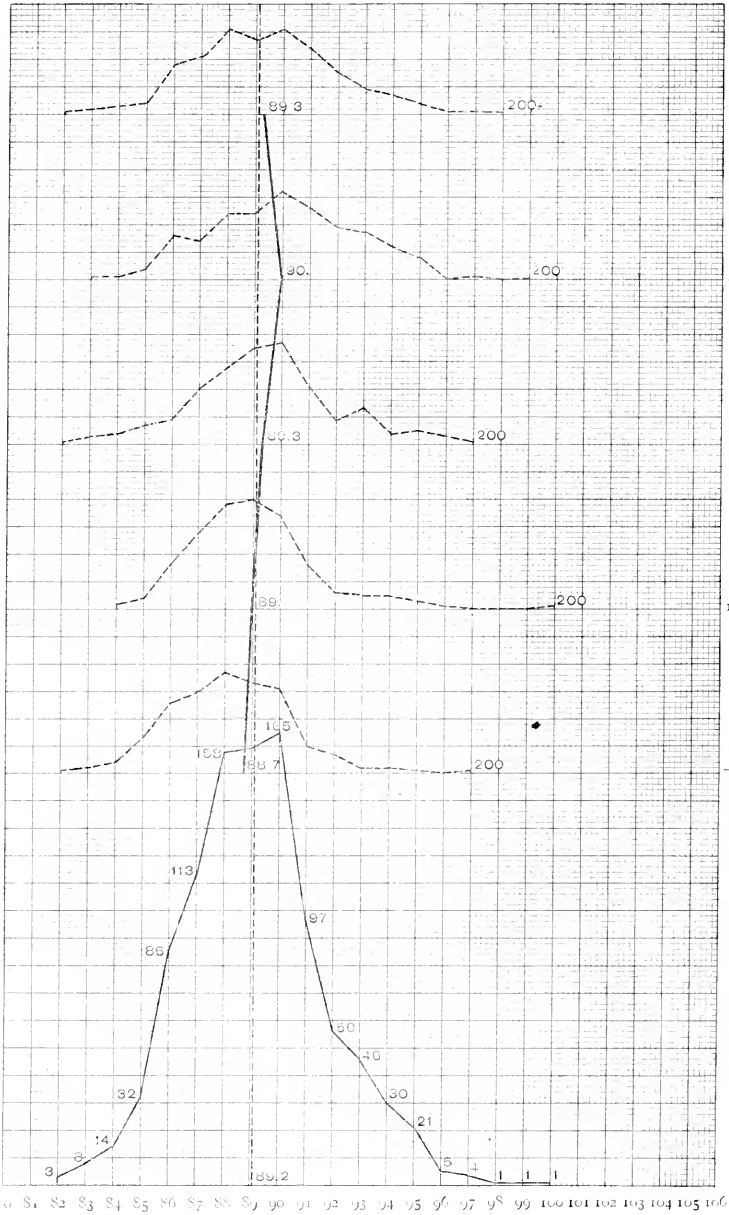


CHART XI. 1,000 LITTORINA LITTOREA. BRISTOL, R.I. (SHINGLE)

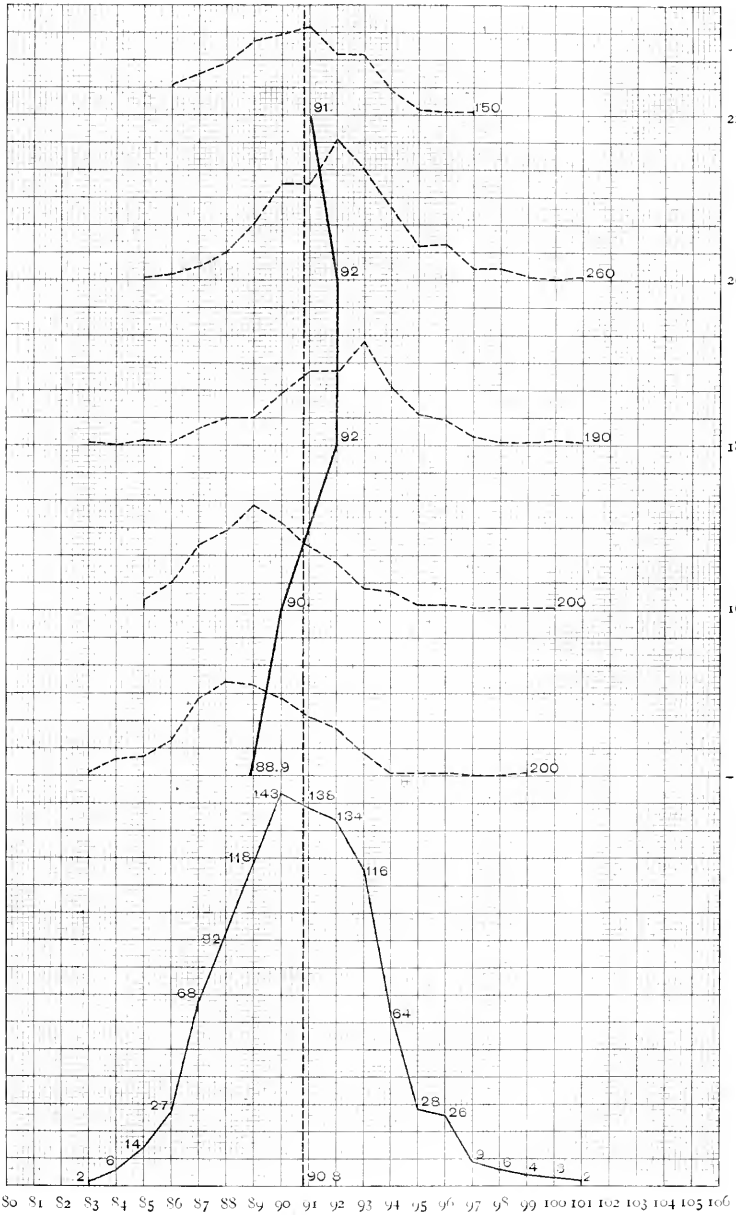
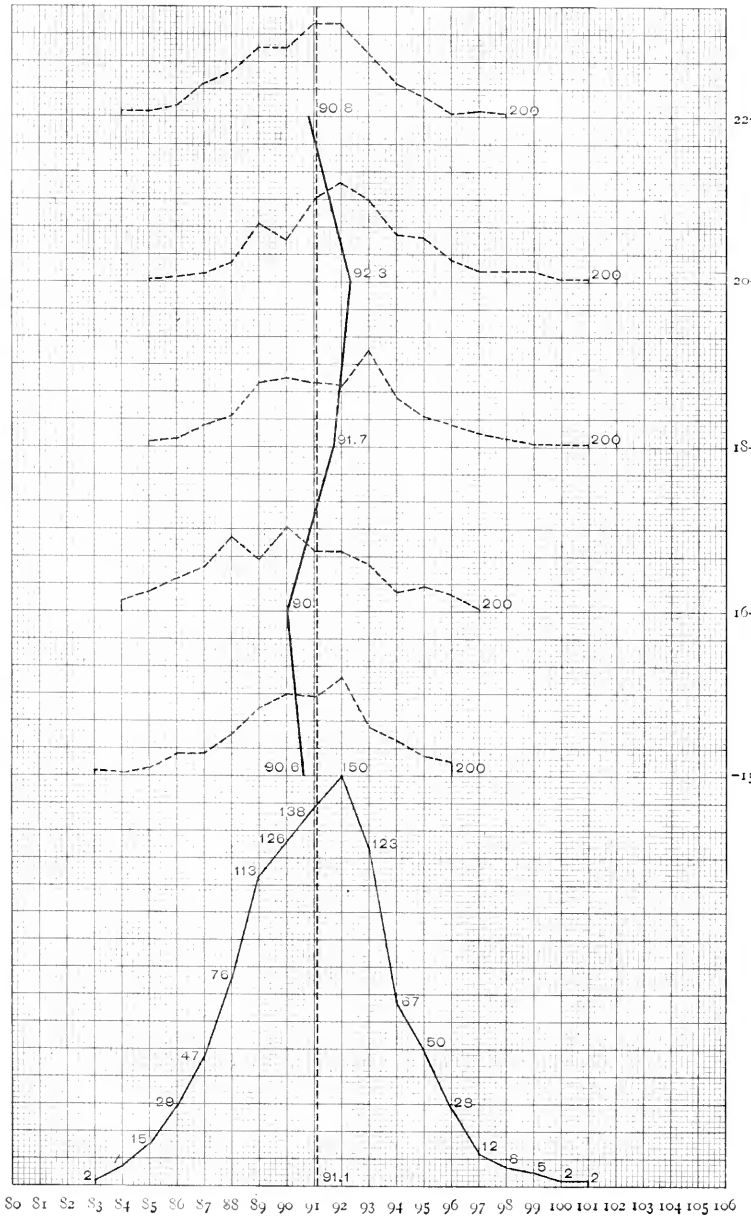


CHART XII. 1,000 LITTORINA LITTOREA. BRISTOL, R.I. (SAND)



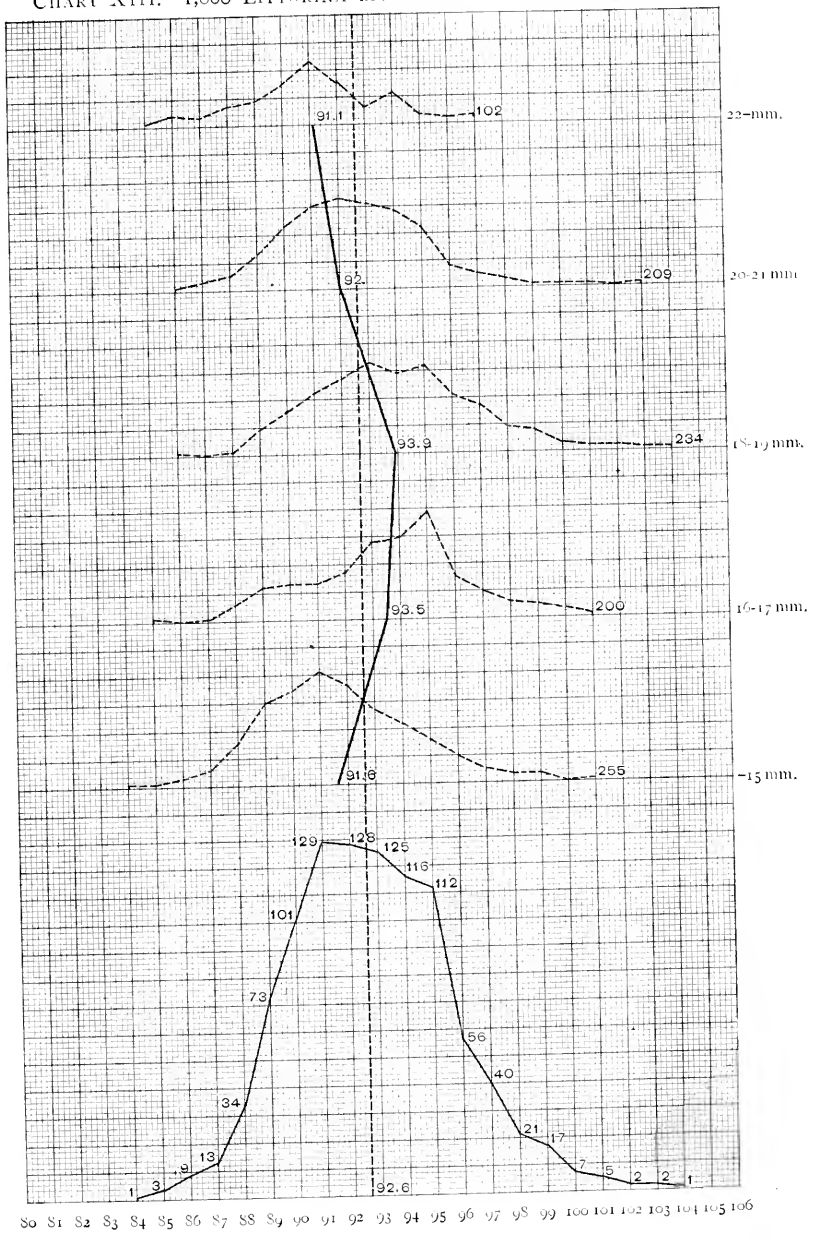
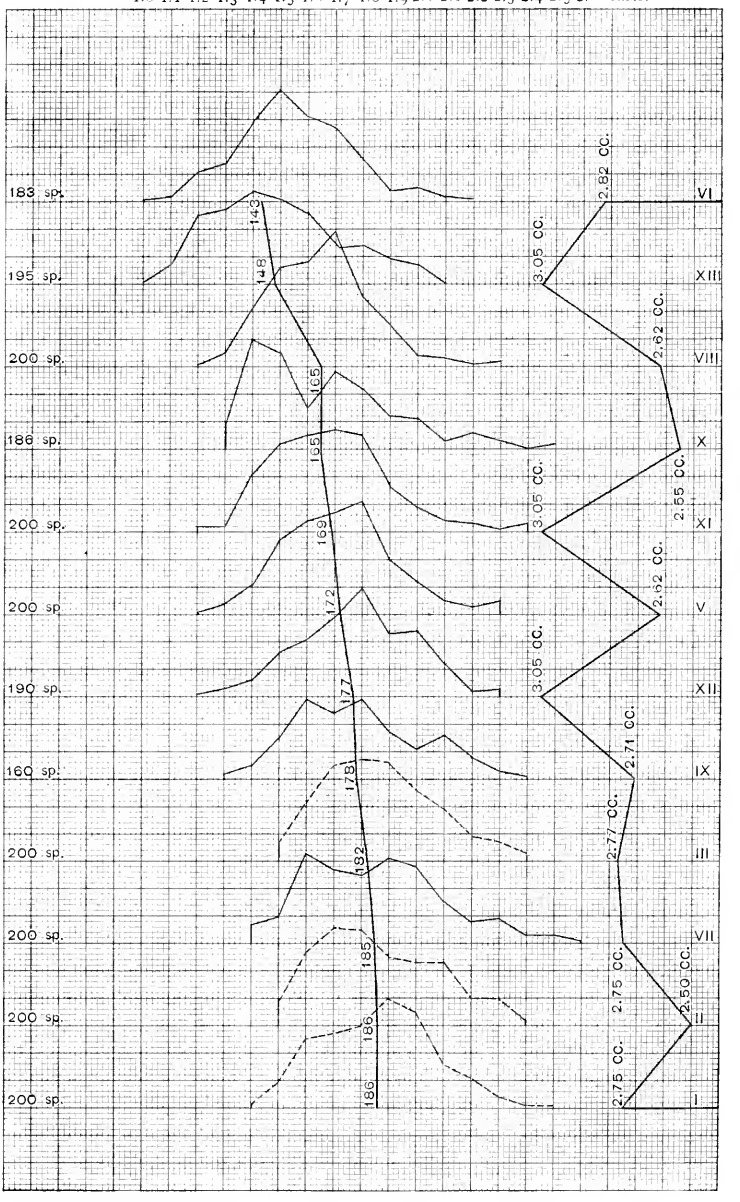


CHART XIII. 1,000 LITTORINA LITTOREA. WARREN RIVER, R. I.
91.1
102
22-mm.
92.
209
20-21 mm.
93.9
234
18-19 mm.
93.5
200
16-17 mm.
91.16
255
-15 mm.
129
128
125
116
112
101
73
56
40
34
21
17
13
9
7
5
3
2
2
1
1
92.6
80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106

CHART XIV. 2.314 L. LITTOREA. ARRANGED IN WEIGHT CURVES.

1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2 2.3 2.4 2.5 2.6 GRMS.



geographical limits, and at least two are admitted to be "perhaps monstrous rather than varietal forms."

Of the 3000 British shells which I have examined, 1000 are from Tenby, Pembrokeshire, southwest coast of Wales, 1000 from South Kincardineshire, east coast of Scotland, and 1000 from the Humber District on the east coast of England — three localities widely separated geographically, and characterized by different geological formations. Ten thousand American shells have been collected from the ten stations indicated on the annexed map, 1000 shells being considered sufficient to represent any given locality.

The First Test of Variability.

