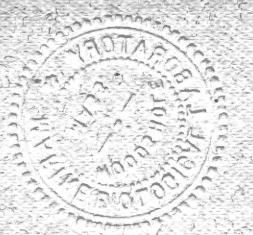

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FOUNDED BY C. O. WHITMAN

EDITED BY

J. S. KINGSLEY
University of Illinois
Urbana, Ill.

WITH THE COLLABORATION OF

GARY N. CALKINS
Columbia University

EDWIN G. CONKLIN
Princeton University

C. E. MCCLUNG
University of Pennsylvania

W. M. WHEELER
Bussey Institution, Harvard University

WILLIAM PATTEN
Dartmouth College

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CRITIQUE OF RECENT WORK ON THE MORPHOLOGY OF THE VERTEBRATE SKULL, ESPECIALLY IN RELATION TO THE ORIGIN OF MAMMALS

WILLIAM K. GREGORY

From the American Museum of Natural History, New York

TWENTY-FIVE FIGURES

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INTRODUCTION

To observers who have followed the trend of zoological research during recent years it is apparent that zoologists have in great numbers turned away from vertebrate comparative anatomy as a thankless task and have come to regard its labyrinths as leading nowhere. The rise of statistical and experimental research has accompanied a reaction against such speculative conclusions as those of Gegenbaur and Dohrn and it has been said that neither comparative anatomy nor paleontology have told us by what steps organs have been evolved but only how they may have been evolved. It has even been hinted that our theories of phylogeny and morphogeny are too much the product of the unchecked imagination, which seizes gladly upon favorable evidence but fails to seek the unfavorable.

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MARCH, 1913

In this country the falling off in the total output of comparative anatomical research has been especially noticeable, and only a few Americans have continued along the old paths. In the morphology of the vertebrate skull, the special topic of this review, it is only here and there, as at Cornell, Tufts and a few other centers, that organized and continuous research has resulted in such fine contributions as Kingsbury and Reid's on the columella auris in Amphibia ('09) or Thyng's on the squamosal ('06).

In Germany, on the contrary, while statistical and experimental investigations have likewise received an extraordinary impetus, the older fields are not left without enthusiastic workers. There the problems of the vertebrate skull still have a human interest; men still take sides over questions of homology and even get to the point of abusing each other in print.

In certain problems of the vertebrate skull which were opened by Cuvier, Owen, Reichert, Kitchen Parker and other pioneers, the eminent morphologist of Freiburg whose recent studies it is my purpose to review has been a prolific investigator. Thanks in no small degree to Gaupp, this subject is no longer in a state of fixity and stagnation, but at least in Germany, has again become mobile.

The nomenclature of the skull, alas, is also passing through a period of unstable equilibrium. The student soon learns that many of the familiar names for the bony elements of the skull, names which have become almost sancrosanct through the prestige of Owen and Huxley, are now being abandoned by certain authors, transferred to other elements, or sometimes even transposed. Squamosal, prosquamosal and supratemporal; prefrontal, lacrimal, adlacrimal and postnasal; prevomer, vomer and parasphenoid; orbitosphenoid, alisphenoid and epipterygoid; these are examples of names affixed to certain Protean elements, the transformations of which in the different Classes have given rise to synonymy and confusion.

PREFRONTAL, LACRIMAL, ADLACRIMAL

The prefrontal of the lizard and other recent reptiles has been shown by Gaupp ('10) to have the same or nearly the same relations with the chondrocranium and with the naso-lacrimal duct as has the lacrimal of mammals. The so-called lacrimal of

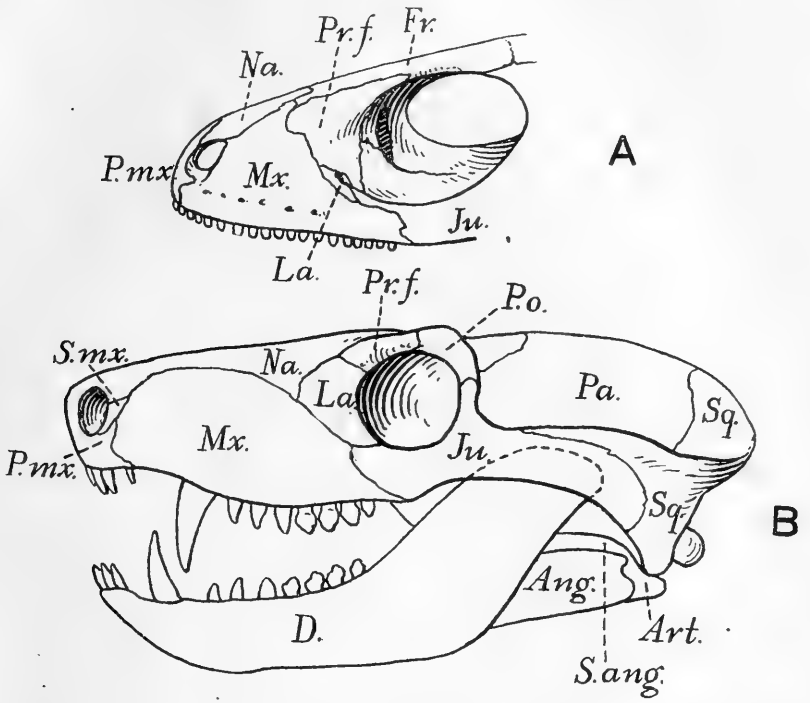


Fig. 1 *A*, The lacrimal region of Lacerta viridis. After Gaupp, 1910, p. 532.

The lacrimal ('adlacrimal') is vestigial, the prefrontal has the appearance and position of the mammalian lacrimal.

B, Skull of the primitive Cynodont Nythosaurus larvatus. After Broom, (1911, text,—fig. 170, p. 899).

The lacrimal is well developed and has the normal relations of the mammalian lacrimal, the prefrontal is separated by the lacrimal from the jugal; it is in contact with the nasal and lacrimal like the anterosuperior portion of the 'frontal' of mammals.

reptiles (which only in the Crocodilia is pierced by the duct) is widely removed from the chondrocranium and in the lizards is an inconstant element. On these and similar grounds Gaupp concludes, with Kober and Jaekel, that the prefrontal of the

reptiles is the homologue of the lacrimal of the mammals and that the reptilian prefrontal should therefore in future be called 'lacrimale'[1] since the mammalian and especially the human skull is taken for the basis of nomenclature. On the other hand, thinks Gaupp, the so-called lacrimal of reptilians and stegocephalians has disappeared in the mammals and should be called either 'postnasal' (Jaekel) or 'adlacrimal' (Gaupp).

There is, however, one objection to this conclusion: it is founded on a comparison between mammals and recent reptiles and leaves the extinct Theriodontia altogether out of account. The lacrimal (adlacrimal of Gaupp) of Cynognathus, Trirachodon, etc., in Broom's recent figures ('11) is similar to the mammalian lacrimal in appearance and position, while the prefrontal borders the orbit superiorly and has neither the appearance nor the position of the mammalian lacrimal.

In no Cynodont is the lacrimal vestigial, in none is the prefrontal in contact with the jugal as it should be if it were about to transform into the mammalian lacrimal. Nor is there any suggestion that the reptilian prefrontal has fused with the 'lacrimal' to form the true lacrimal of mammals; on the contrary the prefrontal may have fused with the frontal to form the superior border of the orbit.

Since the Theriodonts appear to be in every respect closer to the mammals than the lizards are,[2] it seems probable that the resemblances between the prefrontal of the lizard and the lacrimal of mammals have resulted from convergent evolution and that it is incorrect to homologize the reptilian prefrontal with the mammalian lacrimal or to transfer the name lacrimal to the prefrontal.

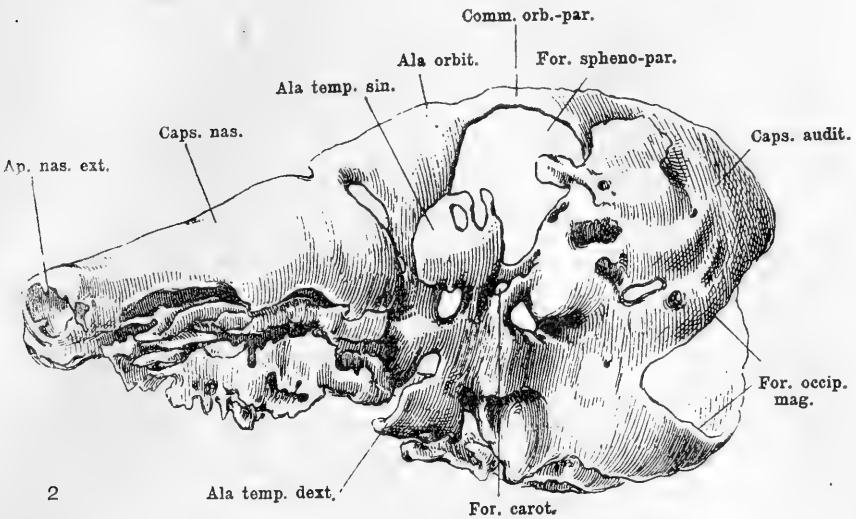
ORBITOSPHENOID, ALISPHENOID AND EPIPTERYGOID

Another pair of elements of the reptilian skull whose customary name and supposed homology have lately been called in question are the 'alisphenoids.'

Gaupp has shown ('02, '11) that, in the cartilaginous cranium of mammals, the alae temporalis (fig. 2), which are replaced by

[1] 'Lacrimale' is etymologically correct (Lat. lacrima, a tear).

[2] See below.



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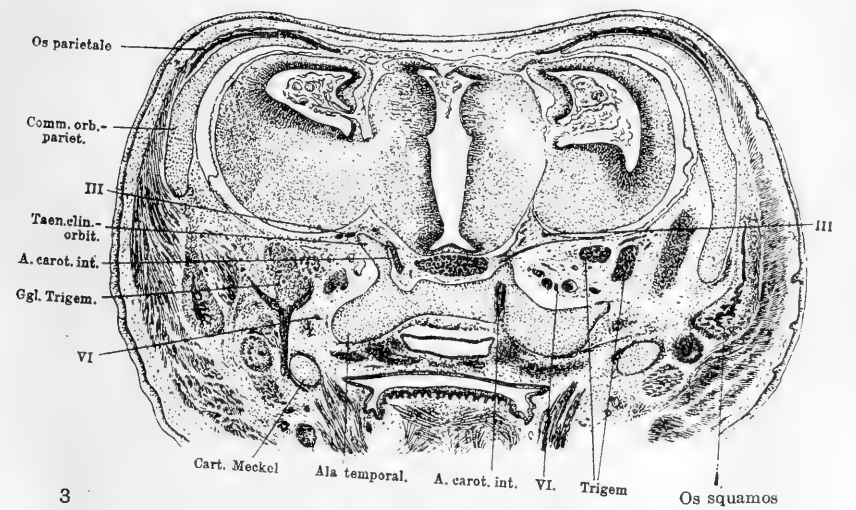

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Fig. 2 Model of the chondrocranium of Talpa europaea, from Gaupp (Merkel u. Bonnet's Anat. Hefte, lxi, 1902, fig. 10, p. 193) after E. Fischer. Seen obliquely from below.

The ala temporalis (cartilage fundament of the alisphenoid) lies outside of the primitive brain case. It is connected below with the basisphenoid. The ala orbitalis (cartilage fundament of the orbitosphenoid) is a part of the primitive brain case; it is posterior to the ethmoid and lateral to the presphenoid.

Fig. 3 Cross section through the sella turcica of a larval Echidna aculeata. From Gaupp (Merkel u. Bonnet's Anat. Hefte., lxi, 1902, fig. 14, p. 201).

The cartilage fundaments (alæ temporalis) of the alisphenoids form no part of the primordial brain case but lie below and outside of the Gasserian ganglia (*Ggl.trigem.*); they are lateral to the carotid canal (*A.carot.int.*) and to the basisphenoid.

the alisphenoid bones, arise as rods or tracts of cartilage lying outside of and below the Gasserian ganglia and separated by them from the true brain cavity (fig. 3). In the embryonic skull these cartilaginous alae temporalis (fig. 3) spring from either side of the basisphenoid, very much as do the basipterygoid processes in the lizard skull (fig. 4); they also hold similar topographic relations with the carotid arteries. For these and similar reasons Gaupp does not hesitate to homologize the alae tem-

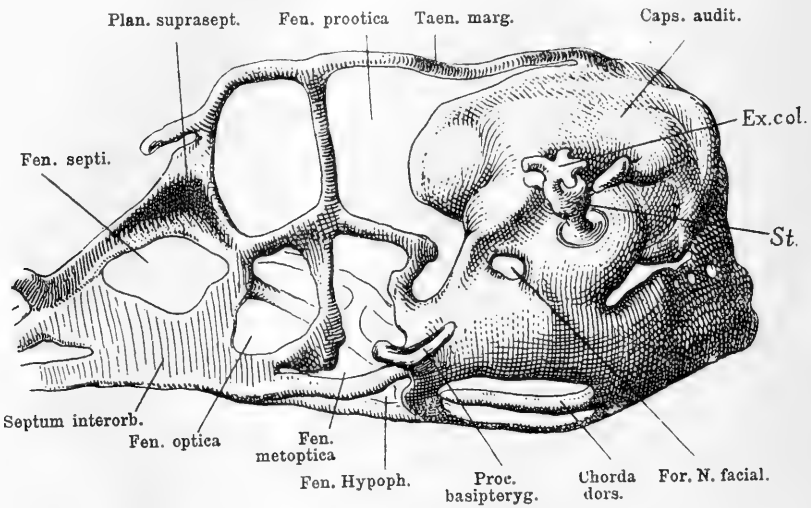


Fig. 4 Chondrocranium of Lacerta agilis. From Gaupp (Merkel u. Bonnet's Anat. Hefte, lxi, 1902, fig. 5, p. 171).

The basipterygoid process, springing from the basisphenoid, lies outside of the primordial brain case and below the Gasserian ganglion, like the ala temporalis of mammals.

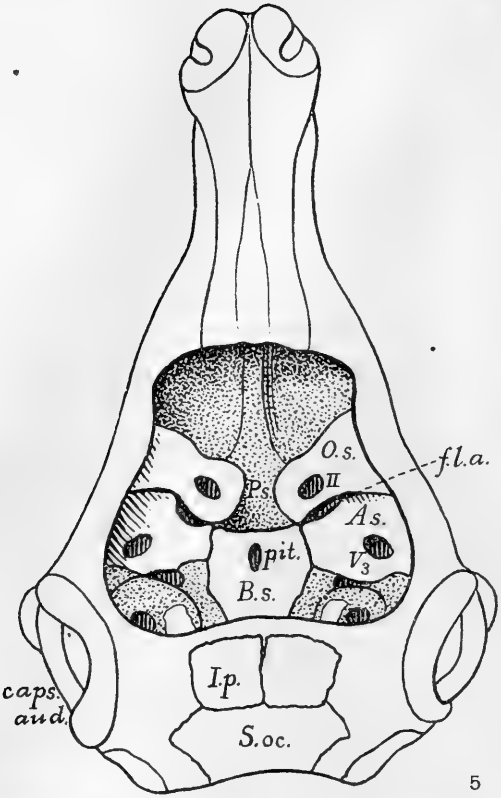
poralis of mammals with the basipterygoid processes of reptiles. These processes he supposes to have become turned upward, so that they embraced the Gasserian ganglia externally; by further upgrowth of the replacing bone they covered the temporal region of the skull. The conclusion is drawn that the mammalian alisphenoids are not represented as such in the reptilian skull, that the pair of bones in the Crocodilia usually called alisphenoids represent some other elements. Accepting these views, Dr. von Huene ('11) applies them in the field of paleontology: "Die

Bezeichnung Alisphenoid dürfte künftig bei keinem Sauropsiden mehr benützt werden. Der bisher so bezeichnete Knochen ist ident mit den Orbitosphenoid."

Last year the present writer had the privilege of studying the skulls of Tyrannosaurus and other Dinosaurs in the American Museum of Natural History in collaboration with Professor Osborn ('12) and Doctor von Huene, and was inclined to accept the view of the latter that the principal elements in the walls of the reptilian skulls are not alisphenoids but orbitosphenoids. Further study, however, has led to the following considerations: In mammals[3] the alisphenoids are lateral to the basisphenoid and pituitary fossa, they connect posteriorly with the proötics and superiorly with the parietals, they are pierced posteriorly by nerve V3, and they lie outside of the Gasserian ganglia and behind the sphenorbital fissure (for nerves III, IV, VI); on the lower surface of the skull they are postero-external to the pterygoids and laterally embrace the basisphenoid; they are also chiefly external to the foramina for the internal carotids.

In Cynodonts (fig. 6) there are a pair of elements showing strong resemblances with the mammalian alisphenoids and so named by Broom. In the internal view of the Cynodont skull as figured by Broom ('11), it is seen that these alisphenoids are anterior to the proötics, lateral to the basisphenoid and pituitary fossa, inferior to the parietals. They also lie in front of the foramen proöticum (for nerves V2, V3); to judge from the relations of the small process running from the proötic upward, inward and forward, it seems probable that the supposed alisphenoids also lay outside the Gasserian ganglia; their anterior border looks much like the posterior boundary of the sphenoidal fissure (foramen lacerum anterius), hence they were probably posterior to the exit of nerves II, III, V1, VI, like the alisphenoids of mammals. On the lower surface of the skull the bones called alisphenoid were postero-external to the pterygoids and embraced the basisphenoids laterally just in front of two openings which Broom identifies as "probably for the carotids." Thus the evi-

[3] See the figures of the skull in embryo marsupials, edentates, insectivores, etc., as figured by Broom, Parker and others (especially Parker, 1885).



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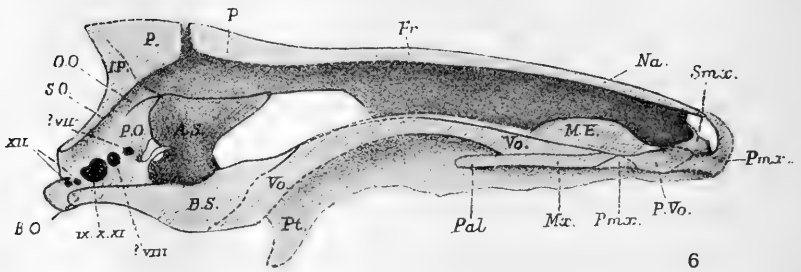

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Fig. 5 Brain case of young Erinaceus europaeus. After Parker (1885, p. 19, fig. 4) Seen from above.

The orbitosphenoid is posterior to the ethmoid, lateral to the presphenoid, anterior to the sphenorbital fissure, (*f.l.a.*), lateral to the basisphenoid and pituitary fossa, anterior to the prootic and chiefly anterior to the foramen ovale (V_3).

Fig. 6 Median section of skull of the Cynodont Diademodon. From Broom (P.Z.S., Dec., 1911, pl. xlvi, fig. 9)

The alisphenoid (*A.S.*) has the same topographic relations as the alisphenoid of mammals. It also appears to be homologous with the alisphenoid of Crocodiles and Dinosaurs. The opening in front of the prootic is believed to be the foramen prooticum (for V_2, V_3). The small spicule of bone that divides this opening is thought by Broom to have been medial to the Gasserian ganglion.

dence for homology with the mammalian alisphenoids is very strong.

Likewise in crocodiles (fig. 7) and Dinosaurs (figs. 8–10) the bones usually called alisphenoids, but called by von Huene 'orbi-

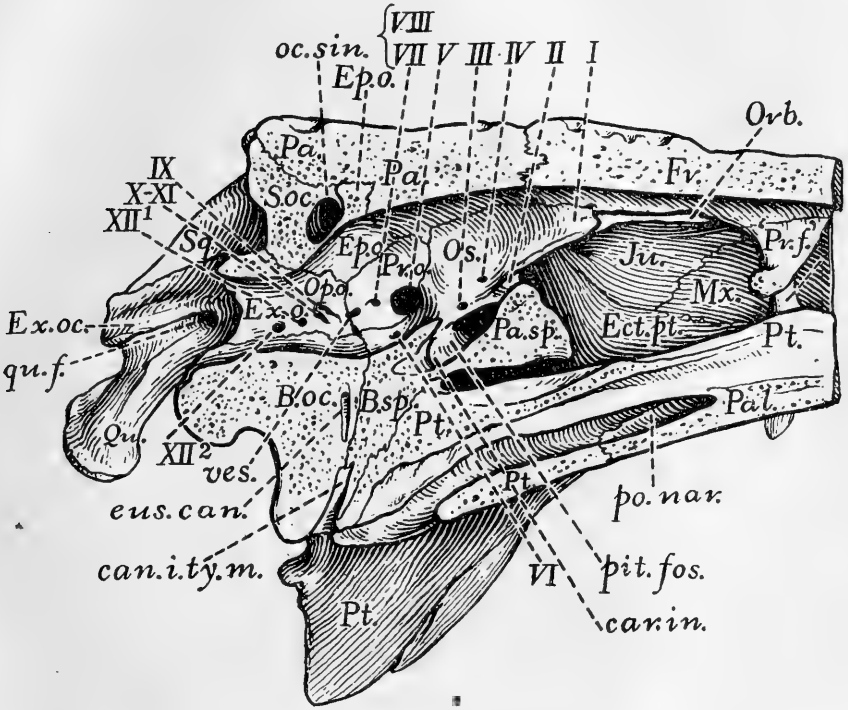


Fig. 7 Median section of the Crocodile skull.
Foramina identified with the kind assistance of Dr. F. von Huene.