A most superficial examination of the complement of shells (1000) from any locality shows that certain individuals are relatively longer and others are relatively shorter (ventricose)

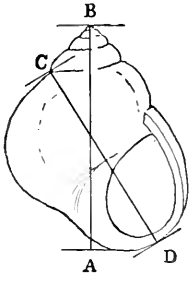


FIG. 2. — Diagram of shell to illustrate the points at which measurements were taken.

than the majority of their companions. The ratio of breadth to length, then, is variable, and may be exactly measured and mathematically expressed. A shell which has a breadth equal to eight-tenths the height may be indicated by 80%; one whose breadth is nine-tenths the height, by 90%; and one whose breadth equals the height, by 100%. The distance from *A* to *B*, Fig. 2, is taken as the height, and from *C* to *D* as the breadth of the shell. These distances were selected because they were more easily measured than the actual diameters. A measuring instrument gave the ratio of breadth to height, *i.e.*, the index of stature, without the labor of computation.

The measurement of 1000 shells from Tenby, Pembrokeshire, Wales, reveals the following facts: The breadth of the most elongated shell is 83% of the height. The breadth of the most ventricose shell is 98% of the height. There are four shells having an index of stature of 84%, four of 85%, twenty-five of 86%, thirty-eight of 87%, etc.

Chart I.[1] — If from these data we construct a "curve of frequency," it is evident that the *location* of the curve upon the base line indicates the general shape of the shells from Tenby. The *length* of the base line, inclosed by the limbs of the curve, is an index of the amplitude of variation in respect to stature of the 1000 shells, and may be numerically expressed as 15 (98 — 83 = 15). The *altitude* of the curve is an expression of conservatism, *i.e.*, it represents the segregation of the shells around a mean; and the flowing trend of the curve is at once an indication that a sufficient number of individuals has been collected to be of statistical value, and that the tension which tends to draw varieties away from the mean is constant.

Chart II. — If we now examine the curve of distribution of 1000 shells from South Kincardineshire, Scotland, we note that it lies further to the left; *i.e.*, the shells are more elongated, though its altitude and flowing trend are not considerably different from those of Chart I. The amplitude of variation, however, is slightly less, being represented by 14 (95 — 81 = 14).

Chart III. — The curve of distribution for 1000 shells from the Humber District, while having practically the same altitude and the same flowing trend as curves I and II, and resting at a mean position upon the base line between the ordinates of 85% and 97%, presents, nevertheless, extreme fixity — the amplitude of variation being indicated by only 12 (97 — 85 = 12). This remarkable constriction of the base line gives a precipitous appearance to the curve which Charts I and II do not have, and indicates a striking paucity in examples which depart, even a little, from the ideal mean.

[1] The subdivisions of the base line from left to right represent the grades of increase in ventricosity expressed in per cents, *i.e.*, by indices of stature. The length of the ordinates represents the number of individuals of each grade.

Let us now examine the curves of distribution of the American shells, and see if a new environment has wrought any change.

Chart IV. — This chart is based on an examination of 1000 shells from the St. Croix River in Maine, and, while it does not rest on the base line in an extreme position, its general trend and the width of its base are very different from any of the British curves. The index of stature varies from 84% to 102%, giving an amplitude of 18 (50% greater than that given by the Humber shells), though the regular trend of the flaring base is still an indication of some continuously active law. If a larger number of shells of the complement have departed from the ideal and have presented more extreme types of elongation on the one side and of ventricosity on the other, the shells of *mean* stature must have been depleted, and the St. Croix curve is consequently less precipitous than any of the British.

Chart V. — This chart is based on an examination of 1000 shells from Casco Bay. The position of the curve indicates a general tendency towards ventricosity. There are but twenty-two shells the index of which is below 88%, while there were among the South Kincardineshire collection 440 examples. The altitude and general configuration of the present curve is not unlike curve IV, and the amplitude of variation of stature, as indicated by the breadth of base, is but slightly greater; *viz.*, 19 (103 — 84 = 19).

Chart VI. — On this chart 1000 shells from Beverly, Mass., have been tabulated. Beverly is located at the mouth of a small inlet, ninety miles southwest of Casco Bay. The curve is typically American, the base indicating an amplitude of variation of 20 (102 — 82 = 20), an amount far in excess of the most variable British shells. It is worthy of note that the right limb of the curve is much less abrupt in its descent and longer in its course than the left, a feature which also distinguishes the other American curves thus far examined. The significance of this character will be considered later on.

We have now examined 3000 British and 3000 American shells, with the result that in every case the latter are more

variable, and, if it be objected that by chance we have selected British shells from localities where they are *least* variable and American shells from localities where they chance to be *most* variable, the objection is met, if the same results follow upon the examination of a more representative collection of shells from either locality. Let us continue, and see if it is possible to find a single American locality where the variation is restricted even to that of the most variable British series.

Chart VII. — One thousand shells from Nahant, a rocky promontory lying about ten miles south of Beverly and bathed by the cold waters of the Atlantic, yield another characteristic American curve, of low altitude and of broad base. The index of the amplitude of variation is 19 (102 — 83 = 19), again in excess of that of the British shells. The right limb is less precipitous than the left, but not so obviously as in previous cases.

Chart VIII. — This chart represents the distribution of 1000 shells from Plymouth, Mass. While the Littorinas of Nahant were subject to the continual beating of the waves, those collected at Plymouth were from the wharves of a sandy harbor, far removed from the boisterous sea. The curve is peculiar in that 213 shells, occurring at the ordinate of 90%, have caused an abrupt break in its contour, and formed a prominent spire to what would otherwise be a characteristic American curve. The ascent of the left limb is again considerably more abrupt than the descent of the right, and the amplitude of variation (17) is clearly American.

Chart IX. — In the warmer waters of the southern shores of New England, at the rocky headland of Seaconnet, Littorina actually swarms. The curve of distribution, though drawn far to the left and indicating tall, elongated shells, has, nevertheless, the characteristic American contour, low altitude, flaring base, and the ascent on the left more abrupt than the descent on the right. The amplitude of variation is as great as it was for the shells from Beverly; *viz.*, 20 (100 — 80 = 20).

Chart X. — Six miles to the west of Seaconnet, and largely of the same geological formation, are the equally rocky promontories of Newport. The temperature and salinity of the water, the oceanic currents, the force of the waves, the facies

of the marine fauna and flora in these two localities are apparently the same, and the Newport curve is not materially unlike the Seaconnet curve, though its amplitude of variation, owing to the lack of individuals presenting extreme elongation, is two degrees less. The ventricose shells are about evenly divided in the two localities.

Charts XI and XII. — Bristol Narrows lies at the mouth of the Kickemuet River, about seventeen miles north of Newport. The water, though sufficiently salt to enable starfish to flourish, is of somewhat less specific gravity than at Newport or Seaconnet. Two sets of shells were examined. One complement of 1000 was taken at a shingle beach, from among stones ranging from the size of one's fist to that of one's head ; the other series was collected at a spot only a few hundred feet distant, where the animals were living upon the sand and mud. The two curves are remarkably alike; they are located at the same place on the base line, between the ordinates of 83 and 101, and thus have the same amplitude of variation; *viz.*, 18.

Chart XIII. — The shells tabulated on this chart were collected at the mouth of the Warren River at a point about three miles from Bristol Narrows. They form a perfectly typical American curve with abrupt ascent, low summit, and sweeping descent. The amplitude of variation is 20, and the curve lies between the ordinates of 84 and 104.

Thus we have examined shells from ten American localities, and in every case we have found that their amplitude of variation is in excess of the most variable British shells, and we conclude that at any locality along the American coast the shells will probably exhibit a greater variation in respect to stature than at any locality along the British coast.

A Second Test of Variability.

It is quite possible that the American shells may be more variable in respect to stature and still be less variable in respect to other characters.

In the previous section it was shown that in each American locality the shells vary through a greater amplitude than in

any British locality; but do the extremes of variation of the American shells from *all* localities equal or exceed the extremes of variation of the British shells from *all* localities? With the data at hand it is possible for us to arrive at a reasonably certain conclusion, though to answer the question with absolute certainty one should have a very large and representative collection of shells from many localities in both countries.

The most elongated shell among the British series was collected at Kincardineshire. Its index is 81. The most elongated American shell was collected at Seaconnet, and its index is 80. While the most ventricose British shell has an index of 98 (*vide* Chart I), the most ventricose American shell has an index of 104 (*vide* Chart XIII). The most extreme cases of variation in stature are, then, presented by the American shells. This is quite a different thing from extreme amplitude of variation in particular localities, and, while it may result from the fact that a larger number of American localities have been examined, it seems hardly probable, from the data at hand, that an equal number of British localities would yield an equal number of such extreme variations.

The British shells from three localities taken at random gather in 17 grades, from 81% to 98%. The American shells from ten localities gather in 24 grades, from 80% to 104%. Moreover, if we can show that the extremes of variation of 3000 American shells from the three localities which offer the *least* variation are further removed than the extremes of the 3000 British shells taken at random, then our position is further strengthened. This we can do, for the combined amplitude of variation of 3000 British shells is 17, while the combined amplitude of variation of the three *least* variable American series is 19.

A Third Test of Variability.

Every one has doubtless observed the difference in the general proportions of the body of the child and the adult. In the former the head is relatively larger, the trunk longer, and the legs shorter. To use a conchological term, the child is more ventricose. Are the smaller shells, from the several

localities, of the same general proportions as the adults? If they are of different proportions, do they differ in a definite way? Is the difference greater in the American than in the British shells?

To answer these questions it will be expedient to divide each complement of shells into five groups: one group including all shells which are less than 15 mm. in height; a second, a third, and a fourth group containing shells ranging respectively from 16 to 17, 18 to 19, 20 to 21 mm. in height; and a fifth group containing all over 21 mm. in height.

On Chart I it will be noted that there are respectively 114, 308, 319, 183, 76 shells in the several groups, and the distribution of the 1000 shells in the several groups indicates, other things being equal, the general size of the shells of a particular locality. Thus, if the shells in a particular locality are small, the lower groups will contain a larger number. If the general proportions of the shell remain the same through the successive stages from infantilism to senility, the curves of distribution of these several groups will lie directly over each other, and a line drawn through their ideal means will be vertical. If the proportions of the shells vary with age, then the line connecting the five ideal means (the curve of growth) will bend, and its trend and its irregularity will indicate the amount of change.

The younger shells from Tenby, Wales (Chart I), are slightly more ventricose than the adults, the curve of growth tending towards the right at the lower part of its course and towards the left at the upper part. The amount of variation, however, is only 1.1 degree ($91.5 - 90.4 = 1.1$). Even less variation is exhibited by the South Kincardineshire shells, while the curve of growth for the Humber District is almost a straight line, varying only .3 of 1%. In all three cases the old shells are less ventricose.

The course of the curve of growth on the first American chart (Chart IV) indicates that the American shells, at least from this locality, are, in this third respect, more variable. Though the older shells still exhibit greater elongation, the curve of growth covers 2.9 degrees, an amplitude more than twice as great as that covered by the *most variable* British

curve. The curve of growth for Casco Bay is also more variable, as it is for Beverly, Nahant, Plymouth, Seaconnet, and, indeed, for all the American localities, without a single exception.

The Fourth Test of Variability.

If we weigh the empty shells of 200 snails from Tenby, Wales, all of approximately the same height (18 to 19 mm.), we will find that they vary in weight from 1.4 grams to 2.5 grams; *i.e.*, the index of their amplitude of variation according to weight is 1.1. Those from South Kincardineshire and those from the Humber District vary from 1.5 grams to 2.4 grams, the index of variation being in both cases .9. The curves of distribution, according to weight, of the shells from the three British localities are represented on Chart XIV by dotted lines, and similar curves of weight for nine American localities are represented by entire lines. (The shells from the St. Croix River were not weighed, as they were cleaned in a manner different from those from other localities, and their introduction would lead to error.)

In every case it will be noted that the length of the base line of the curve of the American shells, even when there are less than 200 (indicated by the figures at the left of the chart), is equal to or exceeds in length the base line of the curve of the most variable British shells. The index of the amplitude of variation according to weight of the most variable British shells is 11, while the amplitude of five of the least variable American shells is also 11; four American localities, however, have an increased amplitude; *viz.*, 12. The *least* amount of variation among the British shells is 9; the least among the American is 11.

The lightest American shell weighs 1 gram; the lightest British shell, 1.4 gram. The heaviest American shell weighs 2.6 grams; the heaviest British, but 2.5 grams. The lightest and heaviest shells are American.

A Fifth Test of Variability.

It will be noted on Chart XIV[1] that the three British curves lie directly under each other. The curves are not only alike in their general configuration, but the ideal weights, as indicated by the vertical curve, are approximately the same. Among the American curves it will be noted that there is more variation both in the contour of the curves and, what is more important, in the vertical curve of ideal means. No manipulation of data or combination of three American localities can be made which will not give an amplitude of variation according to weight which is greater than any combination of the three series of British shells.

A Sixth Test of Variability.

It is, unfortunately, impracticable to express variations in color either by curves or mathematical formulae. Color averages are uncertain and the estimation of extremes difficult.

The bands of color of the British shells are generally clearly defined throughout their entire length, and give a distinct cast to the British shells, whatever their age. The limits of the bands and the edges of the lines are much more irregular and indefinite in the American forms, and the amount of pigment and its distribution in the individual shells is more variable. Thus, the American shells have a mottled appearance and a suggestion of indefiniteness of color that the British shells seldom possess.

[1] On this chart the Roman numerals, at the right, indicate the localities from which the shells are collected, and the Arabic numerals, at the left, indicate the number of specimens used in the plotting of each curve. The lower curve is based on 200 specimens from Tenby, Chart I; the upper curve is based on 183 specimens from Beverly, Chart VI. In the middle of each horizontal curve is a number which indicates the mean weight. The shells from Tenby have a mean weight of 1.86 grams; those from Beverly have a mean weight of 1.43 grams. A vertical curve connects the several mean points, and since it bends to the left, as it ascends, it indicates that the American shells are lighter than the European. The very irregular curve of relative bulks, or displacements, drawn at the right, is intended to show the lack of dependence of the factor of weight upon bulk.

General Questions.

The questions naturally arise: (1) Are the American shells, together with their excessive variability, tending toward the establishment of a new type? (2) Do they offer another example of the principle of "mutation"? or, (3) Do the variations arrange themselves symmetrically around the Old World type?

An examination of the several charts will show that:

(*a*) While the British shells have an average stature of 89.6 $\left(\frac{90.9 + 87.5 + 90.5}{3} = 89.6\right)$, the average stature of the American shells is 91,

$\left(\frac{91.3 + 92.5 + 90.6 + 92.2 + 90.0 + 89.7 + 89.2 + 90.8 + 91.1 + 92.6}{10} = 91\right)$.

The American shells are thus 1.4% longer in proportion to their breadth than the British.

(*b*) While the British shells of 18 to 19 mm. in height have an average weight of 1.85 grams, the American shells of the same height have an average weight of 1.68 grams. The American shells are thus .1 lighter than the British.

(*c*) While the British shells of 18 to 19 mm. in height have an average displacement of 2.67 cc. $\left(\frac{2.75 + 2.50 + 2.77}{3} = 2.67\right)$ — see curve at right of Chart XIV — the American shells of the same height, even though they are lighter in average weight, are relatively and actually larger, for their average displacement is 2.80 cc. (Chart XIV).

$\left(\frac{2.75 + 2.71 + 3.05 + 2.62 + 3.05 + 2.55 + 2.62 + 3.05 + 2.82}{9} = 2.80\right)$

The shells from the Warren River, though extremely light, having an average weight of only 1.48 grams, are nevertheless quite as bulky, that is, their displacement is quite as great as that of the heaviest American shells of equal length.

(*d*) While the color markings of the British shells are laid down with precision, the color markings of the American shells are indefinite and give a characteristic mottled appearance.

Conclusion.

We may then conclude that the periwinkle, subjected to a new environment, and presumably emancipated from many of the restraining influences of natural selection, has become in any and in all American localities:

I and II. More variable in its stature.

III. More variable in its course of growth.

IV. More variable in weight.

V. More variable in bulk.

VI. More variable in the limitations and boundaries of the color patterns.

While presenting these extremes of variation, the American type of *Littorina littorea*, when compared with the European type, is more elongated, lighter in weight, more bulky, and the color markings are less pronounced.

These results are in harmony with and fully corroborative of the conclusions reached from the statistical study of the sparrow's egg.

BROWN UNIVERSITY, PROVIDENCE, R. I.
September 10, 1897.

BIBLIOGRAPHY.

'41. GOULD, A. A. A Report on the Invertebrata of Massachusetts. Cambridge.

'65. JEFFREYS, J. G. British Conchology. London.

'70. BINNEY, W. G. Report on the Invertebrata of Massachusetts. 2d ed. Boston.

79. GRAY, A. F. *Science News*. April 15.

'80. VERRILL, A. E. Note in *Silliman's Journal*. September, 1880.

'80. MORSE, E. S. The Gradual Dispersion of Certain Mollusks in New England. *Bull. Essex Inst.* Vol. XII.

'98. BUMPUS, H. C. The Variations and Mutations of the Introduced Sparrow. (A Second Contribution to the Study of Variation.) *Biological Lectures*. Woods Holl. 1897.

CHART I. 1,000 LITTORINA LITTOREA. TENBY, WALES.

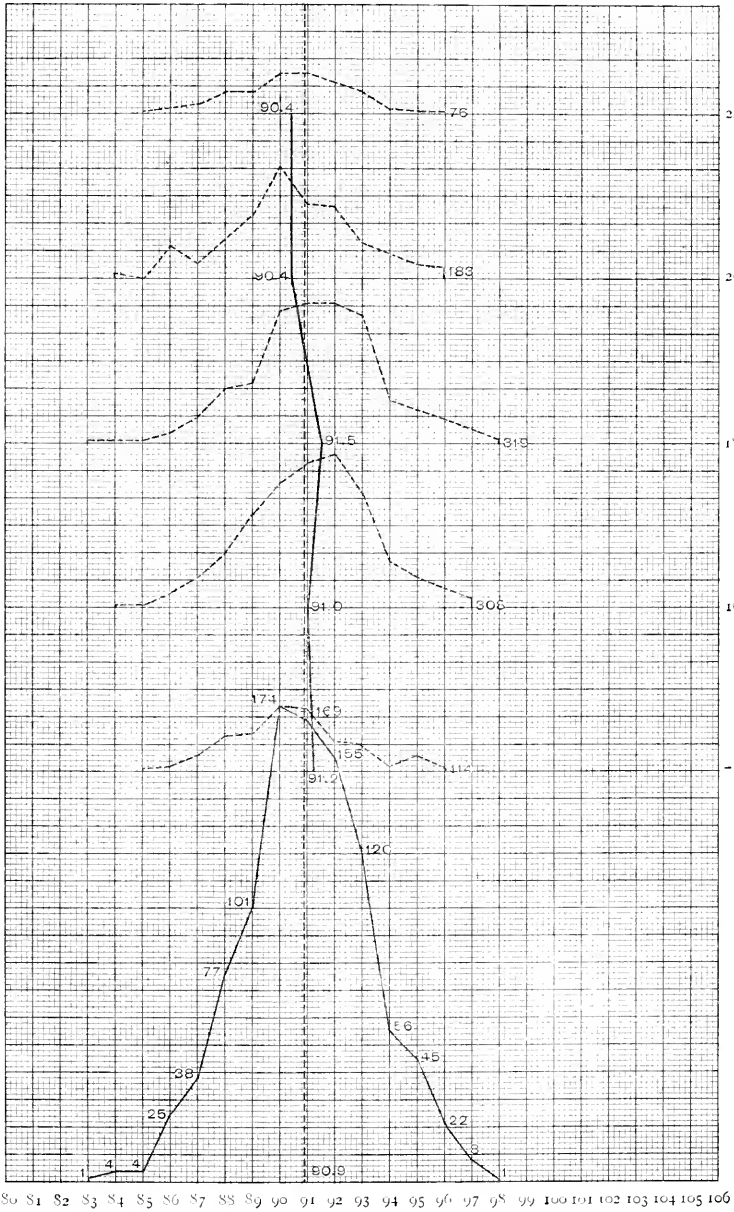


CHART II. 1,000 LITTORINA LITTOREA. SO. KINCARDINESHIRE.

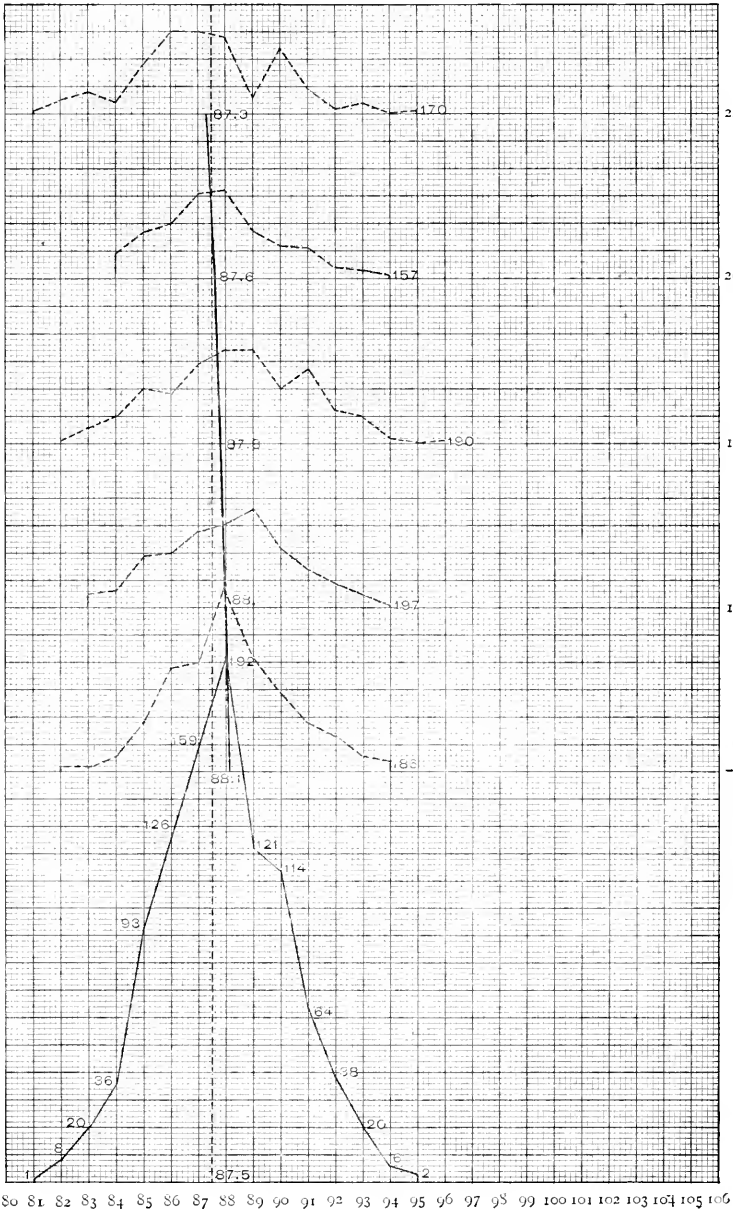
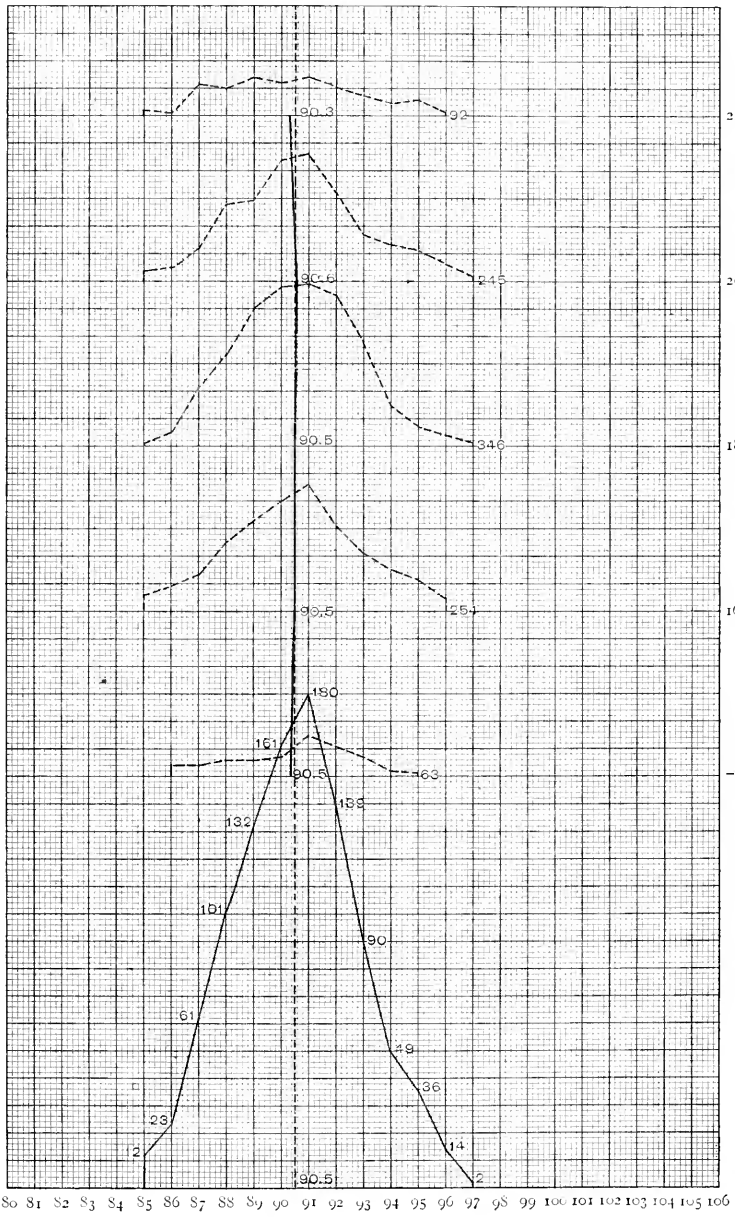


CHART III. 1,000 LITTORINA LITTOREA. HUMBER DISTRICT.



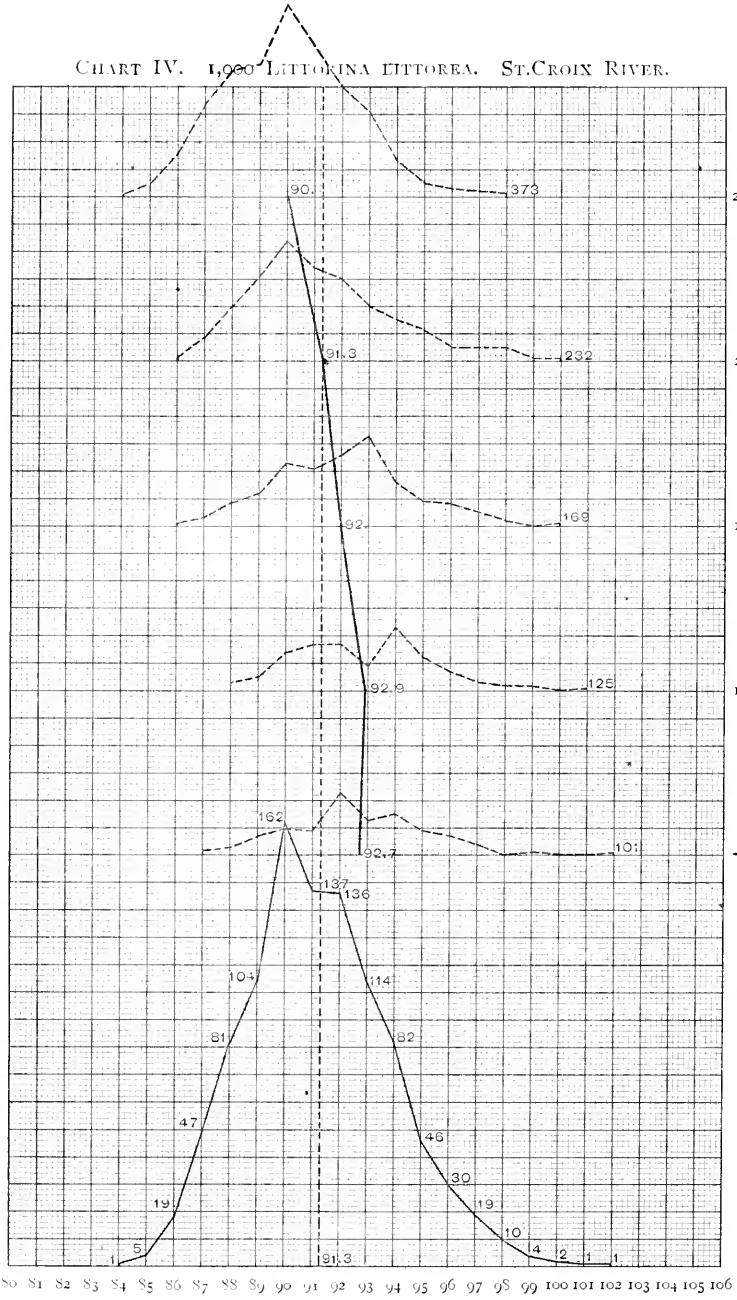


CHART IV. 1,000 LITTORINA LITTOREA. ST.CROIX RIVER.
22-mm.
20-21 mm.
18-19 mm.
16-17 mm.
-15 mm.
90.
373
91.3
232
92.
169
92.9
125
162
92.7
101
137
136
104
114
81
82
47
46
30
19
19
10
5
4
2
1
1
1
91.3
80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106

Volume I. *May, 1898.* *Number 6.*

ZOÖLOGICAL BULLETIN.

ON THE OCCURRENCE OF DISTOMUM OVOCAUDATUM VULPIAN IN AMERICAN FROGS.

W. S. NICKERSON.

In the spring of 1893, the writer found a number of specimens of this trematode inhabiting the Eustachian recesses of the American frog, *Rana clamata*. At that time no satisfactory description with figures of *D. ovocaudatum* had been published, and I was therefore unable to determine positively whether or not the worms which I had discovered were of that species. A comparison of the American worms with specimens of *D. ovocaudatum* preserved in the collection of the Zoologisches Institut in Leipzig has shown me conclusively, however, that the two are identical in all important characteristics. I believe that the occurrence of this species in America has not been reported heretofore.

Previous writers, with the exception of Sonsino, have described this worm as being found only under the tongue of the frog. Sonsino ('93) reports finding it not only under the tongue, but also in the stomach and first part of the intestine, and in a single case in the lung. There is nowhere mention of its occurrence in the Eustachian tubes. In the American frogs, however, this seems to be its normal place of attachment, and in living frogs I have never found it in any other position. In a few cases I have found worms lying unattached in the mouths of frogs which had been killed by chloroform, but in such cases it is probable that the action of the chloroform had caused them to loosen their hold, though I have no means of knowing what their place of attachment had been.

In the rather small number of living or freshly killed European frogs which I have been able to examine in Leipzig, I have found but one specimen of *D. ovocaudatum*, but the fact is suggestive that that one was attached in the Eustachian tube of *Rana esculenta*. That I have not found *D. ovocaudatum* under the tongue of American frogs may be due to accident or oversight. It seems, on the other hand, not improbable that the failure of observers to find it in the Eustachian tubes of the European frogs may be due to the same cause. It would not surprise me if future investigation should show that it occurs as frequently in that position as under the tongue where it has hitherto been observed.

The abundance of this parasite seems to vary greatly from year to year. In the spring of 1893 it was quite abundant in the vicinity of Boston, perhaps as many as one out of every three or four frogs used for laboratory dissection harboring specimens of the worm. During the next two years, although a careful watch was kept for them, not a single specimen was found in the frogs similarly used, although they were collected from the same locality and the number examined was larger. A similar variation for the vicinity of Leipzig has been mentioned by Looss ('94).

There are several points in the anatomy of this species upon which previous writers are not in agreement. One of these is the position of the ovary, which is stated by Sonsino to be upon the left side; Looss found it always upon the right side. In an examination of ten worms with respect to this point, I found that in nine cases the ovary was on the left side (as was the case also in the single Leipzig specimen), in one upon the right. In the same specimens the posterior testis was in seven cases the left one, in three the right. Several other worms had the testes so evenly placed that they could not be counted in either of these lists. From this very small number of observations it would appear that the variations in position of ovary and testes are not strictly correlated.

The egg capsules in this species are terminated at one end by filaments which are described by Vulpian ('59), Creutzburg ('90) and Looss as being from one to one and one-half times the

length of the capsule itself. Sonsino finds in the case of specimens collected in the vicinity of Pisa that the filaments are from four to six times the length of the capsule. The American worms agree in this respect with those of central Europe, and in no case have I found the filaments exceeding in length the measurements given by the French and German observers. It would seem not improbable that the greater length of these filaments in the eggs of Italian specimens may be dependent upon the warmer climate in which they live.