The alisphenoid (marked *O.s.*) aggrees with the mammalian and Cynodont alisphenoid in being anterior to the prootic and foramen prooticum (V), lateral to the basisphenoid and pituitary fossa, inferior to the parietal, and chiefly posterior to the foramina for nerves II, III, IV.

Pa. sp., presphenoid (preformed in cartilage).

tosphenoids' are anterior to the proötics, lateral to the basisphenoid and pituitary fossa, inferior to the parietals and notched or pierced posteriorly by the foramen for nerve V3, they are also chiefly posterior to the exits of nerves II, III, and IV. In the inferior view of the skull the alisphenoids embrace the basi-

sphenoids, are external to the carotid canals and posterior to the pterygoids.

These comparisons, summarized in the following table, offer strong evidence for the view that the bones usually called alisphenoids in the Dinosaurs and crocodiles are rightly so named.

If, however, the large temporal wing-bones of the braincase in crocodiles and Dinosaurs are alisphenoids where are the true orbitosphenoids? In the crocodile Parker ('83) sought the orbitosphenoids in the persistently cartilaginous lateral wings of the chondrocranium, lying above the presphenoid, behind the ethmoids, below the frontals and in front of the foramina for the optic nerves. These topographic relations are exactly similar to those of the orbitosphenoids of mammals, the chief difference being that in mammals, the orbitosphenoids are osseous. In the Cynodonts the region in front of the alisphenoids remained unossified (Broom).

TABLE 1

Topographic resemblances between the 'alisphenoids' of crocodiles, dinosaurs, cynodonts and mammals

	ALISPHENOIDS OF MAMMALS	ALISPHENOIDS OF CYNODONTS	ALISPHENOIDS OF CROCODILES	ALISPHENOIDS OF DINOSAURS
Lateral to basisphenoids and pituitary fossa[1]	X	X	X	X
Anterior to proötics	X	X	X	X
Inferior chiefly to parietals	X	X	X	X
Posterior to presphenoids and orbitosphenoids	X	x	x	x
Anterior to foramen proöticum (for V^3)	X	X	X	X
External to Gasserian ganglia, or to separate trigeminal roots	X	x	—	?
Chiefly posterior to exits for nerves, II, III, IV, VI	X	x	X	X
Postero-dorsal to pterygoids	X	X	X	X
Inferior wings laterally embracing basisphenoid	X	X	X	X
Inferior wings external to canals for carotids	X	X	X	X

[1] X denotes definitely known agreement; x denotes probable agreement.
— denotes disagreement.

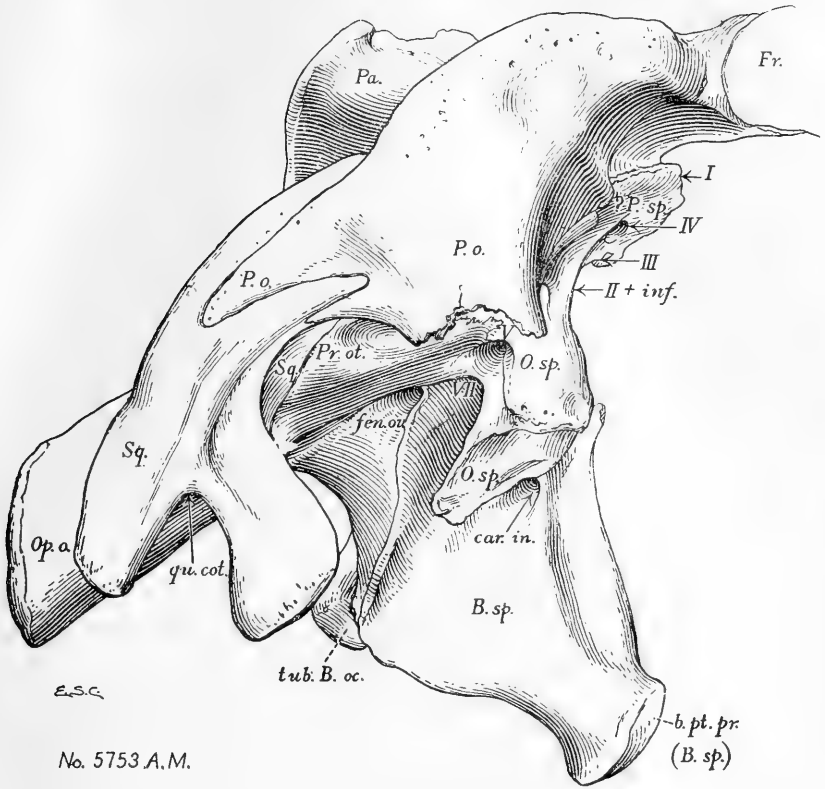


Fig. 8 Brain case of a theropod dinosaur Allosaurus agilis. (Osborn, Mem. Am. Mus. Nat. Hist., n.s., vol. 1, pt.i., text fig. 10, p. 17.) Right side seen obliquely from above. Determination of foramina by the present writer with the kind assistance of Dr. F. von Huene.

The alisphenoid (marked *O.sp.*) is seen to be anterior to the prootic and prootic foramen (V), external to the carotid canal (*car. in.*) and chiefly posterior to the openings for nerves II, III, IV. The lateral wing marked *?P.sp.* may represent the orbitosphenoid.

In the Dinosaurs (figs. 8–10) the orbitosphenoids are probably represented by the lateral wings of the elements called by Dr. von Huene 'presphenoids.' These lateral wings are behind the ethmoids, below the frontals and lateral to the median presphenoid, they are also in front of the openings for nerves II, III, IV. The chief differences then between the true orbitosphenoid wings in Dinosaurs and the orbitosphenoids of mammals is that the latter ossify separately from the median presphenoid whereas

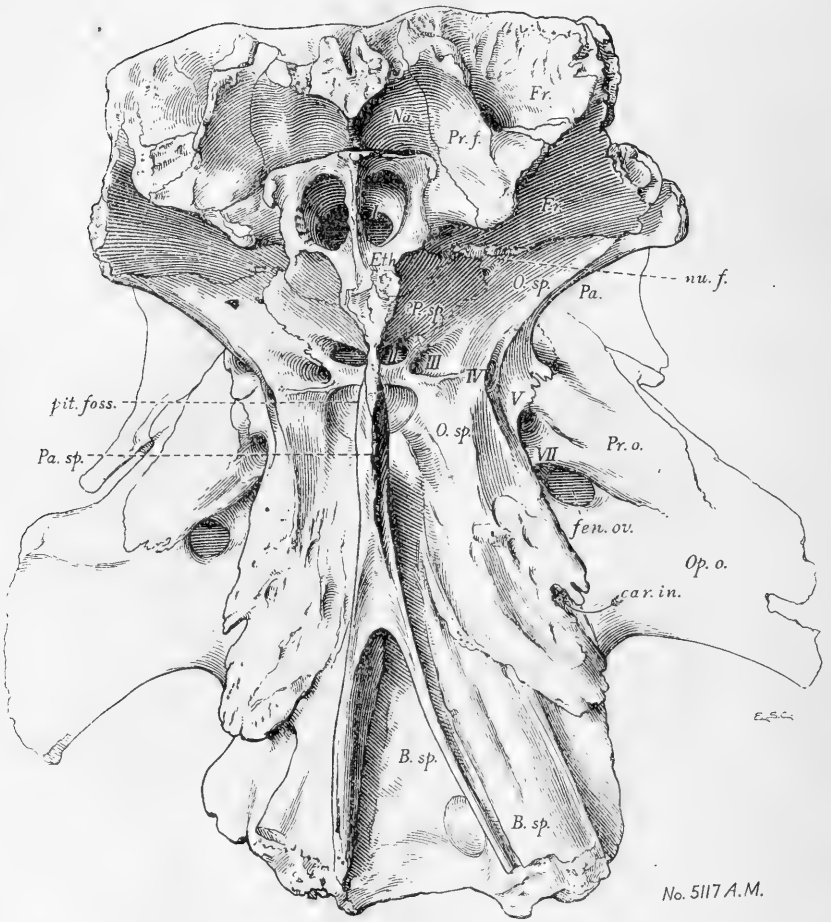


Fig. 9 Brain case of a theropod dinosaur, Tyrannosaurus rex (Osborn, Mem. Am. Mus. Nat. Hist., n.s., vol. i, pt.i., text fig. 8, p. 15). Full front view. Determination of foramina by Mr. Barnum Brown and by the present writer with the kind assistance of Dr. F. von Huene.

The alisphenoid (marked *O.sp.*) lies behind the foramen for nerves II, III, in front of the prootic foramen (V) and carotid canal. The lateral wing marked *?P.sp.* may represent the true orbitosphenoid, which is continuous below with the presphenoid.

in crocodiles and Dinosaurs they are continuous with the presphenoid.

There can be little doubt then, that the mammalian alisphenoids have been derived from 'alisphenoids' of the type repre-

sented in the Cynodonts and that these in turn are homologous with the alisphenoids of crocodiles and Dinosaurs. Whence do these alisphenoids of reptiles and mammals arise? Were they derived through the transformation of basipterygoid processes such as are represented in the lizard (Gaupp)? Or were they derived through the transformation of the elements called epipterygoids in Sphenodon (Broom '09)?

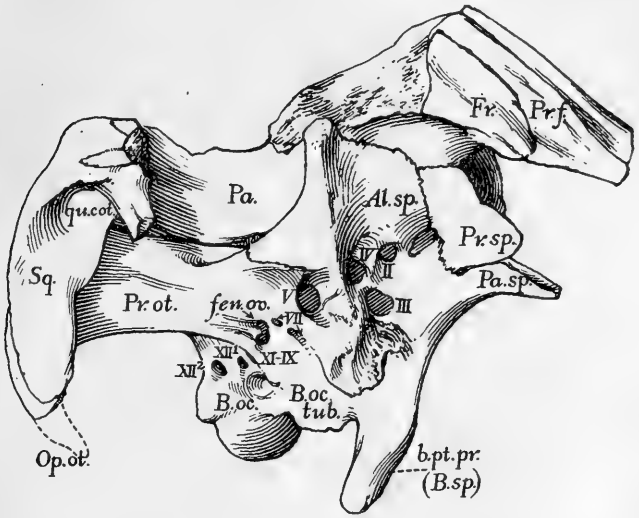


Fig. 10 Brain case of a predentate dinosaur Saurolophus osborni (Barnum Brown, Bull. Am. Mus. Nat. Hist., vol. 31, 1912, p. 134, text-fig. 3).