The position of the genital pore is, as described by Looss, immediately behind the pharynx, not immediately in front of the ventral sucker, as is implied by the statements of Creutzburg and Sonsino. The statements of these authors differ widely also concerning the presence of a penis. Sonsino speaks of a "bursa del pene" lying in front of the ventral sucker, and Creutzburg of a "verstulpbaren Cirrus" contained in the "Samenblase." Looss writes: "In wirklichkeit ist weder von einem Cirrusbeutal noch von einem Cirrus eine Spur vorhanden." My own observations are in entire agreement with the statements of the latter author. The seminal vesicle I have found in some cases filled by a mass of sperm which *in toto* preparations or in the living animal might be easily mistaken for a retracted penis. Sections show, however, that the seminal vesicle is merely a simple thin-walled tube, with no considerable muscular thickening or other modification which can be interpreted as a penis.

The vitellary glands are very aptly described by Creutzburg as "traubige zusammengesetzte Organe." That Looss describes them as clover-leaf-shaped is doubtless due to the fact that he studied them in worms so distorted by pressure as to produce such an appearance. The disagreement must be attributed to the "primitiv und barbarisch" method employed by the latter author. The glands consist of about nine nearly spherical masses clustered upon the vitellary ducts in a manner very suggestive of grapes in a cluster, and the position of these glands in the extreme posterior end of the body affords a most simple means for recognizing the species.

LITERATURE.

'90. CREUTZBURG, N. Untersuchungen über den Bau und die Entwickelung von Distomum ovocaudatum Vulpian. Leipzig. O. Schmidt.

'94. LOOSS, A. Die Distomen unserer Fische und Frösche, pp. 109–111. Taf. 3, Fig. 49. Taf. 6, Fig. 123.

'93. SONSINO, P. Trematodi di Rettili e di Anfibi della Collezione del Museo di Pisa. *Atti Soc. Toscana Sc. Nat. Processi verbali.* Vol. viii, p. 188.

'93a. SONSINO, P. Sul Distomum ovocaudatum Vulpian. *Monitore Zool. Ital.* Anno iv, p. 63.

'59. VULPIAN. Note sur un nouveau Distome de la grenouille. *Compt. rend. et mem. soc. biolog.* Année, 1858. Paris, 1859. pp. 150–152. 1860, Pl. XI, Fig. 4.

CENTROSOME AND SPHERE IN THE EGG OF UNIO.

FRANK R. LILLIE.

In this paper I shall present some observations on the relation of the centrosome to the sphere, and of both to the cytoplasm. It is still undecided, I think, what is to be called centrosome and what sphere in all cases (*e.g.*, see MacFarland,[1] McMurrich,[2] and Haecker[3]); and it is still possible to take either side in the controversy as to whether the centrosome is a unique and permanent organ of the cell or not. If the centrosome is not a permanent organ, it must, at some phase in the life cycle of the organism, lose its identity as centrosome and be replaced. Hence, sufficiently exhaustive observation must reveal lack of continuity. If it can also be shown that centrosomes may arise at various points in the cell, the centrosome loses its place as a unique and permanent organ of the cell. If, finally, any of the products of division of the centrosome become other *formed* elements of the cell, the same conclusion follows. It is this last line of argument on which I shall lay most weight in this paper.

In the *metaphase of the first maturation spindle* both asters possess the following structure (Figs. 1 and 2):

a. In the exact center is a minute black speck, the centrosome, either round or dumb-bell shaped, into which are "inserted" the central ends of some, at least, of the rays.[4]

b. There are two concentric spheres, corresponding to the "medullary" and "cortical" zones of Van Beneden, bounded by microsomes regularly arranged on the rays. The ground

[1] MacFarland, Dr. F. M., "Cellulare Studien an Mollusken-Eiern," *Zool. Jahrbücher, Abth. für. Anat. und Ontogenie der Thiere*. Bd. x, Heft 2. 1897.

[2] McMurrich, J. Playfair, "The Yölk-lobe and the Centrosome of Fulgur Carica," *Anat. Anz.* Bd. xii, Nr. 23. 1896.

[3] Haecker, V., "Ueber den heutigen Stand der Centrosomafrage," *Verhandlungen der Deutschen Zool. Ges. zu München*. 1894.

[4] The sections, 5 μ thick, were stained in Heidenhain's iron haematoxylin and Bordeaux red.

substance of the inner sphere is quite dense, and takes the red stain (Bordeaux red) strongly.

c. The radiating fibers are simply an arrangement of the cytoplasmic network, with all that this implies.

d. Microsomes are found on all fibers of the asters and of the spindle, with the possible exception of the central spindle. They are certainly least conspicuous on the central spindle.

In the *late anaphase of the first maturation spindle* the centrosome has divided into two, and the inner sphere is bounded by a *continuous membrane*, into which the central ends of the

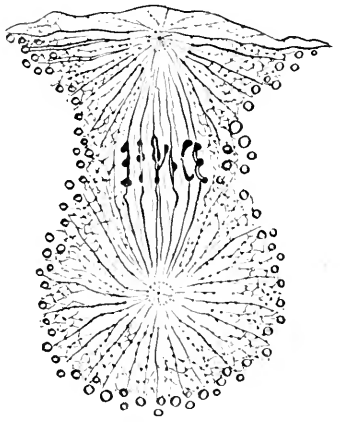


FIG. 1. — First Maturation Spindle of Unio.

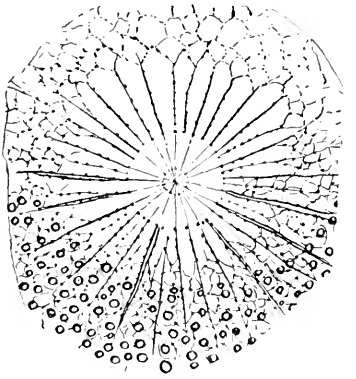


FIG. 2. — Horizontal Section through the Outer Aster of the First Maturation Spindle of Unio in the Stage of Fig. 1.

rays are inserted. This membrane (*cf.* Fig. 6) is produced in part by the fusion of the inner stratum of microsomes, but chiefly by the peripheral accumulation of the ground substance of the inner sphere. The substance of the mantle fibers is heaped up in the cortical zone, and the fibers of the central spindle exhibit large, deeply staining microsomes.

In the *early telophase of the first maturation spindle* the centrosomes are extremely large, and each is composed of a group (at least four) of densely black granules. *The centrosomes are united to the membrane of the inner sphere by a few irregular threads which are not part of the system of radiations* (*cf.* Fig. 6). The rays of the aster and the fibers of the central spindle are studded with enormous closely set black microsomes, the intervening substance staining faintly in Bordeaux.

In the *concluding phases of the telophase*, the inner chromosomes and sphere move towards the surface, drawing out into rays the protoplasm of the degenerating aster. In this stage a new set of fibers radiates in a fan-shaped manner from the sphere to the surface. These radiations then disappear entirely and in place of the two centrosomes but one is found within the inner sphere. In some cases the inner sphere may disappear, and then the centrosome cannot be distinguished from

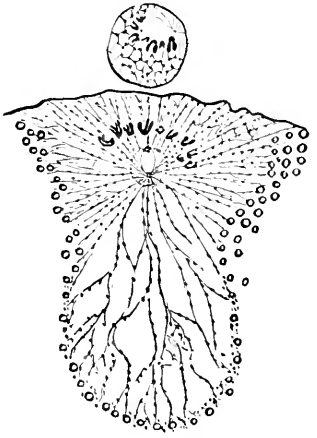


FIG. 3. — Prophase of the Second Maturation Spindle in Unio. Origin of the Central Spindle.

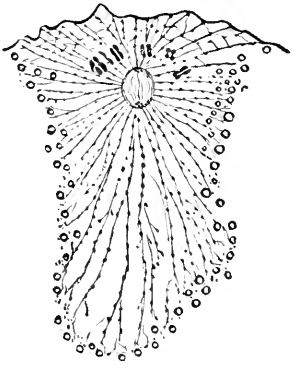


FIG. 4. — Prophase of the Second Maturation Spindle in Unio. Second Stage in Origin of the Central Spindle.

cytomicrosomes. This concludes the telophase of the first maturation division. Without any pause begin

The Prophases of the Second Maturation Spindle. The formation of the second maturation spindle may take place in either of two ways, in both of which, however, the centrosome phenomena are essentially the same. In the first method (Figs. 3–5) the central spindle is formed at the point at which the centrosome remains after the formation of the first polar globule. The central spindle in this method is generally radial in position, though it may lie at any angle. The second method is preceded by the disappearance of the inner sphere, and the horizontally placed central spindle together with the chromosomes sink in towards the center of the egg. The spindle later rotates into position.

In the description of the centrosomes I shall follow the first method entirely. See Figs. 3–5, radial sections through the entire central spindle, and 7*A–E*, horizontal sections through the outer centrosome and spheres drawn on a much larger scale.

The prophase of the second maturation spindle is inaugurated by the origin of a new set of radiations around the sphere (Fig. 3). Within the sphere are two centrosomes united by delicate threads, the beginnings of the central spindle. Each centrosome is composed of several granules. The radiations are generally attached to the sphere, but they can sometimes be traced in part to the centrosomes.

As the spindle elongates, the sphere becomes elliptical, as though stretched by the central spindle, the ends of which abut against it (Fig. 4). The centrosomes have become still more subdivided, and have increased in bulk. The fibers of the central spindle have also increased greatly in number and distinctness. The rays surrounding the sphere are beginning to disappear. They are, plainly, inserted in the elliptical sphere. This is the stage which MacFarland has figured in such detail for Diaulula. He calls the whole sphere the centrosome, because the rays are inserted in it. The history of this body, which was not sufficiently investigated by MacFarland, makes it plain that it is not the *centrosome*, but the *inner sphere*. The insertion of rays is therefore in itself no criterion of a centrosome.

In a slightly later stage the rays have been entirely resolved into vesicular cytoplasm; and the sphere has been stretched out into the peripheral fibers of the central spindle. The centrosomes are yet more subdivided (Fig. 7*A*), and the mantle fibers are just beginning to form.

Now follow some very important centrosome phenomena; when the spindle has elongated a little more the radiations of the asters begin to develop, those of the inner aster before those of the outer in the radial position of the spindle. In a radial section through such a stage, one is inclined at first to think that the inner centrosome has disappeared. More careful observation shows, however (Fig. 5), that the center of

the inner aster is occupied by an extraordinarily minute centrosome lying in a sphere. In the metaphase the outer aster has exactly the same structure (Fig. 7*E*). Later yet the inner sphere of both asters is bounded by a membrane (Figs. 7*F* and 6) formed in precisely the same way as the homologous structure of the first maturation spindle. In this stage the centrosome is much larger than at the metaphase. *What, now, has become of the compound centrosomes of earlier stages, and what is the origin of the concentric spheres?*

Figures 7*A* to *F* illustrate the changes undergone ; 7*A* to *E* are drawn from horizontal sections of the outer aster, and 7*F*

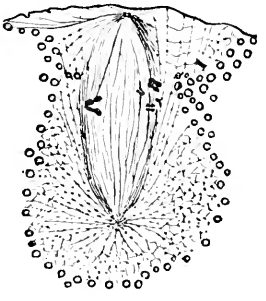


FIG. 5. — Later Stage of the Second Maturation Spindle in Unio.

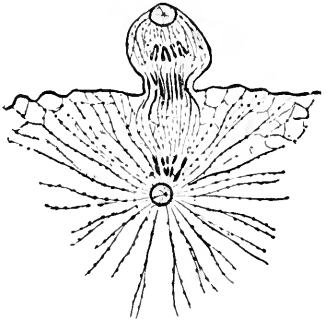


FIG. 6. — Telophase of the Second Maturation Division in Unio.

and 6 from radial sections of the inner aster in the metaphase and telophase of the spindle, respectively. The condition seen in Fig. 7*A* has been reached by growth and division of a single small centrosome ; this figure represents a horizontal section through the outer centrosome of the stage of Fig. 5. The next four figures carry us to the beginning of the metaphase. They show two processes taking place : (1) The subdivision of the relatively large centrosome granules and their distribution in the form of a sphere ; and (2) the increase of the red-staining substance in which the granules are imbedded. The peripherally distributed granules become the stratum of microsomes bounding the inner sphere. One of the granules remains behind as the centrosome of the new inner sphere ; which one of them is, apparently, determined entirely by position. The outer sphere has developed during this process.

The black granules in the inner sphere of Fig. 7*E* are plainly much less in bulk than those of Fig. 7*B*, *e.g.* There is no doubt that a large part of the centrosome granules has been changed into the red-staining substance of the sphere, which is identical in all noticeable respects with the substance from

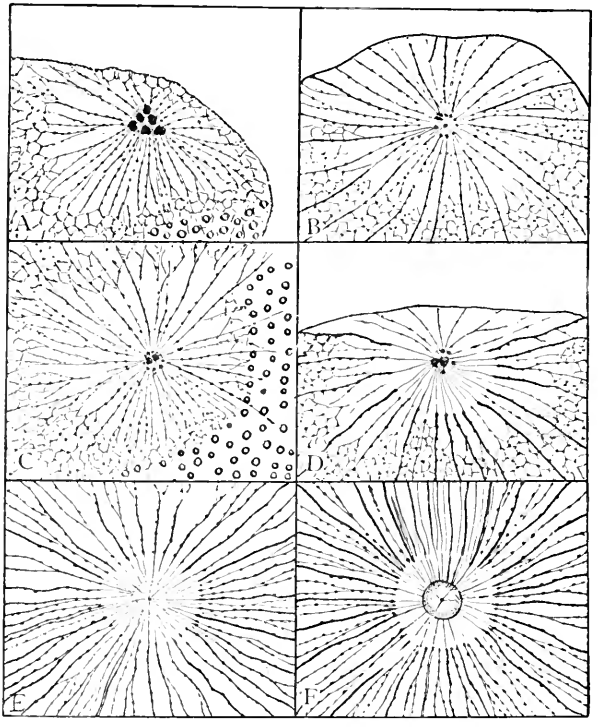


FIG. 7.—*A–E* are horizontal sections through the outer aster of the second maturation spindle, illustrating the phases of the centrosome from a stage slightly before that of Fig. 5 to the metaphase. *B*, *C*, and *D* are not necessarily successive stages, but simply different conditions met with between stages *A* and *E*. *F* is from a radial section of the inner aster in the beginning of the anaphase; this condition of the centrosome follows immediately 7*E* and precedes the stage of Fig. 6.

which the central spindle was formed. And in a later stage one sees the fibers of the central spindle dotted with large black microsomes.

From this description it would seem to follow that the centrosome of one cell-generation becomes the inner sphere of the next; and this is undoubtedly true at times. But I do not

believe that the inner sphere has *necessarily* any such definite morphological value as this would seem to imply. For it may disappear between the first and second maturation divisions, and is then reformed, as the first step in the prophase of the second maturation spindle, from the cytoplasm. The same method of formation may also be observed in other places (*e.g.*, formation of the first cleavage spindle).

Both Van Beneden's and Boveri's conceptions of the structure of the aster appear as phases in the history of the mitosis, though Boveri's "centrosome" is really the inner sphere, and his "Centralkorn" or "centriole" really the centrosome.

In conclusion, I shall sum up the evidence which the study of the egg of Unio has furnished against the theory of the permanency of the centrosome as a unique organ of the cell, combining an earlier paper[1] with this.

1. A sperm amphiaster is formed, but it disappears utterly at the time of the metaphase of the first maturation spindle.

2. Entirely independently of the sperm and egg asters, there arises in the egg of Unio at the time of the metaphase of the second maturation spindle an *accessory aster*, in the center of which is a minute centrosome. This centrosome divides and a small amphiaster is formed, which entirely disappears at the beginning of the telophase.

3. After the formation of the second polar globule the egg centrosome disappears.

4. The two cleavage centrosomes arise independently of any of their predecessors, and apparently separately.

5. Fission products of the centrosome become cytomicrosomes.

Thus the egg of Unio furnishes evidence: in the first place, that the centrosomes are not necessarily genetically continuous; in the second place, that a centrosome may arise in the general cytoplasm (accessory aster); and, in the third place, that products of the centrosomes may become other formed elements of the cell. If this last centrosome phenomenon should

[1] Lillie, Frank R., "On the Origin of the Centers of the First Cleavage Spindle in Unio." See report of the meeting of the American Morphologists in Boston in *Science*, vol. v, p. 114. March, 1897.

be shown to be of general occurrence, it would place in a new light and on a new basis the theory that the cell is composed of self-perpetuating units of a lower rank. So far as these observations go, they tend to confirm Watasé's[1] theory, that centrosomes and cytomicrosomes are homologous structures.

Boveri, Brauer,[2] Van der Stricht,[3] Watasé,[4] Haecker,[5] McMurrich,[6] MacFarland,[7] and Klinckowström[8] have figured structures resembling in some respects the vesicular inner sphere here described. But all have designated them centrosomes. Haecker, and McMurrich have not figured the true centrosome at all.

McMurrich's notice, though brief and incomplete, is of especial interest, because he finds that the vesicular sphere is preceded by a group of deeply staining granules, from which it is probably derived. *How*, he does not know. He figures, moreover, the peripheral microsomes of the inner sphere as nodes in a network. From these nodes the radiating fibers arise. The contents of the inner sphere ("centrosome") are "perfectly homogeneous."

MacFarland's figures are more like mine than any others in the literature of the subject. Moreover, he has followed through exactly the same period with which I have dealt. But his conclusions are radically different, owing to his interpretation of the inner sphere as centrosome. With this interpretation goes his proof of Heidenhain's "Centralspindel und Centrosomen bilden der Genese nach ein Ganzes."

Some of Von Klinckowström's figures of the egg of Prosthe-

[1] Watasé, S., "Homology of the Centrosome," *Journ. of Morph.*, vol. viii, No. 2, 1893. See also *Science*, 1897.

[2] Brauer, A., "Zur Kenntniss der Spermatogenese von Ascaris Megalocephala," *Archiv f. mikr. Anat.* Bd. xliii. 1893.

[3] Van der Stricht, O., "De l'origine de la figure achromatique de l'ovule en mitose chez la Thysanozoon brocchi." in *Verh. Anat. Ges.* 1894.

[4] Watasé, S., "Homology of the Centrosome," *Journ. of Morph.*, vol. viii, No. 2, 1893, p. 434, figure of one of the maturation spindles of the egg of Unio.

[5] Haecker, *v. ante.*

[6] McMurrich, *v. ante.*

[7] MacFarland, *v. ante.*

[8] Von Klinckowström, A., "Beiträge zur Kenntniss der Eireifung und Befruchtung bei Prostheceraeus vittatus," *Archiv f. mikr. Anat. und Entw.* Bd. xlviii, Heft 4. 1897.

ceraeus show a similar vesicular inner sphere ("centrosome" K.). There are generally figured within the sphere a number of granules; but in regard to this point one feels that celloidin sections 15μ thick and stained in borax carmine are inadequate evidence. However, in some eggs in an earlier stage he speaks of a minute "centriole" within the "centrosome."

To the criticism that the centrosome phases shown in Fig. 7*A–F* are pathological, *i.e.*, due to imperfect extraction of the haematoxylin or other action of the reagents, it may be replied: first, that they are found with different killing fluids; second, that the changes are perfectly uniform in all cases, so that, knowing the stage of development of the spindle, one can be certain that a definite stage of the centrosome will be found; third, that, inasmuch as the inner aster develops much more rapidly than the outer, the inner centrosome passes through these phases much more rapidly than the outer. Thus one often finds a spindle in which the inner centrosome has already reached the condition of Fig. 7*E*, while the outer is in the stage of 7*A* (*v.* Fig. 5). Now, 7*E* is a perfectly normal and typical centrosome; hence, if one uses the pathological argument, one must be prepared to assert that one end of a spindle may be pathological and the other normal *in the same section.*

Those who oppose the centrosome theory are often met with the reply, "one positive observation is worth a great many negative ones"; the implication being that one observation in favor of the permanency of the centrosome is worth a great many against it. I think that we are all convinced of the difficulty of proving a negative proposition. Nevertheless, it is a confession of weakness to dismiss all observations incompatible with a theory with the above caption. The observations against the permanency of the centrosome as a unique organ of the cell are now so many that they demand attention. One has a right to ask in detail how the theory is to be upheld against the foregoing observations and against others, such as Mead's,[1] that the two centers of the first maturation spindle are selected,

[1] Mead, A. D., "The Origin of the Egg Centrosomes," *Journ. of Morph.*, vol. xii, No. 2, 1897.

so to speak, from a large number of active ones ; as Morgan's,[1] that any considerable area in the sea-urchin egg may produce an aster, and I may add a centrosome, under the stimulus of abnormal salt content of the sea water ; or Mead's[2] observations in the same direction in Chaetopterus ; or Conklin's[2] observations on the egg of Crepidula, similar to mine ; or of the observations of Strasburger[3] and pupils that karyokinesis in vegetable tissues does not necessarily imply the presence of centrosomes at the poles of the spindle, as it does not in the maturation of Ascaris or of Molgula[3] (Crampton).

UNIVERSITY OF MICHIGAN.
January 6, 1898.

[1] Morgan, T. H., "The Production of Artificial Astrospheres," *Archiv f. Entwickelungsmechanik der Organismen.* Bd. iii, Heft 3. 1896. See also report in *Science* ('98) of the meeting of the American Morphologists in Ithaca in December, 1897.

[2] See report in *Science* ('98) of the meeting of the American Morphologists in Ithaca in December, 1897.

[3] *Jahrb. wiss. Bot.* Bd. xxx.

ON THE MEMBRANA BASILARIS, THE MEMBRANA TECTORIA, AND THE NERVE ENDINGS IN THE HUMAN EAR.[1]

HOWARD AYERS,

PROFESSOR OF HISTOLOGY IN THE MEDICAL DEPARTMENT OF THE UNIVERSITY OF MISSOURI.

THE materials for this investigation consisted of the ears of three human embryos and two adult males. The embryos were two and a half, three and a quarter, and four months old respectively, and since the ear capsules of these five subjects were obtained in a *practically living condition*, it was possible to use with good success both the Golgi and the methylene-blue staining methods in studying them. Both of the adults furnished ears that were normal beyond a doubt ; they came from an electrocuted murderer, on the one hand, and a robber shot *in flagrante delicto* and instantly killed, on the other hand. In the one case the ears were removed at once and preserved in an aqueous solution of corrosive sublimate, while in the other they were studied in their fresh condition. Of the embryonic ears, one was studied in the fresh condition, one was used for serial sections, while the third was used for the dissection of the membranous ear.

This exceptionally favorable adult material has given excellent results, and we may rest assured that the histology is perfectly normal and unaffected by sickness or organic disease, and, since I have used every care to preserve the living conditions in my preparations, and, by previous study of the living cochlea of mammals other than man, thoroughly prepared myself to detect alterations due to reagents, I can assure you that all of the histological characters with which we have to deal have been fixed in death as they were in life. In 1892, after several years spent in the investigation of this subject, I pub-

[1] Read at the meeting of the Association of American Anatomists, held at Ithaca, N. Y., Dec. 28–30, 1897.

lished a monograph on the vertebrate ear in which I devoted special attention to the anatomy and the histology of the mammalian cochlea, with the result that my discoveries necessitated a reconsideration of the prevailing views on the physiology of the ear. Since that time I have made several other contributions to the histology of the ear, mainly on the innervation of its sense organs. The morphological facts are admitted without question by those who have taken the pains to examine my preparations. Most physiologists and some anatomists, however, have not made use of either the facts or the physiological conclusions which necessarily flow from them, and, so far as I know, they have not troubled themselves to find out the facts. Under the circumstances, and especially because of the excellent human material which I have been fortunate enough to secure and to subject to a careful histological analysis, I am glad to bring before the Association of American Anatomists this statement of a few important facts of cochlear anatomy which are essential to a correct knowledge of the cochlea.

Membrana Basilaris.

The membrana basilaris is that part of the connective tissue wall of the cochlear tube which lies under the sense organ and forms its basement membrane. It is far from being the most delicate wall of the cochlear tube, for the membrane of Reissner is much thinner and less resistent. The importance of the basilar membrane to previous investigators was due to the dominant Helmholzt-Hensen piano-string theory of tone perception. But it is neither elastic enough nor thin and homogeneous enough to meet the requirements of this physical hypothesis. According to my latest observations, the human basilar membrane consists of four layers of fibers, three of which run radially, that is to say, from the free edge of the *lamina ossea* to the base of the stria vascularis, being continuous with the periosteum of the former structure and with the connective tissue framework of the latter part. The fourth layer, if it is permissible to call a small number of separated fibers a layer, runs at right angles to the other three ; or spirally, with reference to

the parts of the cochlea. These layers are quite distinct, and are arranged as follows : an upper and a lower layer of fine fibers inclosing between them a layer of fibers. The important imperfect layer of spiral fibers is most apparent upon the upper surface of the basilar membrane. The basilar fibers are the direct product of a part of the connective tissue cells of the embryonic basilar membrane which have been transformed into long cylindrical fibers, for the most part simple, but occasionally branched.

Auditory Cells.

The hair-bearing acoustic cells are cylindrical in shape, those of the inner row being shorter cylinders, so short, in fact, that they become ovoidal. They are surrounded and supported by the peculiarly modified non-nervous cells of the organ of Corti. The hair cells are much shorter than the supporting elements, and do not reach the basement membrane, or, as it is called, the basilar membrane, a fact of much significance in view of certain physiological hypotheses.

The hairs arise from the top of each cell as a slender bundle, the fibrils growing from all parts of the cell cap, not forming, as some assert, a crescentic or horseshoe-shaped outline upon the cell cap. Each cell bears on an average two dozen delicate, flexible filamentous hairs, which sweep inwards from the cell to end free in the endolymph above the *limbus spiralis*. The whole hair is thus supported by, or floats in the endolymph, and all the hairs from the aggregate of hair-bearing cells are so closely placed that they exert a capillary attraction upon each other, and thus, when they are loose from the tops of the cells, they remain adhering in the form of a long band or ribbon which has been called the *membrana tectoria* or damper, from its supposed rôle in auditory physiology. The long hairs are the percipient elements in the cochlea instead of the connective tissue fibers of the basement membrane of the sense organ, and the ear thus agrees with the eye, the nose, and other sense organs in the disposition of its percipient, recipient, and transmitting apparatus.

The Nerve Endings in the Ear.

The fibers of the cochlear nerve, when traced from the twisted cone of medullated fibers in the modiolus outward to the cochlear ganglion, are found to occasionally unite with or give off another fiber, which is not to be regarded as a collateral, since such fiber extends to the hair cells at the periphery in the organ of Corti. In doing so its fibrils do not pass through but around the ganglion cell, through which all the fibrils of the regular nerve fiber must pass. On gaining the ganglion cell the regular nerve fiber issues from the peripheral border of the cell as a single fiber (bipolar cell) or as from two to six distinct nerve fibers (multipolar cell), all of which then take their way towards the organ of Corti, branching as they go. These fibers may leave their radial course at any point and pass at right angle to their former course for greater or less distances (spiral nerve fibers). However, all nerve fibers leaving the cochlear ganglion sooner or later attain the organ of Corti, where they terminate in the bases of the hair cells (first method) or in a sub-acoustic nerve net from which fibers are given off to the hair cells (second method). There is thus formed a compound nerve net disposed in two layers, one above the other, immediately beneath the hair cells, which net serves to connect together hundreds of hair cells in different regions of the epithelial ridge in which the hair cells lie imbedded. Inter-epithelial or free nerve ends may occur, but I have never seen them ; all such cases are apparent, not real, so far as my observations go. The intra-cellular endings are genuine and real, and here, as elsewhere, one positive fact of observation is worth many negative observations. The facts I have stated above are all statements of my positive observations on the basilar membrane, hair cells, hairs, and nerve ends of the human ear.

UNIVERSITY OF MISSOURI,
December 10, 1897.

NEW AMERICAN SPECIES OF THE GENUS ATAX (FAB.) BRUZ.[1]

ROBERT H. WOLCOTT.

THE author had the pleasure of presenting at the annual meeting of the Nebraska Academy of Sciences, November 27, a paper embodying the results of a study of this genus. In it were embraced the descriptions of seven new species, and, since the printed report of the society's proceedings will not appear for a few months, it is deemed desirable to publish at once descriptions which will be sufficient for ready identification, and will preclude the possible publication of synonyms.

The collections studied were made in twelve localities in Michigan, one in Wisconsin, five in Nebraska, and two in New York, the latter by Mr. R. H. Johnson, of Harvard College, and through his kindness transmitted to the author, who also acknowledges the assistance of Mr. Bryant Walker, of Detroit, Mich., in the identification of the mussels, and his indebtedness to Dr. F. Koenike, of Bremen, Germany, for specimens of European species. The collections involved the examination of 1125 specimens of Unionidae, belonging to 36 species, and resulted in the preservation of 4500 mites, divided among 12 species, of which 7 are new and of which 2 more are recorded for the first time from this country.

The species represented are *A. crassipes* (Müller), *A. pectinatus* n. sp., *A. intermedius* (Koenike), *A. abnormipes* n. sp., *A. indistinctus* n. sp., *A. serratus* n. sp., *A. fossulatus* (Koenike), *A. stricta* n. sp., *A. arcuata* n. sp., *A. ypsilophorus* (Bonz), *A. tumidus* n. sp., and *A. ingens* (Koenike). The validity of the genus *Cochleophorus* Piersig is recognized and the species referred to it excluded from this paper. The new species may be characterized as follows :

[1] Studies from the Zoölogical Laboratory, The University of Nebraska, Lincoln, under the direction of Henry B. Ward, No. 23.

I. *A. pectinatus* n. sp.

Related to both *A. crassipes* (Müller) and *A. figuralis* (Koch), and bearing resemblances to each, but rather closer to the latter than to the former. Of the form of *A. figuralis*, but smaller, the males averaging .7 mm., the females .8 mm. in length ; legs somewhat shorter, the first pair shorter than the body, the second and third about one fourth longer, and the fourth two thirds longer. No spines on first pair of legs set into the side of projecting sockets, and all the spines shorter than those of *A. figuralis*. Palpi much thicker than first pair of legs and very long, equalling one third the length of the body ; chitinous papillae one fourth segment short. Claw of first pair of legs peculiar, being expanded dorso-ventrally and flattened laterally, forming a broad plate, the ventral margin of which is deeply pectinate, thus suggesting the specific name (Fig. 1). Claws of the other legs long, slender, and simple. Sexual area of female similar to that of *A. crassipes*, — three acetabula on each of four plates ; in the male the two plates on either side united into one, upon which the six acetabula are arranged in two groups of three.

FIG. 1. — *A. pectinatus* ♂ — distal segment first pair of legs. × about 165.

A free-swimming form, of which six specimens were dredged in Lake St. Clair, Mich., over a bed of Chara, at depths of from six to twelve feet.

II. *A. abnormipes* n. sp.

A small species, the body of the male pyriform in shape, deeply emarginate posteriorly, .5 mm. long ; of the female very slightly pyriform, with the posterior margin rounded, .6 to .7 mm. long. Surface of the body marked by lines dividing it into minute hexagonal areas. Palpi rather slender, somewhat less than half the length of the body, with a long fourth segment and a broad fifth segment, the lateral surface of which is quadrate in outline with the ventral distal angle produced, and the distal margin with two curved claw-like projections (Fig. 2).

The epimera cover most of the under surface of the body, leaving but a narrow space between the second and third, and between those of opposite sides. Legs short and thick, the fourth pair longest and only one fifth longer than the body; those of the female slenderer and relatively even shorter than those of the male. The three distal segments of the fourth leg of the male are peculiarly modified, the fourth being compressed laterally through the distal two fifths of its length, and in this compressed portion a bunch of six very large spines, exceeding in length the fifth segment, in two rows on the anterior surface, and on the posterior surface about nine moderately stout spines; the fifth segment is short, at its base narrower than the preceding segment, tapering toward the tip, with two very heavy, curved blunt spines on the extensor surface and a row of spines along the flexor side, and with a bunch of fine hairs at the distal end; the sixth is very slender and rather long. The claws are strongly bent and with an accessory tip on the convex side at a distance from the principal tip equalling one sixth the total length. One half of the sexual area of the male is situated on either face of the groove in the posterior surface, the opening being at its bottom; the acetabula are five, in two groups — two anteriorly, three posteriorly — on a single plate. In the female this plate is divided, the posterior portion with three acetabula being the larger.

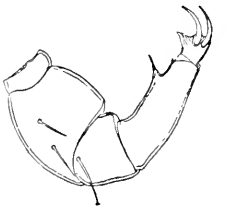


FIG. 2. — *A. abnormipes* ♀ — outer side left palpus. × about 125.

Of this species over 500 specimens have been collected from a number of species of Unio, at Lake St. Clair and Grand Rapids, Mich., Oshkosh, Wis., Chautauqua and Cheektowaga, N.Y.