The alisphenoid (*Al. sp.*) articulates with the prootic; it lies in front of the prootic foramen (V) and chiefly behind the foramen for nerves II, III, IV. *Pr.sp.*, orbito-sphenoid.

In the palatal aspect (fig. 11) of the skull of Gomphognathus (Broom '11, pp. 921–922) the pair of alisphenoids are seen to form a part of the pterygoquadrate series, in so far as they lie between the pterygoids and the quadrates. Likewise in embryo mammals the cartilaginous alae temporalis are interpreted by Broom ('09) as remnants of the cartilaginous pterygoquadrate bar of reptiles.

Broom's 'alisphenoid—epipterygoid' hypothesis is greatly strengthened by the evidence offered both in the chondrocranium

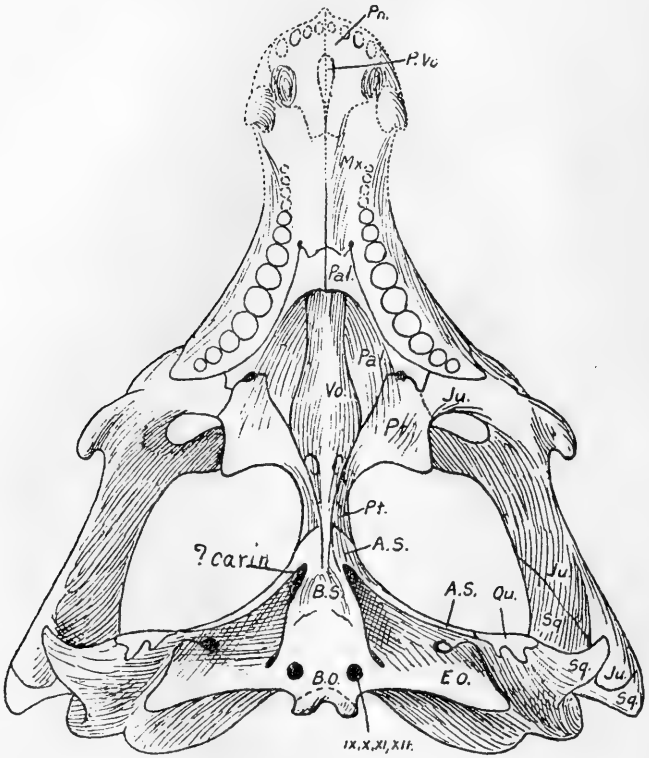
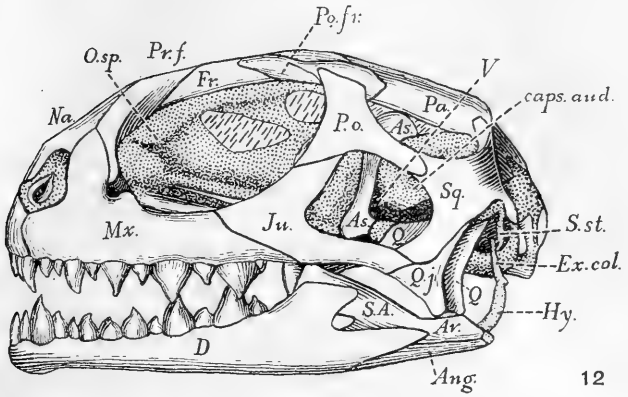


Fig. 11 Under view of skull of Gomphognathus minor. (Broom P.Z.S., 1911. p. 911, text-fig. 177)

The inferior branch of the alisphenoid covers the region of the basipterygoid processes of the basisphenoid. It is external to the supposed carotid canal and is continuous with the pterygoid [Watson].

and in the adult skull of Sphenodon (fig. 12). There the bones usually called epipterygoids have close topographic similarities to the alisphenoids of mammals, Cynodonts, crocodiles and Dinosaurs: viz., they are lateral to the basisphenoid and pituitary fossa, anterior to the proötics, inferior to the parietals, anterior to the proötic foramen (for the trigeminus), and fill the gap between the true orbitosphenoid and the proötics. They differ from the alisphenoid of crocodiles and Dinosaurs in retaining their ancient connection below with the pterygoids (as do also the 'epipterygoids' of the Permian Pelycosaurs, as well as the alisphenoids of Cynodonts). Their cartilage fundaments (fig. 13)



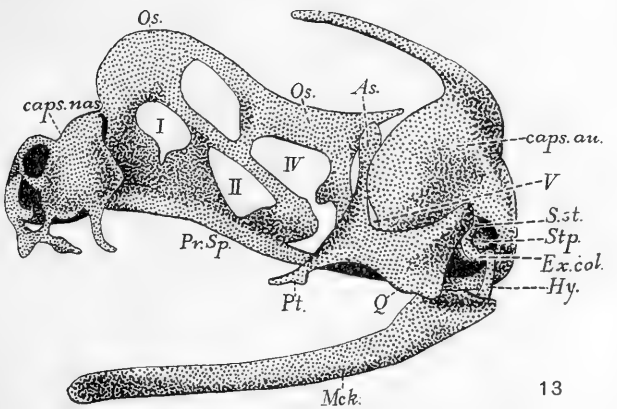


Fig. 12 Developing skull of Sphenodon punctatus. (Howes and Swinnerton, Trans. Zool. Soc., vol. 16. p. iv., fig. 4. Lettering modified.)

The alisphenoid (epipterygoid) has essentially the same topographic relations to the prootic (*caps. aud.*), prootic foramen (V) and parietal (*Pa.*) as the alisphenoid of Crocodiles, Dinosaurs, Cynodonts and mammals.

Fig. 13 Developing chondrocranium of Sphenodon punctatus. (Howes and Swinnerton, Trans. Zool. Soc., vol. 16, pl. III, fig. 8. Lettering modified.)

From the pterygoquadrate cartilage springs a dorsal branch, the fundament of the epipterygoid, or alisphenoid. Its topographic relations with the auditory capsule and with the true orbitosphenoid are the same as those of the alisphenoid of Crocodiles and Dinosaurs.

(Howes and Swinnerton '01, pl. 3, fig. 4) on either side of the skull are vertical rods, which are dorsal processes of the pterygoquadrate cartilage; these vertical rods lie quite outside and below the trigeminal roots. The bases of these epipterygoid rods fuse

(fig. 14) with the basipterygoid processes of the basisphenoid. If, in the Cynodonts, the cartilage fundaments (alae temporalis) of the alisphenoids (epipterygoids) had fused below with the basipterygoid processes, then, like the basipterygoid processes of lizards and like the alae temporalis of mammals, they would have been below the trigeminal roots and external to the openings for the carotids.

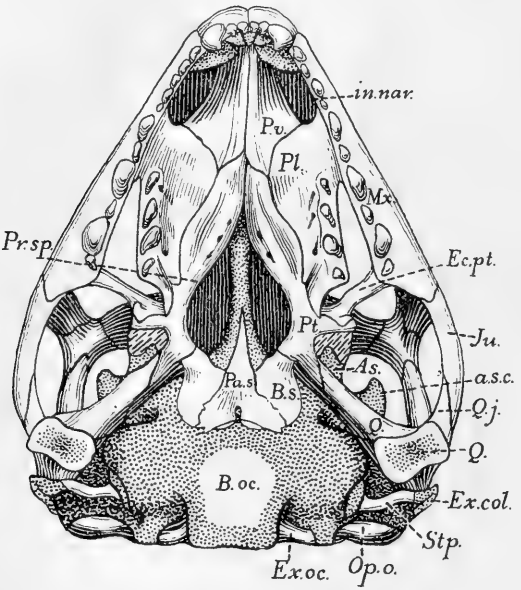


Fig. 14 Under surface of developing skull of Sphenodon punctatus. (Howes and Swinnerton, Trans. Zool. Soc. vol. 16, pl. iv, fig. 16. Lettering modified.)

The cartilage fundament of the alisphenoid (*A.s.*) is continuous with the basipterygoid process, below the postero-external branch of the pterygoid. This fact is shown in several of the stages figured by Howes and Swinnerton as well as in two wax models in the American Museum of Natural History which were made by Dr. Dahlgren from serial sections of Sphenodon embryos.

In brief it is not difficult to conceive that all the parallel relations noted by Gaupp between the alae temporalis of mammals and the basipterygoid processes of lizards are due, first to the derivation of mammals from Cynodont-like reptiles retaining certain primitive characters in common with the lizards, and secondly to the fusion of the cartilage fundaments of the pterygo-quadrate bars to the basipterygoid processes of the basisphenoid.

REPTILIAN LOWER JAW

The nomenclatural history of certain bones of the reptilian lower jaw (fig. 15) has been very intricate and confusing. It is desirable—though hardly to be expected—that stability be gained by a speedy adoption of the following names, which have been selected with great learning and discretion by Gaupp ('11, pp. 124–128) and which in large part have long been used in Germany.

Articulare Cuvier: preformed in cartilage as the articular region of Meckel's cartilage. Remaining unossified in recent Amphibia.

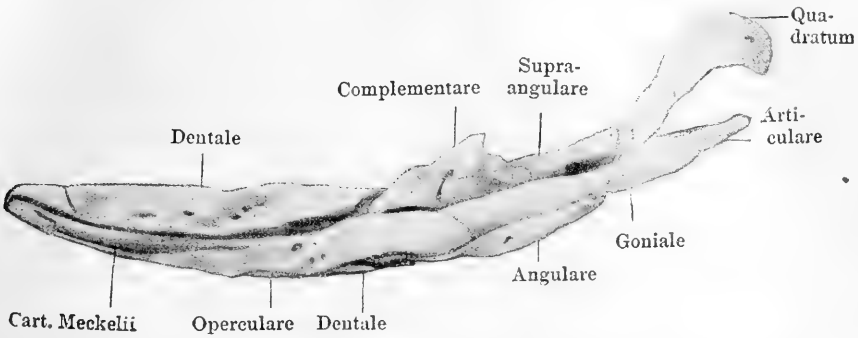


Fig. 15 Model of the lower jaw and quadrate of an embryo Lacerta agilis, medial aspect. (Gaupp, Anat. Anz. Bd. 39, 1911, p. 105, fig. 7.)

Goniale Gaupp (dermarticular Kingsley, postopercular Gaupp, prearticular Williston): the dermal medial extension of the articular, bordering below the entrance to the 'primordial canal' for Meckel's cartilage; occupying the medial posterior part of the jaw. Present in lizards, snakes, turtles; very large in Amphibia, where it is usually called the angular.

Angulare Cuvier: on the lower border of the jaw, lying between the dentary and the articular, articulating anteriorly with the splenial (operculare). Absent or reduced in recent Amphibia.

Supraangulare Cuvier: (supraangular) on the upper posterior border of the jaw, chiefly on the outer side, above the angulare and goniale. Absent in recent Amphibia.