III. *A. indistinctus* n. sp.

With the preceding and the following this species forms a group of three closely allied species. Of this form only females have been collected, so that there is an absence of the marked structural peculiarities which the males of the others possess, but differences exist sufficient to separate these from

the females of the other species. The body is regular in outline and measures about .8 mm. in length, with the same division of the surface into areas as in *A. abnormipes*. The palpi are relatively stouter (Fig. 3), while the epimera cover, as in that species, most of the ventral surface. The legs are relatively long and moderately stout, the fourth two fifths longer than the body, while the accessory tip on the claw is one third the length from the distal end. Each lateral sexual plate is in two parts, with the acetabula three and six, respectively; in one instance four and five on one side, three and six on the other.

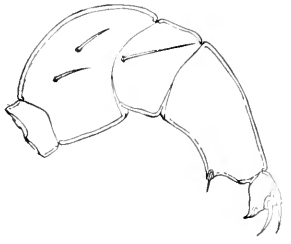


FIG. 3. — *A. indistinctus* ♀ — outer side right palpus. × about 125.

Specimens were taken at Lake St. Clair, Mich., and confused with the following species until mounted and subjected to a careful microscopical examination, when the differences became apparent.

IV. *A. serratus* n. sp.

The third and largest member of this group just referred to, averaging in length about 1.1 mm., and with a regular outline. Surface of the body marked as in preceding forms. The palpi are relatively much stouter (Fig. 4), while the legs are a trifle more slender and not so long proportionately. The fourth leg is only one eighth longer than the body in the male, and in the female, where all the legs are shorter, it is even less than the body length. In the male a larger or smaller number of the stouter spines on all the legs are serrate along both margins; on only the basal segment of the first, on each successive leg more, till on the fourth are serrate spines on each segment, and on the distal segment is a row of very prominent, flattened,

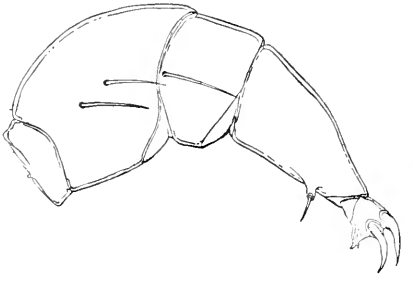


FIG. 4. — *A. serratus* ♂ — outer side right palpus. × about 125.

lanceolate spines with both edges sharply serrate. On the fourth segment of the posterior legs are three heavy club-shaped spines. In the females serrate spines are present, but less numerous and not so prominent. The claws have the accessory tip at two thirds the distance from the base. The epimera cover only the anterior two thirds of the ventral body surface, and the spaces between them are wider. The sexual area is broader than long, and in both sexes there are two plates on either side, in the male the number of acetabula varying from 12 and 25, respectively, to 15 and 31, in the female from 8 and 12 to 6 and 17.

Twenty-eight specimens from Lake St. Clair and Grand Rapids, Mich., and Cheektowaga, N.Y., taken from *Unio occidens* Lea, *U. coccineus* Hild., *U. undulatus* Barnes, and *U. alatus* Say.

A. fossulatus (Koenike).[1]

The male differs from the female described by Koenike in the greater length of the legs in proportion to the length of the body, the somewhat larger epimera and narrower spaces between them, and in the fact that the acetabula are placed on two plates, one on either side of the sexual opening, instead of being imbedded in the surface of the body.

V. *A. stricta* n. sp.

With the preceding were collected numbers of an Atax, which apparently was *A. fossulatus*, differing on ordinarily close observation only in the fact that the five acetabula were in one line, instead of the two posterior being side by side. At first looked upon as males of *A. fossulatus*, the contrary was only perceived when careful microscopical examination of mounted specimens revealed the true males of that species and led to a minute examination of several specimens. The differences are found to be slight, but constant. The body is broader and averages less in length, the legs are slighter, the distal

[1] Koenike, "Nordamerikanische Hydrachniden." *Abhdlgn. d. naturwiss. Ver. Bremen*, Bd. xiii, Heft 2, p. 221, Taf. 111, f. 68–71. 1895.

segments are nearly uniform in thickness from base to tip, instead of tapering as do those of *A. fossulatus*, and the first pair are hardly stouter than the others, while in *A. fossulatus* they are considerably heavier. The claws are relatively longer and slenderer, and all but those of the first pair seem to be simple instead of bifid. The epimera are relatively shorter and broader, and the acetabula are placed one behind the other in two curved lines, as referred to above.

This species has been taken at Grand Rapids, Mich., and Milford and Lincoln, Neb., — 176 specimens altogether, — and the Nebraska specimens are peculiar in that the acetabula are smaller and more closely crowded together, while the whole sexual area is smaller than in the Michigan specimens.

The writer has been uncertain whether this is a distinct species or a variety of *A. fossulatus*, but for the present, at least, has decided to consider it as separate, though closely allied.

VI. *A. arcuata* n. sp.

This species is equal in size to *A. fossulatus* and *A. ypsilophorus*, between which it seems to be intermediate, and is of the same elongated elliptical form. The palpi resemble very closely those of *A. ypsilophorus*, as do also the epimera in size and relationship to each other. The legs are very long, in the male the fourth nearly half as long again as the body ; they are slender and the spines are small and weak, while they are characterized especially by the curved form of the terminal segment, the curvature being only moderate in the first pair, but in the fourth amounting to a deflection of 30°. The distal segment also tapers toward the tip, but just at the tip is broadly expanded to receive the short, thick, bifid claw (Fig. 5). The sexual area is toward the tip of the body and resembles very closely in structure that of *A. tumidus* ; the general proportions are about those of *A. ypsilophorus*, but

FIG. 5. — *A. arcuata* ♀ — anterior surface distal segment, posterior pair of legs. × about 125.

the acetabula, instead of being toward the margin, are over the greater part of the plate, and are larger than in that species, while in the middle of the outer margin, on either side, are two much larger than any of the rest. The number of acetabula in the male varies from 25 to 30, in the female from 30 to 35.

Two hundred and ninety-five specimens were obtained at Charlevoix and Grand Rapids, Mich., in various species of Unio.

VII. *A. tumidus* n. sp.

Very similar to *A. ingens*[1] in form and size, the females of both showing a tendency to enormous distension when filled with eggs, in the relative length of the legs, in the habit of depositing these in loose masses in the mantle and gill cavities, and also in color, the internal structure of each suggesting no trace of the Y-shaped mark, but the color being a light brown with numerous fine white vermiculate lines. The palpi are very similar in the thickness of the fourth segment and in the presence of four inconspicuous papillae at the tip. The legs are feeble in proportion to the size of the body, as are those of *A. ingens*, but, unlike that species, the claws are not simple, but are of the type of *A. ypsilophorus*, though relatively much smaller and heavier (Fig. 6). The sexual area also shows a marked difference from that of *A. ingens* and agrees in position, form, number of acetabula, etc., almost precisely with that described for *A. arcuata*.

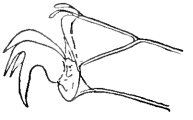


FIG. 6. — *A. tumidus* — distal end of distal segment, fourth pair of legs. × 280.

Taken at Lake St. Clair, Ann Arbor, Charlevoix, Intermediate Lake, and Grand Rapids, Mich., in *Margaritana deltoides* Lea, *Anodonta fragilis* Lam., *A. edentula* Say, and *A. ovata* Lea, thus an Anodonta parasite, while *A. ingens* is a Unio dweller. Few specimens were taken in any one locality.

[1] Koenike, *l.c.*, p. 219, Taf. 111, f. 65–67.

REGENERATION AND LIABILITY TO INJURY.

T. H. MORGAN.

It is not uncommon to meet with the statement that there exists a relation between the power of regeneration of a part and its liability to injury. Certain well-established facts in regard to the regeneration of internal organs are entirely overlooked or disregarded in the attempt to show that the above relation "explains" the origin of regeneration. It would be interesting if those who hold that "there is no such thing as a general power of regeneration; in each kind of animal this power is graduated according to the need of regeneration in the part under consideration," — it would be interesting if such persons would show how such a thing could arise by means of "variation" and "survival of the fittest."

True, Weismann has given rather an elaborate explanation of his view of the matter; but I cannot believe that the chapter on regeneration in the Germ-Plasm will convince any one that the phenomena are in any way explained. For myself, I fail to see by what nice mechanism the power of regeneration is graduated according to the need of regeneration of each part. The "Natur-philosophie" seems not yet dead.

The following pages contain an account of some experiments made at Woods Holl, Mass., at the Marine Biological Laboratory during the summer of 1897, on the power of regeneration of the different appendages of the common hermit crab (*Eupagurus longicarpus*). A part of the animal is protected by the appropriated shell of a mollusc, and the appendages are modified in connection with the peculiar habitat of the animal. The anterior appendages are exposed, and some of them are not infrequently lost; while the appendages protected by the shell do not seem to be often injured The results are treated under the following headings:

I. *Description of the Appendages.*

II. *The Percentage of Appendages Lost under Natural Conditions.*

III. *Experiments on Regeneration.*

IV. *Conclusions.*

FIG. 1. Third pair of walking legs. — FIG. 2. Next to last thoracic leg. — FIG. 3. Last thoracic leg. — FIG. 4. First abdominal appendage of male (belonging to second segment). — FIG. 5. Second abdominal appendage of male. — FIG. 6. Third abdominal appendage of male. — FIG. 7. Telson and sixth segment with last pair of abdominal appendages.

I. Description of the Appendages.

The eyes, antennae, antennules, and mouth parts (maxillae and maxillipeds) are not sufficiently different from those of other Decapoda to call for special comment. The first three pairs of walking legs are large and strong (Fig. 1), and protrude from the shell when the animal moves about.

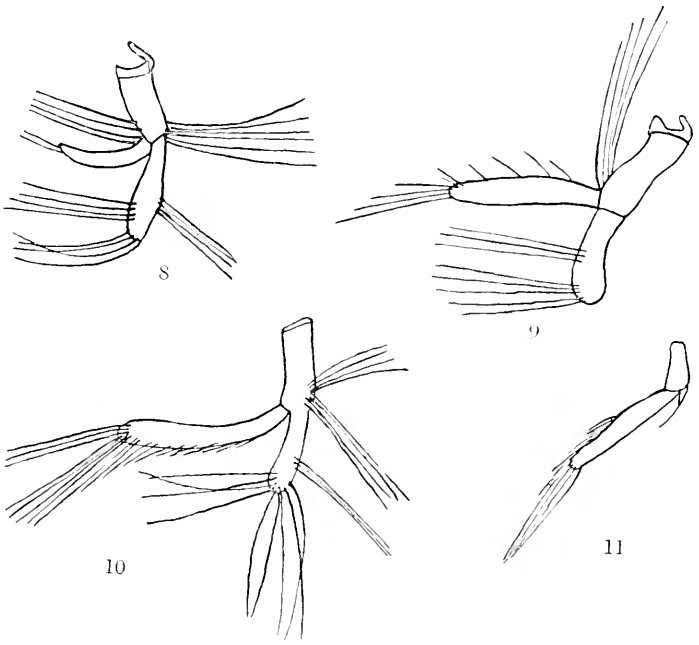


Fig. 8. First abdominal appendage of female (belonging to second segment). — Fig. 9. Second abdominal appendage of female. — Fig. 10. Third abdominal appendage of female. — Fig. 11. Fourth abdominal appendage of female (= third abdominal appendage of male). (Same scale as last figures.)

The fourth (Fig. 2) and fifth (Fig. 3) pairs of legs are small and weak. They are brought to the edge of the shell when the crab is active, but take no part in locomotion. They may be of service in bracing the animal against the shell.

The anterior abdominal appendages are entirely absent on one side of the body, — the side toward the axis of the shell. Those on the other side are said to represent in the female the second, third, fourth, fifth, and sixth appendages (Figs. 8, 9,

10, 11, and Fig. 7). In the male the anterior abdominal appendages are smaller (Figs, 4, 5), and only the second, third, fifth, and sixth appendages are present. The abdominal appendages of the female (second, third, and fourth) carry the eggs during the breeding season. These appendages are supplied with long hairs, to which the eggs are attached. In the male the hairs are not so well developed.

The last abdominal appendages (Fig. 7) are hard and strong — the left one much larger than the right. These appendages seem to play an important part in anchoring the abdomen in the shell. So securely is the abdomen fastened that it will often pull apart before the appendages loosen their hold.

Are any of these appendages rudimentary? A statement that this or that part is rudimentary implies a knowledge of the descent of the animal possessing such structures. A simple reduction in size, moreover, is not a criterion, because a smaller appendage may be in certain cases a more perfect adaptation than a larger one. For instance, most zoölogists would admit, I think, that the fourth and fifth pairs of thoracic legs of the hermit crab have been reduced in size, but how far this reduction is due to degeneration and how far to new adaptation is difficult or impossible to say. In comparison with most other Decapoda these appendages seem reduced, and they have lost their locomotor function.

In the abdominal region the evidence in favor of a reduction or rudimentary condition of the appendages of the male is stronger. On one side the appendages have entirely gone, and on the other side they are small and weak, and the third appendage (on the fourth segment) has completely disappeared. In other related forms the two anterior abdominal appendages of the male have also entirely gone, or are represented by only a few tufts of hairs.

The terminal appendages, on each side of the telson, do not appear reduced in comparison with the similar appendages of other Crustacea, but one is nearly twice the size of the other (Fig. 7).

II. The Percentage of Appendages Lost under Normal Conditions.

The anterior end of the body is exposed when the crab is moving about. This portion of the body is covered by a thick, hard cuticle. The parts enclosed in the shell are softer, excepting the telson and the sixth abdominal appendages. We should anticipate that the exposed portions of the body, despite their greater strength, would be more often injured, and such is the case. I have made an examination of a number of animals, and have found that quite often one of the first three pairs of walking legs has been lost. The antennae, too, are often broken at the end.

The first three thoracic legs can be thrown off near the basal joint. Autonomy is known to take place also in other Decapoda (see papers by Fredericq, Réaumur, Goodsir, Chantran, Brook, Andrews, Herrick). This has been looked upon as an adaptation for regeneration! The fourth and fifth pairs of thoracic legs cannot be thrown off.

The following observations show the percentage of individuals that have lost, under natural conditions, one or more appendages.

Of 47 individuals (collected June 12), 5 had lost one of the first three walking legs.

Of 73 individuals (June 14), 13 had lost one of the first three legs.

Of 68 individuals (June 17), 3 had lost one of the first three legs.

None of these were examined in respect to other appendages. If we reduce the figures given above to percentages, we find 10.6, 17.8, and 4.4 per cent, respectively. That is, out of 188 individuals, 21 (or 11 per cent) had lost an appendage.

In order to ascertain whether the other appendages were also lost, a hundred individuals were collected in September; they were killed and then removed from the shells, and all the appendages carefully examined. The following results were obtained:

The eyes were present and uninjured in all the individuals. The antennae and antennules were also present. In some cases the ends of the antennae were broken off. The third maxillipeds were present and uninjured. One of the chelae was absent or regenerating in five individuals, and one of these had lost both right and left chelae (6 per cent). A second leg was absent in one case (1 per cent), and a third leg in two cases (2 per cent). In all, therefore, 9 per cent had lost one of these legs. In not a single case were the fourth or fifth legs missing or injured.

In the abdominal region the third abdominal appendage (belonging to the fourth segment) was absent in one female, and in one male the second abdominal appendage (belonging to the third segment) was absent. In another male the broken proximal end of the second abdominal appendage was present, and in another the first abdominal appendage was reduced in size, and the second also was smaller than the normal, but the third appendage (representing the fifth segment) was normal. The two cases — one male, the other female — where an appendage was absent may be the result of individual variation; although it is also possible that the appendage was accidentally pulled off in removing the crab from the shell. In the latter case some evidence of tearing or breaking ought to appear, but no such evidence was found. The individual in which the first and second abdominal appendages are smaller than normal may be the result either of variation or of incomplete regeneration. The evidence, therefore, furnished by an examination of the abdominal appendages is conflicting, and it is possible that the appendages may be occasionally lost.

The sixth abdominal appendages were present in all cases.

Forty of the individuals were females and sixty males.

Summary. — The results, taken as a whole, show that the first three walking legs are most often lost. The last two thoracic legs were not absent in a single case examined, and the same statement holds for the last abdominal appendages. The second (♂) and third (♀) abdominal appendages were absent in one case each, but whether from individual variation or from loss during life is not known. Yet, since in one case small

abdominal appendages were found, it may be that these appendages are sometimes lost.

Shall we find, then, in the regeneration of these different appendages any correspondence between the power of regeneration and the liability to injury or loss of a part?

III. Experiments on Regeneration.

Two series of experiments were made, — one lasting through parts of June and July and another in August and September.

The crabs, after the removal of one or more appendages, were kept in aquaria with running water. They were fed every day or two.

In the first series of experiments the following operations were made: From one series one or more of the walking legs were removed on June 12. On July 2 the ten crabs were killed and examined.

(1) The right and the left first legs were both beginning to regenerate. The tip of a second and of a third leg had been cut off, but had not regenerated.

(2) The right first leg had a new bud.

(3) The right first leg had a long new part.

(4) The left first leg had a moderately long new part.

(5) The right first leg, removed very close to body, had not regenerated, nor had the tips of the third and fourth legs that had been cut off.

(6) The right first leg and the third left leg had not regenerated (very small individual).

(7) The first right leg had a very little (if any) new tissue, and the left second leg had a long new part.

(8) The left second leg and the right second and third legs had long new parts.

(9) The left second leg had regenerated a small new part.

(10) The tip of the left third leg had not regenerated.

Summary. — In three cases the leg had not regenerated; in one of these the leg had been *cut off* very near to the body, and in the other two cases two legs of the same crab, presumably *thrown off* after injury, had not regenerated. Seven new legs

were forming, and three others had the beginning of a bud. In three cases in which the tip of the leg had been cut off no new part formed. As these legs were in constant use, any new tissue would, perhaps, even if it formed, be worn off.

From another series one of the last two thoracic legs or the first, second, or third abdominal appendage was cut off on June 12. On July 2 the crabs were killed and examined. Thirty-one individuals were present at the end of the experiment, and of these five were regenerating the fourth leg, and four showed as yet no signs of regenerating the lost leg; six were regenerating the fifth leg; three were regenerating the first abdominal appendage, but nine showed no signs of regeneration in this appendage; two were regenerating the second abdominal appendage (♂), and four were not regenerated (♂).

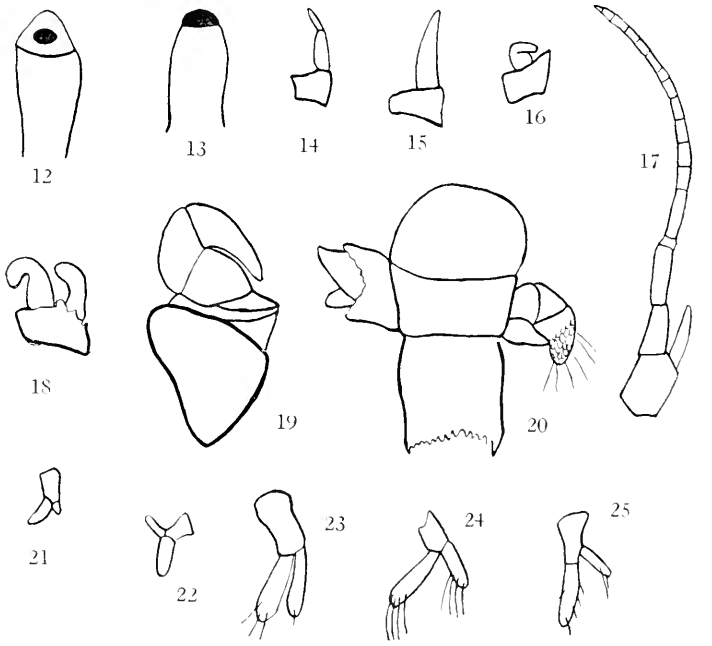
Summary.—The results show that the small fourth and fifth legs possess the power of regeneration. The abdominal appendages (first, second, and third) have also the power of regeneration, but the percentage of those in which the process takes place is smaller than in the case of the thoracic appendages. Whether this difference is due to the longer time necessary for these appendages to grow again or to some difference connected with the place at which they were cut off is not clear. Whether a molt is necessary for the reappearance of the abdominal appendages I do not know. It may be that they do more rarely regenerate, and this in turn may be connected in some way with the amount of food supply brought to the region from which they arise. At any rate, the positive result in those cases where regeneration took place shows that these appendages still possess the power of regeneration.

In another experiment one of the last abdominal appendages, the right or the left, was cut off on June 14. On July 2 all, with one exception, were regenerating. Eight individuals had a new, or regenerating, left appendage, and the right smaller appendage was regenerating in three cases. In a single case the absent right appendage had not regenerated.

In the second series of experiments, made on August 18 and brought to a close on September 15, the eyes, antennae,

antennules, and maxillipeds were cut off; and I also repeated the experiment of removing one or more of the first three or four (♀) abdominal appendages.

Eyes. — In ten individuals the pigmented tip of the eye was cut off, and in ten others the eye-stalk was cut off at or near



FIGS. 12, 13. Eyes regenerating from distal end of stalk. — FIGS. 14, 15 Antenna-like structures regenerating from base of old eye-stalk. — FIGS. 16, 17. Antennae regenerating. — FIG. 18. Maxilliped regenerating — FIG. 19. First walking leg regenerating. — FIG. 20. Left last abdominal appendage regenerating. — FIGS. 21, 22. First and second abdominal appendages regenerating. — FIGS. 23–25. First, second, and third abdominal appendages of male regenerating. (Drawn to same scale as preceding figures.)

its base. Eight individuals that had the tip of the eye cut off were alive at the end of the experiment, and seven of these had a new pigment spot at the end of the old stalk (Figs. 12, 13). In some cases a sharply defined, oval, pigmented body was present, and two of these, when cut into sections, showed that a new eye was in process of development.

In the other ten individuals, in which the eye-stalk had been cut off near its base, five had regenerated a new part, and five had not; but in this experiment the new organ was antenna-like (Figs. 14, 15). Herbst also found that when the eyes of

Palaemon and Sicyonia were removed sometimes an eye and sometimes an antenna reappeared. Chantran, in 1873, also noticed that if half of the eye-stalk of the crayfish were cut off a new eye appeared. If the entire eye were excised, it was not regenerated. It is interesting to note that in the hermit crab the new eye came in when the stalk was cut off near its outer end, and an antenna-like structure appeared in five out of ten cases in which the stalk was cut off near its base.

Antennae. — Nine individuals had begun to regenerate a new antenna from the old basal joint. Five of these had long segmented antennae, one-half or one-fourth as long as the fully formed antenna (Fig. 17). Four of the nine had smaller buds, showing generally evidences of segmentation (Fig. 16). Only one of the ten survivors had not regenerated at all.

Antennules. — One had completely regenerated; two, on the same individual, were two-thirds the full length; three had new short buds, and one of these individuals had also lost the other antennule, but had not regenerated it. A sixth individual had not regenerated. Four individuals died during the time of the experiment.

Maxillipeds. — The third and sometimes the second maxilliped were cut off from one side. In all eight survivors (of ten operated upon) the parts removed had begun to regenerate. In two cases the third maxilliped was nearly as long as the normal appendage. In five cases it was represented by two small outgrowths from the basal joint (Fig. 18). In one individual a very short bud was present. The second maxilliped had been cut off in five of the above cases. In four individuals it was represented by two new processes growing out of the basal joint. In one case the new maxilliped was half the normal size. A part of the first maxilliped had been cut off in one instance and was regenerating.

Abdominal Appendages. — Of twenty-four individuals from which the abdominal appendages had been cut off, fourteen were alive on September 15. Only two had regenerated the first abdominal appendage (Figs. 21, 23); while ten other individuals from which the same appendage had been cut off had not regenerated. Two had not had this appendage cut off.

Two individuals (the same that regenerated the first appendages) regenerated the second appendages (Figs. 22, 24). Both were males. Eleven others had not regenerated. The third abdominal appendage of a male (belonging, therefore, to the fifth abdominal segment) had regenerated (Fig. 25). This was also on one of the individuals that had regenerated the first two appendages. The same appendage had been removed from six other males and from two females, and had not regenerated. In three cases the third abdominal appendage (of the fourth segment) of three females had been removed and had not regenerated.

How far the regeneration of these appendages is dependent on an ecdysis I do not know. The more anterior appendages of the body may develop quite far before the animal changes its exoskeleton.

Summary. — The experiment shows that the abdominal appendages have the power of regeneration, although they do not regenerate as readily as do the more anterior appendages, or as do the last abdominal appendages. The positive evidence showing that the anterior abdominal appendage regenerated in several cases is, I think, of much greater weight than the negative evidence that they did not do so as frequently as the other appendages during the short time of the experiment.

In regard to the relative usefulness of the abdominal appendages little can be said. In the male they are small and weak, and one, the third, present in the female, is absent in the male. In the female the first three abdominal appendages carry the eggs. They are, therefore, essential for the existence of the race; yet there is no difference found between the power of regeneration of the three anterior egg-bearing appendages of the female and the two corresponding appendages of the male.

IV. Conclusions.

The results of the experiments show that the more anterior appendages regenerate quickly. A large percentage of the parts removed had begun to regenerate even during the short

time of the experiment. An examination of the frequency of loss of the appendages showed that from 9 to 11 per cent of the crabs living under natural conditions had lost one of the first three pairs of walking legs. The eyes, antennules, maxillipeds, and especially the two last pairs of thoracic legs do not seem to be often injured, at least, not in all the individuals that I have examined. Nevertheless, these parts regenerate as quickly and in as large proportion as the three walking legs. Moreover, the last abdominal appendages that are used to hold the abdomen in the spiral shell regenerate as readily as the more exposed anterior appendages. It is improbable that these strong, hard appendages on the end of the soft abdomen can be often injured inside the smooth shell; and when changing shells the crabs take great precaution to expose the abdomen as little as possible. In no cases of the hundred individuals examined, and in none of the other individuals that have passed through my hands, have I found these appendages injured or missing. It is, therefore, of some importance to find that these appendages regenerate quickly, and in as large proportion as the thoracic legs.

The abdominal appendages have disappeared on one side in both sexes, and those on the other side that remain have shifted their position high up on the abdomen. In the male the appendages (particularly one of the branches) are smaller than in the female, and the third appendage of the female has disappeared in the male. Since the eggs are carried by the anterior abdominal appendages of the female, these must be essential to the existence of the species; yet in the experiment these appendages did not regenerate oftener in the female than in the male.

The question of the degeneracy, and at the same time of the uselessness, of a part is fraught with difficulty, for we have, in the first place, very little evidence (and that little rests only on probability) as to whether a part has been reduced during the evolution of the species; and, in the second place, we cannot tell, even if a part be admitted to be reduced in size, of how much use such a part may still be to the animal. Therefore, although we find the last two pairs of thoracic legs and the

anterior abdominal appendages of the male still capable of regeneration, we do not know with certainty that the parts are degenerate. The question is further complicated by the amount of food material that is brought to the part of the body from which the appendage springs. If the amount is small, we can readily imagine that the regeneration may be retarded, or not even started, although the cells might be potentially capable of regenerating the missing part. It is not improbable that the smaller 'reduced' appendages would be most likely to suffer in this respect.

There is still another factor that must be taken into account, *viz.*, the place at which the appendage is cut off, for it is probable that, while an appendage may regenerate at one level, it may not be able to do so at another. Perhaps, for instance, had the abdominal appendages been cut off nearer to the body, a larger or smaller percentage would have regenerated.

In regard, however, to the problem of the frequency of injury of a part and its capability to regenerate, the preceding results, I think, speak with sufficient clearness. No such relation is found to exist. The advocates of such a view overlook a very vital part of the problem. If, for instance, it were found, as the result of a large number of observations, that those animals or parts of animals that were most subject to injury had most highly developed the power to regenerate lost parts, it would by no means follow, as Weismann and other Darwinians claim, that this result must have come about by what they call a process of natural selection. They overlook the possibility that unless these animals had from the beginning the power to regenerate they could not continue to live under the adverse circumstance. The animal would be then either entirely destroyed or else confined to other locations where the danger did not exist. Many persons confuse this statement with the theory of natural selection, but the two views are as wide as the poles apart.

We need not, perhaps, be greatly concerned with the argument that attempts to make plausible a connection between accidental injuries and the power of a part to regenerate, for there are known already a number of remarkable cases of

regeneration of internal organs, and these organs can rarely or never be injured. If, then, such organs have the power of complete regeneration, why need we seek for special explanations for those cases in which the organs happen to be more or less exposed to injury?

BRYN MAWR COLLEGE, BRYN MAWR, PENN.,
January 15, 1898.

OBSERVATIONS ON THE PARASITISM OF ANODONTA PLANA LEA BY A DISTOMID TREMATODE, AT CHAUTAUQUA, NEW YORK.

HENRY LESLIE OSBORN.

THE materials on which these observations are based were collected at Chautauqua Assembly on the south shore of Lake Chautauqua, New York, during the months of July and August of 1895, 1896, and 1897. The animals are very abundant in the semi-muddy bottoms near the shore, and can easily be seen and watched *in situ* and reached and collected with the hand from a boat. They are found in company with several species of Unio, and it is not always possible to tell from the boat whether one has found this genus or Unio. The studies were made partly in the Biological Laboratory of the Chautauqua College of Liberal Arts and partly at Hamline University, Saint Paul, Minn. Most of these shells of Anodonta exhibit on the inner surface a more or less extensive vermilion-yellow-ochre coloration, in the form, apparently, of a foreign material laid down at the expense of the nacre. This led me to study the case carefully, and brought to light the fact that the red-colored cases of Anodonta are infested by a distomid parasite which lives in the space between the mantle and the shell, and is apparently the agent chiefly concerned, directly or indirectly, in the production of the red coloration. A somewhat careful examination of the materials at hand has brought certain facts to light which seem of interest as new or little known.

The fact that shells of Anodonta are reddened has been known since 1839, when Lea[1] was misled into describing shells thus diseased from Ohio as a new species, *Anodonta salmonea*, in language which so closely agrees with the case of the Chautauqua shells as to leave no doubt of the identity of the two. But so far as I have been able to ascertain, the colora-

[1] Lea, Trans. Am. Phil. Soc. N.S., vol. vi, p. 45. Pl. XIV, Fig. 41. 1839.

tion has never before been traced to its cause, nor have the flukes in this situation been noticed and described, and hence a careful account of the matter is desirable. I am indebted to Mr. Chas. I. Simpson of the United States National Museum for the identification of the Anodonta, and for the information about Lea's work, and to Dr. W. S. Nickerson of the University of Minnesota for suggestions in connection with the identification of the fluke.

I. THE EXTENT OF THE COLORATION.

The amount of the deposited salmon-tinted material is very variable indeed. I will describe an extreme maximum case at the outset. The shell in this instance has an extreme length of 62 mm. The entire inner surface, excepting the pallial line and the muscular impressions, is salmon colored; this includes the area between the pallial line and the borders of the shell, and all of the hinge area. The only points at which the bluish native color of the shell can be seen are the muscular impressions and the pallial line. The shell is thicker than normal, and the red is shown to be an abnormally thick deposit by the fact that the muscular impressions and the pallial line, instead of being flush with the inner surface, are beneath the general level, the pallial line forming a very conspicuous furrow, as in many of the Unios with their much thicker shell. The red layer, moreover, is not smooth, but minutely warty-roughened, and in many instances increased by folds. Its luster is dull or in places distinctly pearly, especially toward the borders of the shell, *i. e.*, in the newest portions of the deposit.

An extreme minimum stands in marked contrast with this maximum case. Such a shell (described from one actual case) is 75 mm. long; its general inner surface is perfectly normally bluish tinted, with mother-of-pearl luster, thin and perfectly smooth. The only indication of the red coloration is a small, thin, very slightly granular area of red 5 mm. across, which lies directly beneath the umbo and is of the same extent and position in both valves. Between these extremes of little and much coloration I have found every intermediate amount.

Moreover, the color is not indiscriminately scattered as if merely accidentally, but it is very definite in its distribution in two respects; namely, in the single valve and in both valves.

First in one valve it appears to be a law that the location of the coloration varies with the amount of the red material. Thus (*a*) in case of a very small deposit this is found directly beneath the umbo of each valve and in no place else; (*b*) in case of a larger deposit the color is found in a patch which extends from the umbo downwards and forwards towards the ventral border of the shell; (*c*) in case of still more red there is a second narrower patch running down posteriorly from the umbonal area toward the ventral border of the shell; (*d*) in cases of still more coloration these areas are filled in between so that in some cases the red crosses the shell above, but not ventrally, and in others it extends to the pallial line, or even beyond this to the very margin of the shell (see Fig. 1).