Dentale Cuvier (dentary Owen): the main antero-external bone of the jaw, bearing the principal row of teeth. In recent Amphibia often extending backward to the posterior end of the jaw.

Operculare Cuvier (splenial Owen): on the inner side of the jaw, opposite the dentary, articulating posteriorly with complementare (coronoid) goniale and angulare. Absent in most Cryptodira and certain other Chelonia: tooth-bearing in certain recent Amphibia. Wrongly named presplenial by Baur in lizards and crocodiles.

Complementare Cuvier (coronoid Owen): lying chiefly on the inner side, between the supraangular and operculare (splenial). Not present in recent Amphibia.

These elements in recent reptiles and Amphibia are very clearly illustrated in the fine series of figures published by Gaupp[4] representing embryonic and adult conditions.

The critical element for the understanding of the lower jaw of recent reptiles and amphibians is the goniale. This element was recognized by Baur ('95) in the lizard as a 'dermogenous' process of the articular, but he greatly confused the subject by calling the same element in the turtles the 'angular,' by applying to the true or Cuvierian angular the name 'splenial' and by renaming the true splenial 'presplenial.' This strange blunder was set right at last by Williston ('03), Kingsley ('05) and Gaupp. The recognition of the goniale as an element distinct both from the articular and the angular is of great importance, not only in clearing up the morphological relations of these elements in amphibians and reptiles, but also in bringing additional evidence for 'Reichert's theory' of the origin of the mammalian auditory ossicles (see p. 23).

MAMMALIAN LOWER JAW

From the side of 'neontology' (embryology plus comparative anatomy) the manifold evidence bearing on the origin and morphological relationships of the mammalian lower jaw has been ably arranged by Gaupp (1911, III) in favor of the following conclusions:

1. The mammalian mandible is the homologue solely of the reptilian dentary bone. The ascending ramus including the coro-

[4] 1911, pp. 104–117, figs. 5–16; pp. 437–455, figs. 1–23.

noid process and mandibular condyle is in all probability homologous with the 'ascending process' of the reptilian dentary. This ascending ramus was originally a process for the insertion of muscles. It became differentiated into two parts, an anterodorsal branch (coronoid) for the temporal muscle and a posteroinferior branch (condylar process) for the external pterygoid muscle.

2. The joint between the lower jaw and the skull in mammals is solely a squamoso-dentary joint which arose in front of the old quadrato-articular joint and in which neither of the old elements (quadrate, articular) participate. In contrast with most

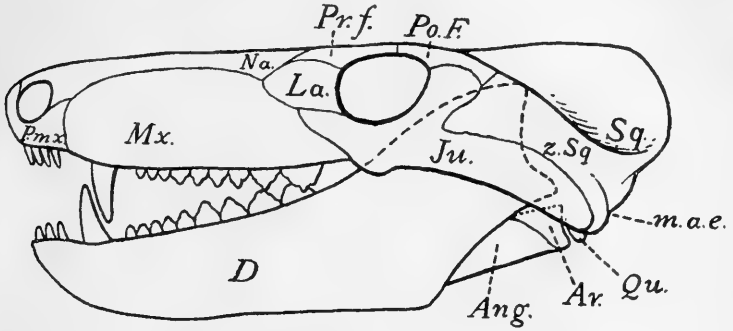


Fig. 16 Scheme showing approximate relations of lower jaw to skull in Cynognathus.

m.a.e. auditory groove.

other joints, the squamoso-dentary joint is thus a secondary attachment of two elements which formerly had no connection. This is also indicated in the ontogeny (see paragraph 6 below).

3. The quadrate and articular elements of the old reptilian jaw articulation no longer function as such in the mammalian skull but have become transformed into accessory auditory ossicles (incus, malleus, see p. 24).

4. Thus the special peculiarity of the mammalian as compared with the reptilian lower jaw is that in the mammals the anterior tooth-bearing element has become completely separated from the posterior half of the jaw and the two halves now subserve two widely different functions (mastication, audition).

5. The *detachment* of the anterior or dentary portion of the reptilian jaw from the posterior portion is a process for which analogies are offered among scarid fishes, Mosasaurs and Caprimulgus. According to Gaupp's view (pp. 657–658) this detachment has arisen in connection with a change in the muscular mechanism for the opening of the mouth. In reptiles, the jaw is depressed chiefly by the depressor mandibulae, which is finally lost in mammals. In mammals the jaw is depressed by the muscles of the floor of the mouth cavity, assisted by the external pterygoid muscles. An increase in size and strength of these muscles, which at first merely assisted the depressor mandibulae and which are all inserted in the dentary bone, would lead, thinks Gaupp, to the detachment of the dentary from the bones composing the hinder half of the jaw. The diminution of the depressor mandibulae muscle may, in turn, be ascribed to the reduction of the quadrate and to the transformation of the skull in the auditory region, under the influence of the rapidly enlarging brain.

6. The secondary *attachment* of the ascending ramus of the dentary to the squamosal again finds its analogue among the scarid fishes, where an ascending process of the dentary bone has formed a joint with the supramaxillary. The first step in this process in the ancestors of the mammals was probably the formation of a simple glandular cavity (Schleimbeutel) between the connective tissue on the lower surface of the squamosal and the connective tissue covering the ascending process of the dentary (see paragraph (1) above) at the place where the external pterygoid muscle was inserted. The meniscus or interarticular disc (1911, pp. 659, 626–629) represents a separated portion of the connective tissue covering the condyle (Gaupp, Lubosch), is continuous with the fibers of the external pterygoid muscle and has nothing whatever to do with the quadrate (with which element Broom ('90) had sought to homologize it).

The cartilaginous epiphysis ('accessory cartilage') of the condyle, which is very large in embryonic stages, is not a portion of the primordial chondrocranium, but is purely secondary, like the cartilaginous areas in many other dermal bones (Gaupp '07).

(Fuchs had tried to show—'09, pp. 237–242—that the cartilaginous epiphysis of the mandible was derived from the articular portion of Meckel's cartilage, and that the meniscus represented the distal portion of the quadrate, a view which may now be regarded as having been thoroughly disproved by Gaupp.)

7. The connection between the ascending ramus of the dentary and the squamosal was at first loose and mobile (Gaupp '11, p. 658). The temporary 'fixation' of the dentary upon the squamosal as a fulcrum was effected by the muscles which were inserted on the dentary, serving as 'active ligaments.' The new joint at first acted only as a force-resolving mechanism (? with reference to the direction and strength of the several components of the muscular pulls), while in higher types such as the Mustelidae, which have acquired a hinge-joint, motion and the direction of pressure are greatly limited.

8. The increase in size and backward growth of the ascending ramus of the dentary and its final contact with the squamosal are to be ascribed to three influences or conditions: (*a*) the general upward and backward trend of the ascending ramus itself, which would favor further development in the same direction; (*b*) the progressive diminution of the quadrate, a process which may on other grounds be confidently predicated of the ancestral mammals; (*c*) the transformation of the skull as a whole in the auditory region (diminution and basal displacement of the auditory capsule due to the broadening of the brain). As a result of these conditions the contact of the squamosal and the dentary took place in front of the old quadrato-articular joint, as is evidenced by the relations of the auriculo-temporal nerve and by the detrahens muscle of monotremes (Gaupp '11, p. 657).

9. The development of the new jaw articulation must have taken place in forms possessing a zygomatic arch such that the hinder half was composed of the squamosal ('11, p. 657).

Those who look scornfully at the theoretical deductions of comparative anatomy as mere flimsy plausibility, unverifiable hypotheses, will doubtless see in Gaupp's conclusions only a confirmation of their sceptical opinions. But those, who by patient study succeed in gaining a fair insight into these complex

matters, will realize that Gaupp has developed a perfectly consistent body of doctrines, resting upon many independent lines of evidence and offering a highly probable explanation of the two-fold problem of the lower jaw and of the auditory ossicles.

It seems truly remarkable that these elaborate conclusions, developed with practically no aid from palaeontological evidence, should now be found to be entirely consistent with it. 'Neontologists,' as a rule, have been so busy gathering and sifting the intricate facts furnished by recent forms, they have devoted so much energy to the elaborate embryological technique, they have heard so much and so often about the fragmentary nature of palaeontological evidence, that until very recently they have failed to realize the critical importance in the problems under consideration of the Theriodont reptiles of the Permian and Triassic. Palaeontologists, on the other hand, with the passing of Owen, Cope and Baur, have for the most part ceased to contribute to neontological research on the vertebrate skull and with few exceptions, have taken little or no part in discussing the origin of the mammalian lower jaw and auditory ossicles.

The first effective application of palaeontological evidence to the lower jaw problem was made by Dr. R. Broom ('04) who pointed out the marked approach toward mammalian conditions exhibited by the Theriodonts and suggested that the ascending ramus of the dentary grew backward until the condylar region came into contact with the squamosal. The next year Gaupp ('05) without reference to the Theriodonts, suggested the same view, but his explanation of the manner in which the new joint arose was based largely on the conditions in the lizard, and these conditions as elsewhere shown (Gregory '10) are essentially unfavorable to the origin of the mammalian type of articulation. Gaupp ('11, pp. 619–623) now accepts the lower jaw of Cynognathus (fig. 16) as virtually offering the fulfilment of the hypothetical conditions for the jaw of the ancestral mammal.

MAMMALIAN AUDITORY OSSICLES

In all the field of vertebrate morphology there is perhaps no more remarkable theory than that associated with the name of Reichert ('37). This theory deals with the origin of the auditory ossicles in stapediferous vertebrates; it holds that these ossicles represent the transformed elements of the visceral arches of fishes and in particular that the quadrate of reptiles is the homologue of the mammalian incus, the articular of the malleus. Even before Reichert, Gaupp tells us ('11, p. 123) the homology of the malleus with the articular had been suggested by J. F. Meckel ('20) and the homology of the incus with the quadrate by C. G. Carus ('18). Like every other great theory, this one had to undergo a long period of opposition and during the preceding century it evoked an extensive literature. The whole subject was carefully reviewed independently by Gaupp ('99) and by Kingsley ('00), and both of these authors strongly supported the Reichert theory.

Since then Gaupp has further strengthened this doctrine in his work on the development of the skull of Echidna ('08) and in several later works ('05, '09, '10, '11). Interest has been added to the subject by the polemical opposition of Fuchs ('09) to Gaupp. Many other workers, such as Bender, Drüner, Lubosch, Kjellberg, Schulman, Toldt, have also made valuable contributions to the subject.

In general the most important evidence for homologizing the quadrate and articular of reptiles with the incus and malleus respectively of mammals lies in the parallel, essentially identical, topographic relations and mode of development of these two sets of elements in the two classes. This parallelism in topographic relations and in development although marked by wide adaptive differences, is so fundamental, so extensive, so complex, that the possibility of these resemblances being accidental or due to convergent evolution seems practically excluded.

To recall only a few points in this complex evidence[5] the malleus of mammals is developed as the proximal or articular portion

[5] For a fuller discussion, see Gregory, "The orders of mammals," 1910, pp. 125–143.

of the primary lower jaw; the developing incus has every appearance of representing the quadrate and has similar relations with reference to the stapes (p. 28), to the chorda tympani nerve (fig. 21), to the squamosal (p. 26), to the inner ear (p. 27) and to the tympanic cavity (p. 25).

To this remarkable series of parallel relations in mammals and reptiles, Gaupp has recently made known an important addition. The malleus of mammals is a composite structure consisting first of a portion preformed in cartilage (comprising the main mass of the bone and the handle, or manubrium) and secondly of a membranous portion forming the anterior process

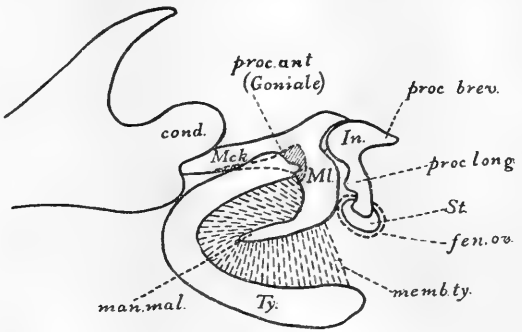


Fig. 17 Developing lower jaw and auditory ossicles of Tatusia hybrida. (Based on Parker, 1885, pl. 5, fig. 3 and pl. 6, fig. 3a.)

The malleus (*Ml.*) is seen to form the articular region of the Meckelian cartilage (*Mck.*); the dermal bone ensheathing it below, forming the anterior process of the malleus, is homologized by Gaupp with the reptilian goniale. The handle of the malleus (*man. mal.*) is inserted in the tympanic membrane which is stretched on the tympanic annulus (*Ty.*).

(processus anterior s. Folianus, fig. 17). The cartilaginous portion of the malleus has long been regarded as the articular of reptiles; the dermal portion is therefore regarded by Gaupp as the goniale or prearticular which in the reptiles forms the medial internal extension of the articular. Moreover, both the goniale of reptiles and the anterior process of the malleus of mammals are sometimes pierced by the chorda tympani nerve. For these reasons Gaupp regards the malleus of mammals as the homologue of the gonio-articulare of reptiles.

The evidence favorable to, or consistent with, Reichert's theory offered by the Theriodont reptiles, has until recently remained

unappreciated. Kingsley ('00), Gadow ('01), Gaupp ('11) and others have held that the Theriodonts, being monimostylic, were definitely excluded from the mammalian ancestry, because, from embryological evidence, the mammals are inferred to have descended from forms with a freely movable quadrate.

The Theriodonts were apparently first considered as favoring the 'quadrate-incus' doctrine in 1909–1910 by the present writer ('10) in the following conclusions:

1. In Therocephalians and Cynodonts the progressive enlargement of the ascending ramus of the dentary and the progressive reduction of quadrate, articular and angular were regarded as adaptively correlated processes, tending on the one hand towards the formation of a new squamoso-dentary joint and on the other hand to a decrease in suspensorial functions of the old quadrato-articular joint.

2. From the conditions in Cynognathus, Trirachodon, etc., it seemed plain that the new joint, when established, must have been not far in front of the old joint (fig. 16); that there was more or less slip between the dentary and the angular ('10, p. 137); and that the new and the old joints long functioned together, all these relations being prophesied, as it were, although not attained in known Cynodonts.

3. A certain groove in the base of the skull of Cynognathus (fig. 18) was shown (loc. cit., p. 121) to have identical topographic relations with the auditory groove of mammals; it was, therefore, probably homologous with that structure and hence it was fair to assume that the tympanic cavity and tympanic membrane were closely associated with this groove and consequently lay below the reduced articular and quadrate (loc. cit., p. 122, fig. 113, *mb. ty;* p. 141).

4. From these inferred relations of the tympanic cavity and membrane in Cynognathus, and from the fact that in ontogeny the tubo-tympanal cavity grows up around the auditory ossicles which arise outside of it, it was suggested ('10 a, p. 126, fig. 3 B; '10 b, p. 600) that phylogenetically this upgrowing of the tubo-tympanal sac (fig. 19) around the vestigial quadrate and articular may have caused them to share in its vibrations and thus to

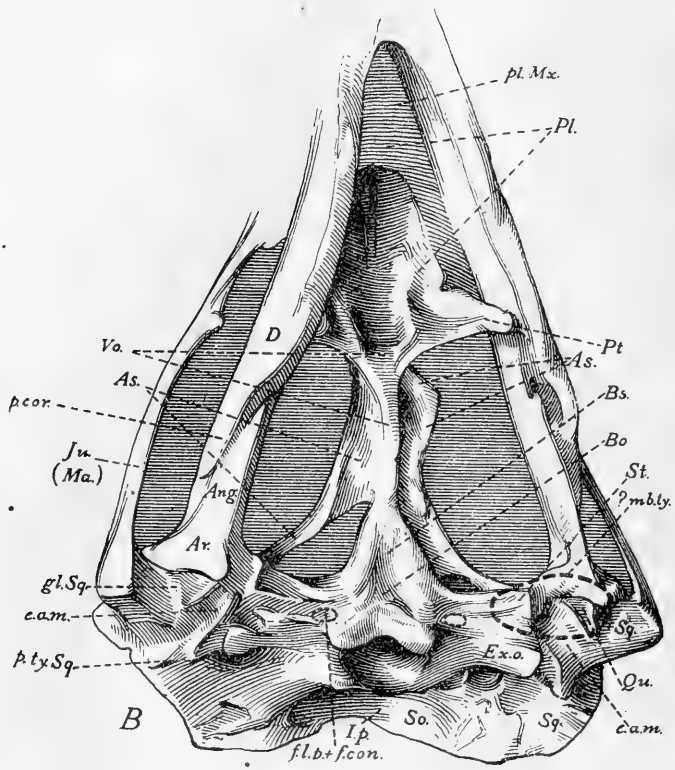


Fig. 18 Base of the skull, with lower jaw attached, of Cynognathus platyceps. Drawn from a cast of the type.

The stapes (*St.*) is apparently displaced; according to Broom's figure (P.Z.S., 1904, vol. 1, pl. xxxv, fig. I) it should be in contact with the quadrate.

e.a.m., auditory groove; *p. ty. Sq.*, post-tympanic process of squamosal.

take on an incipient auditory function before their old suspensory function had ceased. It was also suggested that the Weberian apparatus of Siluroid fishes offers a somewhat analogous case: where a tense vibrating sac had literally pressed into its service elements that had subserved originally a totally different function.

5. It was pointed out ('10 a, p. 139) that the minute quadrate of Gomphognathus (fig. 11) resembles the incus of mammals: (*a*) in being a very small flattened bone located between the

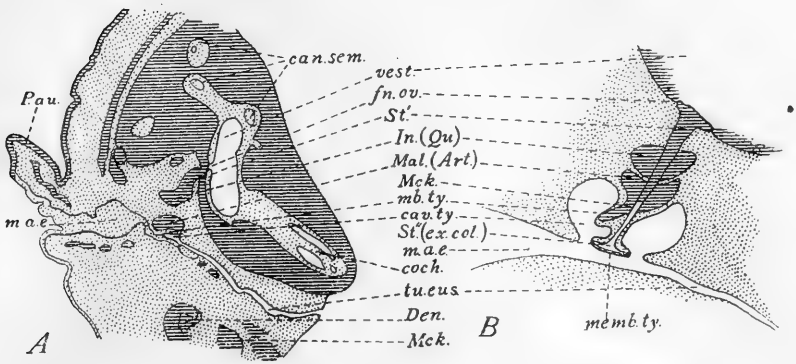


Fig. 19 Relations of the auditory ossicles to the auditory capsule and tubo-tympanal cavity.

A, Cross section of the auditory region in a human embryo of three months. After Minot (lettering modified).

The developing ossicula (*St.*, *Mal.*) lie above the incipient tympanic cavity (*cav. ty.*) which is merely a dilatation of the Eustachian tube (*tu.eus.*), the supposed homologue of the spiracular gill cleft.

m.a.e., external auditory meatus; *mb.ty.*, tympanic membrane, separating the external auditory meatus from the Eustachian tube.

B, Hypothetical scheme of the relations of the stapes (*St'*) to the reduced quadrate and articular and to the tubo-tympanal cavity in a pro-mammalian stage.

The quadrate and articular should have been lowered to the region where the stapes joins the extra-columella. The essential idea is the upgrowth of the tubotympanal cavity around the quadrate and articular.

proötic and the zygomatic branch of the squamosal; (*b*) in articulating with the articular (=malleus) by a convex-concave joint.

6. The fact that the quadrate is attached to and partly covered by the squamosal in Cynodonts (fig. 16) considered by all neontologists an insuperable objection to relationship with the mammals was clearly recognized ('10 a, p. 139); but it was hinted that as only the dorsal prolongation of the quadrate was covered by the squamosal, antero-posterior pressure on the lower end of the quadrate would tend to loosen the upper end from its squamosal attachment and thus to transform a monimostylic into a streptostylic condition.[6]

[6] It also seems reasonable to infer that as the new squamoso-dentary joint was being established the old quadrato-articular joint would be more or less wrenched by the pull of the temporal and other muscles. The matter is further discussed below, p. 36.

7. The streptostylic condition of the incus (quadrate) in mammalian embryos was held ('10 b, p. 600) to be a caenogenetic result of its secondary function as an accessory auditory ossicle.[7]

In the brief time that has elapsed since this application of Reichert's doctrine to the conditions observed in Cynodontia, considerable collateral evidence has become available and certain doubtful questions appear to be nearer to solution. Gaupp's remarkable studies on the lower jaw of vertebrates (see pp. 18–21) have practically demonstrated that the mandibular joint of mammals is a secondary joint connecting the squamosal and the dentary; hardly less rigorous is his proof that the malleus represents the gonio-articulare, the incus the quadrate, of reptiles. On the other hand, all of Broom's recent work ('11) has brought cumulative evidence for the view that the Cynodonts are phylogenetically very near to the ancestral mammals.

First in importance among the points discussed but left in doubt in the writer's earlier paper is the homology of the rod-like bone (fig. 18, *stp.*) in Cynodonts which Broom formerly identified with the mammalian tympanic. It has, however, every appearance of being the bone usually called stapes in Permian reptiles (e. g., Dimetrodon, Case, '07, pl. 19, fig. 2; Labidosaurus, Williston, '10, pl. 2). It also has the appearance of being homologous with the true stapes of Sphenodon. Gaupp ('11, p. 641) thinks it highly probable that the doubted element is a stapes and that, as in Dimetrodon, its outer end was in contact with the quadrate.