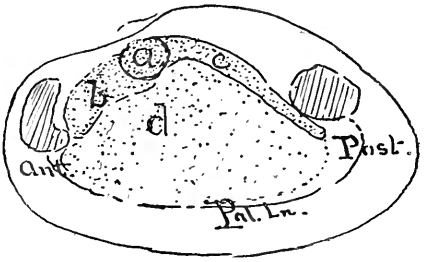


FIG. 1. — Inner surface of valve of Anodonta. The dotted lines indicate areas affected by the color-deposit.

It will be noted that these differences are not progressive stages observed on a single individual, but they are different cases that are observed. They may, however, indicate a progress in the growth of the coloration; if so, then we should have to say that the process begins at the umbo, then spreads in a narrow line downwards and outwards, first anteriorly, then posteriorly, and that later it spreads over the whole shell from these points. While I do not know of any actual evidence to show that such is the case, still the facts indicate some such order.

Not only is the coloration of the single valve thus definite, but it is an even more remarkable fact that the effect is uniform on the two valves; that is, the amount and position of the coloration is always bilaterally symmetrical. If one valve has a small spot at the umbo, then so has the other; there is

always an anterior strip in both valves if there is in one, and so on. In some cases the amount of the red color is so great that the shell lining is thrown into folds; in such a case we find folds on both sides, but they are not symmetrical, and in this minor point the symmetry is slightly lost.

It will be interesting to inquire whether the amount of the coloration is correlated with the age of the host. I suppose that we are justified in using the size of the shell as a criterion of the age of the animal, and if so, then we must conclude that the amount of the coloration is not in any way correlated with the age of the shell. It would be possible to find all sizes of shells with every stage of amount of color, — small ones in which there was little and much, and largest ones in which there was much. I have tabulated here a few measurements and amounts of coloration :

	Extreme Length of Valve.				
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
No red present	23	38	44	47	
Small umbonal patch	27	44			
Anterior patch no posterior	70	86			
Anterior and posterior patches . . .	28	61	64	72	80
Red general	49	50	67	73	

I am not able to state the percentage of Anodontas in which the red coloration is found; it is very prevalent, but not universal. Speaking very roughly, I should give it as my belief that at least 75 per cent of the animals at Chautauqua are affected, and I feel tempted to put the estimate even higher, for it is not at all frequently that one runs across a specimen in which the shell is absolutely normal.

It is an interesting and striking fact that the presence of the parasites is practically confined to the Anodontas. There are several species of Unio found in company with Anodonta, and these have all of them been carefully examined for the fluke. A single case of it has indeed been found,[1] — a specimen of *Unio edentula*, in which the shell exhibited the red coloration and flukes were found which seemed to be identical with those so commonly found in *Anodonta plana*. This case

[1] By Mr. R. H. Johnson.

is, however, the only one found after opening dozens, if not hundreds, of Unios.

II. Nature of the Coloration.

In order to determine, if possible, the exact relation of the color deposit to the shell, I made a vertical section of the shell, by sawing out a piece and then grinding it down to the necessary thinness on a stone. A view of this section is given in Fig. 2. The three usual layers of the shell are seen; *viz.*, the epidermis, on the outside; the prismatic layer beneath this, in which vertical prisms are distinctly visible; and the inner nacreous layer. The nacreous layer, however, is rather distinctly divided into two portions, a deeper, normal layer closely laminated and not colored, and an outer layer chiefly composed of the same material, but containing the red coloration in addition. The red is not a separate and distinct material mixed with an equally separate nacreous stuff, but the two are so intimately blended that it is impossible to show the red coloration in a black and white drawing. It thus appears to be rather a modification of the nacre, or at any rate a material dissolved in it, than any separate deposit.

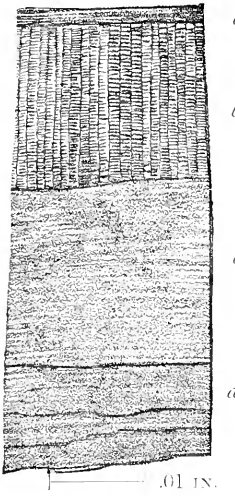


FIG. 2. — Cross-section of shell of Anodonta through a thick deposit of red material, showing its nacreous character. *a* = epidermis; *b* = prismatic layer; *c* = nacre; *d* = deposit due to the fluke.

Surface views of the red deposit were also studied. They were obtained by first decalcifying the shell in dilute hydrochloric acid, after which the lamination of the nacreous portion became very distinct and permitted me to tear off very thin pieces of considerable size. Such pieces under the microscope do not show any distinct colored material; they are structureless membranes which either are colorless or else they are salmon tinted. But in no case was I able to find any distinct and separate deposit. These facts are correlated with the

nacreous luster of the shell over the points of red coloration, and they indicate, not a separate layer of red deposited on the nacre, but the secretion of some soluble material which is poured out with the nacreous secretion and hardened with it.

III. Relation of the Parasites to the Shell.

The parasites concerned in the red coloration are found attached by their suckers to that surface of the mantle which is toward the shell. Their home is thus in a perfectly closed chamber out of direct contact with the water, which is the home of the host and remote from sources of food such as are usual to parasites of their kind. Though I have carefully searched for them in other portions of the Anodontas, I have never been able to discover any in any other part of the host. I have not been able to find any evidence to show that the parasites enter the host with the in-going water and then bore through the tissues to find their place of residence. I have examined the surface of the mantle carefully to find any modification which its surface might have undergone, but without finding anything that could be assigned to the flukes. I have also sectionized the mantle for indications of flukes, but have not found any traces of them there. I am, therefore, inclined to believe that they reach their home by inserting themselves at the margin between the shell and the mantle.

The parasites are found in the red-colored shells almost without exception, and are always found directly at the points of coloration. In some cases there are a very few flukes, and these are umbonal in position or there is a line of them in the anterior line of coloration. Usually in shells with much color there are a great many flukes, and generally the flukes are found to agree in number and position with the red. I have found some highly colored shells in which there were no flukes, and concluded that they had migrated. I have also examined shells in which there was no coloration without finding any flukes. So that it can be said that the flukes are constantly present in colored shells, and that they are located at the points of coloration. I have found a very few shells

with much color and no flukes; these cases have not been numerous. I have interpreted them to mean that the flukes have migrated in search of another host.

The constant occurrence of the flukes in correlation with the coloration seems to be sufficient evidence to justify the conclusion that they are the cause of the coloration, but when one attempts to go beyond this point and determine the exact relation that exists, one reaches a realm of speculation. It seems to me probable that the red color is a modification of the secretion of the mantle due to the irritation by the flukes. I have not been able to find out why the flukes collect as they do, and am inclined to suppose some attraction, perhaps of food, which draws them to the points where they are habitually found.

IV. Facts about the Parasites.

The parasites present some points that are of interest to the student of the trematodes. Many specimens were examined alive under compression, and many others were studied in total preparation of specimens that had been fixed in corrosive sublimate solution and carried up through the alcohols and cedar oil to Canada balsam. One specimen was sectioned serially and the series carefully studied; the following description rests on these three modes of study.

A view showing the points observed on the living specimen and on the total preparation is shown in Figs. 3 and 4. The total length of the body in the living fluke was variable, according to its state of contraction, and it presented every condition between a broad and short form and a very long and slender one. The length of the preserved specimens is .04 of an inch. A living specimen under slight compression from the weight of the cover glass measured .054 inches.

All the structural features of the parasite are indicated in the accompanying text-figures, so that only a brief mention of them is necessary. The outline of the body is broadly elliptical and there is no distinction into regions. The outer skin is everywhere, except the suckers, entirely simple, and I did not see any conspicuous cuticle; there are no spines of any kind visible in any portion of the skin.

Two very distinct suckers are present; the anterior one surrounding the mouth is considerably smaller than the ventral, and located at the extreme anterior end of the body. The ventral sucker is very large and prominent. It is on the level

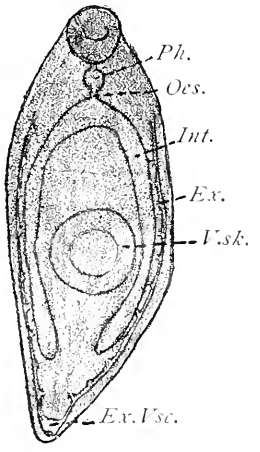


FIG. 3

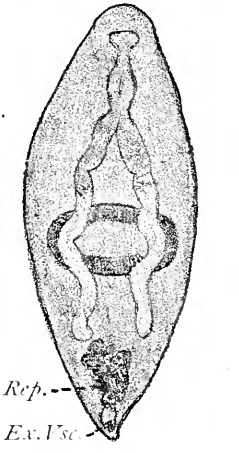


FIG. 4.

FIG. 3. — Drawing of the distomid, from life.

FIG. 4. — Drawing of the distomid, from a total preparation in balsam after corrosive sublimate and borax-carmine.

of the surrounding surface, is slightly wider than half the diameter of the body at its level, and is located very slightly behind the center of the ventral surface.

The body wall is as usual; its musculature can be seen in sections, greatly increased in amount at the suckers, and bounding the customary parenchymatous filling of the body cavity.

The alimentary organs are considerably differentiated. The pharynx closely follows the mouth; its wall is much thickened, and internally it is distinctly lined with nucleated columnar cells. The alimentary passage divides immediately behind the pharynx, without any intermediate oesophagus, into two intestinal tubes. These are entirely simple, and present an anterior portion lined with taller glandular cells shading posteriorly into a part, bounded by a flat-celled epithelium. So that in total mounts the anterior portion is dark and the hinder portion is clear and bladdery.

The excretory system consists of a terminal pore and a vesicle, into which in the living specimen two lateral passages are seen to lead. These lateral vessels (Fig. 3) in living specimens were traced forward where they were lost. They were ciliated so as to produce a current toward the terminal pore, and small side branches were seen which were lost in the parenchyma. Indications of a second similar and probably excretory vessel were seen at the hinder end of the body in compressed living specimens, but I was not able to see its connection (if it has one) with the more distinct lateral vessel already mentioned. In the preserved specimens (Fig. 4) the only portion of the excretory system that was visible is the terminal vesicle and the pore. The lateral vessels do not show; doubtless they are too delicate and similar to the parenchymatous cells to be distinguishable from them. I have not been able to recognize any nerves, and there are no noticeable sense organs on the surface of the body.

The reproductive system has not developed; there is, however, in the posterior end of the body in front of the vesicle of the excretory system a mass of undifferentiated cells. They are clearly seen in total preparations where they are deeply stained, and in contrast with the looser surrounding tissues. The cells of this mass are seen in sections as spherical objects with a large central nucleus, and the mass is clearly a mass of undifferentiated cells. Their future destination is not determined, but it seems very probable that it is the "Anlagen" of the reproductive organs. The gonads in some of the distomids are posterior in position, though the passages may run forward and open anteriorly.

V. Theoretical Points.

Since the reproductive organs are not as yet developed in these flukes, it is impossible to identify them by means of anatomical data; and since the case has not been reported heretofore, no light is thrown on the matter from the work of other observers. There seems, however, no doubt that the animal is of the family Distomidae, and it seems not unlikely

to belong to the genus Distomum. It seems to be a case of arrested development of the final form. The inactivity of the germinal tissue is indicated by the fact that no advance is noticed during the six weeks in which they have been observed, and by the fact that mitotic figures are not seen in the presumably germinal tissue noted above. The form of the body is clearly that of the final stage, and not a larval form. There is no evidence that I know of to show what the earlier stages are, but they are apparently passed in some other host; and the same is true of the final mature form, the Anodonta seemingly serving as a sort of halting place in which the flukes pass a certain period. It is also peculiar to find a distomid in this stage of its life history in an invertebrate host. The long list of hosts for distomids given in Bronn, '93, does not mention a single instance in which the mature form of the fluke is found in an invertebrate, though Aspidogaster is so found; but in that case there is only one host. It is also unique, as far as I have been able to learn, to find a distomid that is not located parasitically on some portion of the alimentary tube or on some hollow organ connected with it. Here, however, the fluke has no such relation. I have inferred from the restriction of the fluke to Anodonta, when there are so many allied animals at hand, that there is a close relation between the two, doubtless for the benefit of the fluke, and that when the case is better understood we shall find that the residence in Anodonta in this situation is an essential middle part of a life history, both ends of which are at present unknown or unrecognized in connection with this part.

I shall hope during the coming summer to make further observations upon these parasites and to plan for Anodontas being sent to me at regular intervals during a year, so that careful examinations can be made extending over a longer period. Also it is hoped to make examinations of the mussels at a number of different points on the lake. In the meantime it seems to be worth while to publish the results thus far obtained.

HAMLINE UNIVERSITY BIOLOGICAL LABORATORY,
January 28, 1898.

THE STRUCTURE AND ORIGIN OF THE EXCRETORY ORGANS OF LIMULUS.

WILLIAM PATTEN.

The coxal gland of Limulus has long been regarded as a ductless gland of uncertain significance, but we are now able to demonstrate that even in the adult crabs the organ is provided with a duct several millimeters in diameter and three or four inches long.

Its development also has been carefully studied, but the structure described as the developing gland proves to be the developing duct; the embryonic gland has been heretofore entirely overlooked.

In the adult the duct is so thin walled that it is not readily seen, and is very difficult to dissect. But it may be readily injected with celloidin or asphalt, so that the mass fills the duct and penetrates the lobes of the gland.

The coils of the duct may then be dissected out in the usual way, or they may be isolated by dissolving the surrounding tissues with caustic potash. When the duct is isolated by either of these methods it is seen to run straight forward along the dorso-lateral margin of the plastron, then back again, and, after many coilings, open into a large, irregular chamber, or end sac, situated in the middle of the fifth nephric lobe, *cal*. The duct arises as a tubular outgrowth of the ventral wall of this sac, which represents a remnant of the fifth coelomic cavity; the distal end of the duct finally unites with a short ectodermic ingrowth, readily distinguished in the adult, which opens at the base of the fifth leg, *ec.p*.

The secretions from the gland are collected by gradually widening anastomosing tubules. Each of the four lobes of the gland have many separate openings into the large tubules of the longitudinal stolon, *st*.; the large tubules empty into the coelomic space, or end sac, and from these a single nephric duct carries the secretions to the external opening at the base of the fifth leg.

The glandular portion of the kidney is developed from six pairs of segmentally arranged "anlagen." Omitting all details, it may be stated that a part of the fifth coelomic cavity persists as the thin-walled chamber or end sac mentioned above. The other coelomic cavities of the thorax break down after producing, by a thickening of their neural walls, paired masses of finely granular cells. These cells become hollow and unite end to end to form irregular groups of anastomosing tubules. The

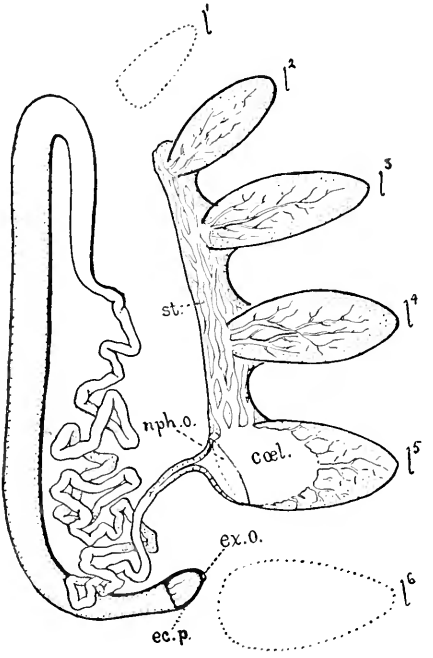


FIG. 1.— Diagram of the excretory organs of an immature Limulus.

tubules derived from the walls of the first and sixth coelomic cavities disappear. The remaining ones form the four lobes of the adult kidney. The longitudinal tubules of the stolon are formed in a similar manner, by the union of outgrowths from each cluster of cells. Many tubes on the periphery of the gland retain the embryonic condition in the adult. But in the center of the lobes and in the longitudinal stolon the nuclei of the tubules multiply rapidly, giving rise to a lining endothelium of flattened cells.

The kidney of Limulus is, therefore, derived from segmentally arranged groups of excretory cells. Each group of cells probably emptied originally into its corresponding coelomic cavity, and from there to the exterior. The separate external openings have now disappeared, and the organs are united by longitudinal tubules which finally open by a single duct, or coelomic funnel, to the exterior.

Many of the details of the above account were worked out in the biological laboratory at Dartmouth College by Miss Annah P. Hazen. They will be fully described and illustrated in a joint paper that we hope will appear at an early date in the *Journal of Morphology*.

HANOVER, N. H.,
January 17, 1898.