Dr. Broom, in a letter to the writer dated July 20, 1911, stated that he had decisive evidence showing that the doubted element is stapes and not tympanic. In Broom's figure ('11, p. 7, pl. 46, fig. 8) of the very primitive Cynodont Bauria this supposed stapes runs out toward the quadrate; its distal end is imperfect, but Broom restores it in contact with the quadrate. The stapes is represented as reaching nearly or quite to the quadrate in Cynognathus (Broom, '04, pp. 490–498, pl. 25) and Oudenodon (Broom), Dimetrodon (Case), Labidosaurus (Williston), as well

[7] In view of the radical change of function some caenogenetic conditions in modern ontogeny are, from all analogies, to be expected.

as in modern snakes, chameleons, tortoises and some urodeles (Kingsbury and Reid) and caecilians (Kingsley). If, as now appears probable, the stapes touched the quadrate in Cynodonts, then it is clear that stapes, quadrate, articular, already formed a connected train of bones (fig. 23). Thus would be met Gadow's objection ('88) that " . . . . . the incus cannot be the homologue of the quadrate because of the impossibility of intercalating the quadrate as an incus into the ossicular chain as a link between the stapes (hyomandibula) and lenticulare (sympletic) and the malleus (articulare)." But the quadrate (incus) was not 'intercalated' in the chain; it was there, from the time that the hyomandibular (stapes) became attached to it.

Very obscure and difficult is the complex of questions involving the origin of the handle of the malleus, of the tympanic membrane and tympanic bone (annulus tympanicus), the fate of the reptilian extracolumella and angulare.

In typical reptiles the tympanic membrane is stretched on or near the quadrate, squamosal and articular. Between the inner and outer layers of the tympanic membrane is inserted the extracolumella (fig. 20), which is joined to the true stapes; this extracolumella, like the stapes itself, is believed to be a derivative of the hyoid arch; from the extracolumella springs a dorsal process, the suprastapedial, or intercalare; the ascending hyoid is generally attached either to the extracolumella or to the parotic process of the opisthotic. In mammals the handle of the malleus (manubrium mallei) is inserted into the middle layer of the three layered tympanic membrane; the extracolumella, if present, is not recognized as such and is not connected with the stapes; the hyoid is attached to the periotic.

Kingsley ('00) held that the malleus is a compound element, that the manubrium (fig. 21) in ontogeny arises "distinct from the body of the malleus, that it is at first, like the extracolumella a separate element developing in the tympanic membrane and only later uniting with the rest of the structure." Kingsley therefore concluded that the manubrium mallei had been derived from the extracolumella of the reptilian ossicular chain, a view endorsed also by Fuchs. Hammar and Fuchs have also found

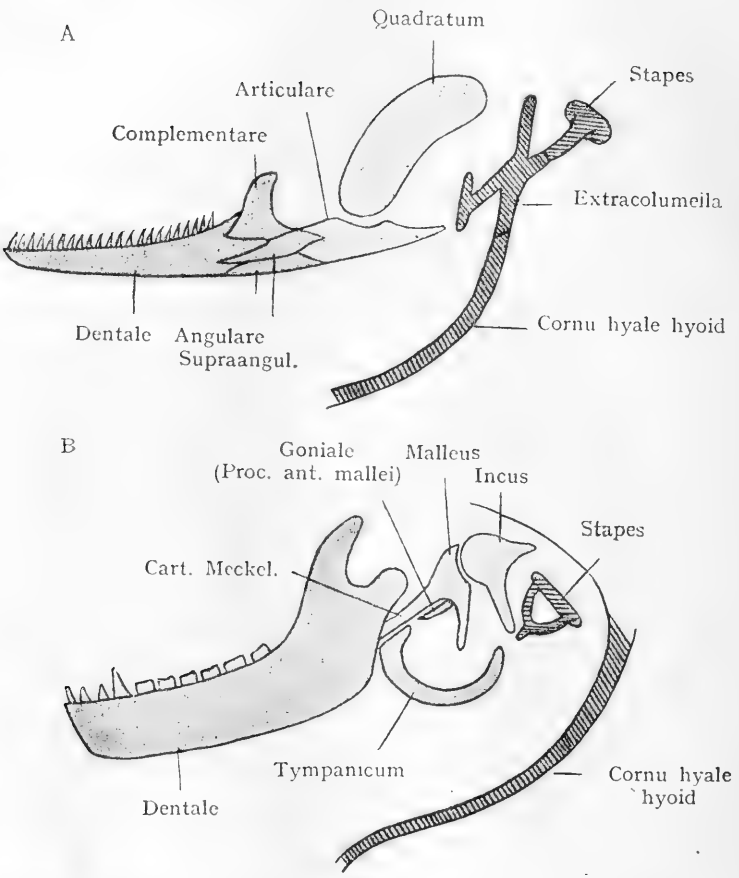


Fig. 20 Schematic representation of the relations of lower jaw and ossicula auditus in (*A*) saurian embryos and (*B*) mammal embryos (Gaupp, Verhandl. des VIII. Int. Zool. Kongr. zu Graz, 1910, Fig. 9, p. 231).

Primordial parts of the mandibular arch white, dermal bones of the Meckelian cartilage gray, stapes cross hatched, 'ventrohyal' obliquely hatched. In Sauropsida the upper section of the ventrohyal is represented by the columella, in the mammals the upper end of the ventrohyal is connected with the auditory capsule (Gaupp).

that the manubrium arises independently or at least begins to chondrify peripherally (Gaupp). Gaupp ('09, p. 96; '11, pp. 458–459) however, will not admit that the manubrium is the homologue of the extracolumella; he is undecided whether it represents the down turned retroarticular process of the articular or

a new cartilage (analogous in newness to the ethmoturbinal cartilages), or a derivative of the hyoid.

The reptilian extracolumella and suprastapedial (figs. 12, 20, 22) have been homologized by many authors (including Peters, Cope, Baur, Dollo, Gadow '11, Broom '07) with the mammalian incus and malleus. This is a 'common sense' theory, whose advocates regard the supposed transformation of the quadrate into the incus as an impossibility. The gist of their contention as presented by Gadow, is that the fenestra ovalis of the inner ear and the membrana tympani are fixed points, between which,

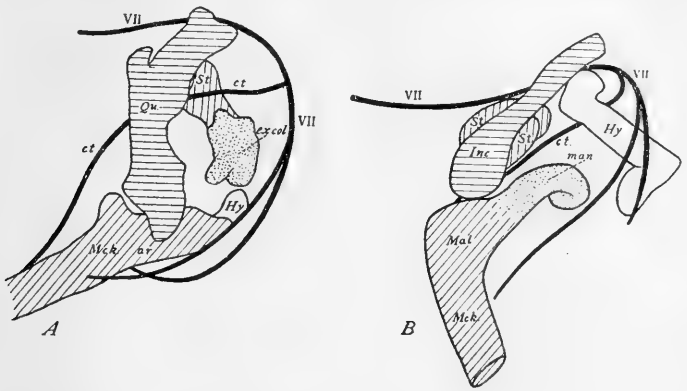


Fig. 21 Schematic representation of Kingsley's view of homologies in ossicula and jaw parts (*A*) of embryo lizard, (*B*) of embryo mammal (pig). According to this view the manubrium mallei has been derived from the extracolumella. *c.t.*, chorda tympani.

in the reptile, lies the columella-extracolumella chain and in the mammal the stapes, incus and malleus; that the ossicular chain of Sauropsida is consequently homologous as a whole with that of the mammal and that it is impossible to conceive the intercalation into the mammalian chain of new elements, such as the quadrate and articular. But as shown above (p. 29), the quadrate and articular, according to the best evidence available, were not 'intercalated' into the chain, they were functionally already a part of it.

In support of the hypothesis that the mammalian ossicular chain is homologous with the extracolumella + stapedial rod of

reptiles Gadow and Broom ('07) took as primitive the conditions revealed in early embryos of the crocodile (fig. 22). Here the extracolumella is continued downward as a strand of cartilage (the 'ceratohyal' of Parker) which is in turn continuous with the Meckelian cartilage behind the articular region. Gadow points out the parallel between this so-called ceratohyal and the malleo-meckelian connection of embryo mammals. He says:

> The whole string, whether cartilaginous or ligamentous, which connects the downward extracolumellar process with the articulare, is *of course*,[8] homologous with the continuation of Meckel's cartilage into the malleus of foetal and young mammals.

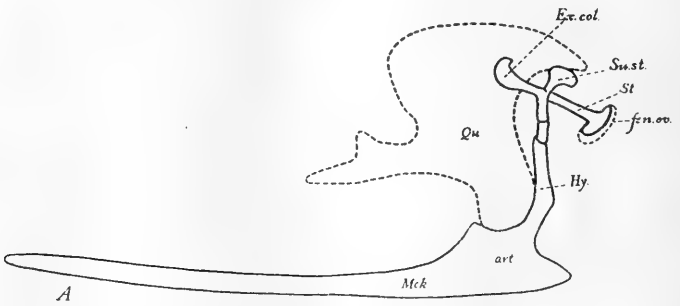


Fig. 22 Lower jaw and auditory ossicles of an embryo Crocodile. After Parker (lettering slightly modified).

The hyoid (ceratohyal Parker) is secondarily fused with the posterior part of the articular region of Meckel's cartilage; the hyoid is connected above with the extracolumella and suprastapedial; the stapes (*St.*) fits into the fenestra ovalis.

But, apart from the suspicion that conditions in the crocodile are highly specialized (in correlation with the peculiar Eustachian diverticula, etc.), a comparison of the 'ceratohyal' of the crocodile with the hyoid of developing lizards leaves no reason to doubt the homology, which is indeed endorsed by Versluys ('03), after the most thorough researches. Again, in early stages (figs. 12, 13) of Sphenodon (Howes and Swinnerton '01), the ascending branch of the hyoid is closely appressed to Meckel's cartilage and has every appearance of being homologous with the 'cera-

[8] Italics mine.

tohyal' of the crocodile. But, if this homology be granted, the very superficial resemblance to the malleo-meckelian connection in mammals is purely accidental and of no homological significance. Versluys indeed finds ('03, p. 177) that this secondary connection by means of the hyoid, is the only way in which the Sauropsid extracolumella is ever connected with the Meckelian cartilage. In brief there can now be little doubt that the malleo-meckelian rod of mammals represents solely the first or mandibular visceral arch and has nothing to do with the second arch which is the source of the extracolumella and hyoid cornu (cf. Howes and Swinnerton, p. 49). Versluys ('03, p. 177) concluded, in opposition to Peters and others, that the extracolumella and suprastapedial, instead of giving rise to the malleus and incus have practically disappeared in mammals and are only represented by certain transitory embryonic vestiges connecting the stapes and hyoid ('03, Taf. 11, figs. 3, 4.)

With regard to the origin of the mammalian tympanic membrane, it seems likely that at least some of the Cynodonts already approached mammalian conditions. In the remarkably mammal-like genus Sesamodon of Broom (fig. 25) the auditory groove (doubtless homologous with that of mammals) indicates essentially mammalian conditions for the tympanic cavity and membrane. On the other hand, in the far more primitive Cynodont Bauria there is little hint of mammalian structures and the tympanic membrane, if differentiated, was probably stretched as in reptiles behind the squamosal and articular.

The stapes of Bauria is supposed to have touched the quadrate; but conceivably it may also have been connected with an extracolumella; just as in embryo lizards the stapes-extracolumella chain touches the quadrate (Versluys '03, Taf. 8, fig. 8); the hyoid was perhaps still connected with the extracolumella.

The essential feature of a primitive auditory chain is a jointed system of rods, subjected to pressure at opposite ends but kept tense by muscular pull and by direct fastening to adjoining bones. In both mammals and reptiles the outer end of the chain is connected with the tympanic membrane. But is the tympanic membrane homologous in the two classes?

Great is the need for decisive evidence on this question, but before accepting Gaupp's suggestion ('11, p. 659) that the mammalian and reptilian membranes were differentiated altogether independently, we may put forth the following purely provisional hypothesis embodied in figure 23: that in the most primitive Cynodonts, such as Bauria, there was an extracolumella, resting against a tympanic membrane behind the squamosal, which had been differentiated out of the tissue lying between the endodermal epithelium of the tympanic cavity and the epidermis;

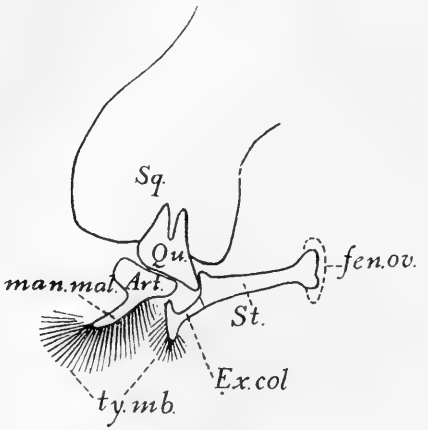


Fig. 23 Hypothetical scheme showing the reptilian extracolumella and the mammalian manubrium mallei (= ? proc. retro articularis) both functioning at the same time.

that, with the spread of the tympanic cavity (see p. 25) the differentiation of the future tympanic membrane also spread, until it included the stretched skin on the posterior end of the jaw, below the quadrate and articular and above the angular; that concomitantly with the reduction of the quadrate and articular (p. 25) and the detachment of the angular and goniale from the dentary (p. 20), the newly differentiated portion of the tympanic membrane became functionally more active than the old 'reptilian' portion; that, in this way the old membrane together with the extracolumella became vestigial, while the new membrane became altogether free from the dentary, but remained

fastened both to the angular, which gave rise to the tympanic bone, and to the retroarticular process of the articular, which gave rise to the manubrium of the malleus. With the reduction of the 'reptilian' tympanic membrane the hyoid became separated from the extracolumella (as it does in many lizards) and migrated to a new insertion point on the periotic (but by what path is not clear).

Such an hypothesis or series of hypotheses seems to embody the best available evidence concerning the origin of the manubrium, the origin of the tympanic bone and the fate of the extracolumella. While this matter is still unfortunately in the speculative stage, the evidence tending to show that the tympanic bone has been derived either from the supraangular (van Kampen '05) or preferably from the angular (Gaupp '11, pp. 100, 461) seems of far greater value than the evidence cited by Gadow[9] to show that the tympanic bone has been derived from the reptilian quadrate.

ORIGIN OF MAMMALS

From the foregoing pages it will be evident that the most prominent neontologists have looked almost exclusively to Lacerta, Sphenodon, Echidna, Lepus and other recent forms for answers to the intricate problems of skull morphology. Gaupp, in his luminous address ('10) before the Eighth International Zoological Congress, explicitly defends this procedure on the ground that only the recent types afford us an insight into the highly important morphology of the chondrocranium. From various reasons contemporary neontologists have shown a disinclination to extend their morphological studies and conclusions to the extinct types. Although the skull morphology of Cynognathus has been known in its essential facts for many years, it is only recently that Gaupp has discussed the Theriodont lower jaw, which he now recognizes as a fulfilment of his neontological prophecies.

[9] For a criticism of Gadow's view, see Gregory, The orders of mammals, pp. 128–129.

While recognizing the mammalian tendencies in the Theriodont lower jaw Gaupp still refuses ('11, p. 635) to admit the closeness of the relationship between Theriodonts and mammals:

Damit ist natürlich nicht gesagt, dass die Saüger unmittelbar an Cynognathus-ähnliche Formen anzuschliessen sind; eine solche Vorstellung halte ich bei den mancherlei hohen und einseitigen Spezialisierungen der Theriodonten geradezu für ausgeschlossen. Aus dem Gebiete des Schädels nenne ich hier nur die feste Verkeilung des Quadratums mit den benachbarten Schädelknochen und seine Entfernung von der eigentlichen Ohrgegend durch einen weit nach der Seite vorspringenden Fortsatz (Crista parotica, Proc. paroticus), wie ihn auch Rhynchocephalen und Saurier besitzen. Demgegenüber ist der Amboss (das Quadratum) der Saüger beweglich und dicht neben der Ohrkapsel gelagert, und die Crista parotica ist auf die niedrige Crista facialis reduziert,

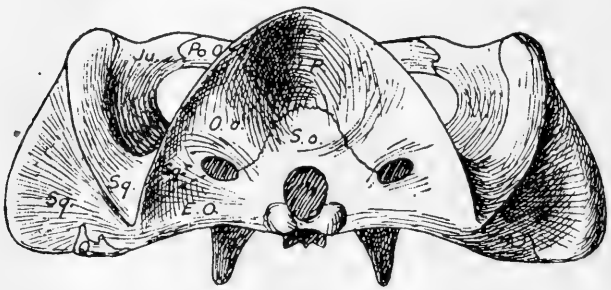


Fig. 24 Occipital view, skull of Gomphagnathus minor. (Broom, P.Z.S. 1911, text-fig. 178 p. 912).

unter der der N. facialis verläuft. Indessen kann es uns einstweilen genügen wenn sich überhaupt im Kieferapparat Einrichtungen realisiert finden, die uns einen Hinweis darauf geben, in welcher Weise die Ausbildung der Saügerverhaltnisse möglich war.

Now what are these "manifold high and one sided specializations" of the Theriodonts which exclude them from immediate ancestry of the Mammalia? The first mentioned and traditional objection is the "fast wedging of the quadrate by neighboring skull bones." But we have tried (p. 27) to show that this "fast wedging of the quadrate" is a matter of slight morphological importance, that the quadrate in Gomphognathus (fig. 24) with its projecting lower end, is already in a way to become movable. In the remarkably mammal-like genus Sesamodon

(fig. 25) the dentition according to Broom ('11, p. 916) indicates "an articulation for the lower jaw which permits of some degree of antero-posterior movement." Does not this antero-posterior movement imply a functionally streptostylic quadrate?

The second point raised by Gaupp to exclude the Theriodontia from mammalian ancestry is the

> wide separation of the quadrate from the true auditory region, through a long lateral parotic process (of the quadrate) as in Rhynchocephalia and lizards, whereas in mammals the movable incus (quadrate) lies close to the auditory capsule and the parotic process is reduced to the low facialis ridge (of the incus), beneath which runs the facial nerve.

But because the Cynognathus quadrate retains certain primitive reptilian characters, exhibited also by the quadrate of Rhynchocephalia and lizards, is that a good reason for excluding the Theriodonts from the ancestry of the mammalia? Why not then exclude all reptiles that possess a quadrate, that is to say, all reptiles whatsoever? It is of course entirely consistent with the 'Theriodont theory' that the lower Theriodonts, i. e., the Therocephalians, should have a large and typically reptilian quadrate, while the higher Theriodonts, e. g., Gomphognathus of the Cynodontia, have a reduced quadrate with a reduced parotic process.[10]

What other "high and one-sided specializations" are there, common to all Theriodonts (i.e., Therocephalia + Cynodontia) and not simply generic, which would exclude Theriodontia in their *ordinal* characters from being the morphological archetypes of the Mammalia? Are they excluded because they retain such primitive reptilian characters as a pineal foramen (lost in Sesamodon) separate prefrontals, postorbitals, 'reptilian' pterygoid and the full complement of upper and lower jaw bones? Are they excluded because some of them, in combination with certain generic specializations, such as the grinding dentition and enlarged squamosals of Gomphognathus, have also acquired many characteristically mammalian characters? What could be more mammalian except the mammals themselves, than Gomphognathus, in the details of its palate, pterygoids, vomer, alisphe-

[10] Cf. figure 11, Broom, 1911. The long parotic process of the 'alisphenoid' is entirely separate, according to Broom, from the parotic process of the quadrate. [But see Postscript below.]

noids, occipital condyles, interparietal, posttemporal canal (cf. Echidna), etc., than Sesamodon, with its opossum-like zygoma and auditory groove, its infraorbital canal, its nostrils, its lower jaw, its dentition?

The mammalian affinities of the Theriodonts are thrown into even clearer emphasis when we compare other extinct reptiles with the mammals. How wide is the structural gap between mammals, on the one hand, and Pelycosaurs, Cotylosaurs, lizards, Rhynchocephalians, Chelonians on the other. And if we extend our comparisons to the post-cranial skeleton of the Theriodonts[11] we again find that, after setting aside generic specializations

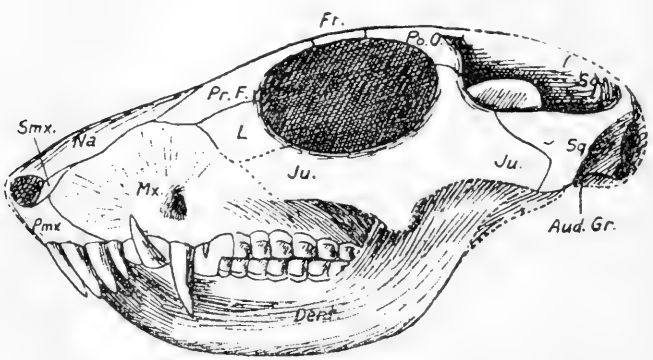


Fig. 25 Skull of Sesamodon browni, the most mammal-like known Cynodont. (Broom P.Z.S. 1911, p. 914, text-fig. 179.)

we have left a great majority of characters favoring the view that the mammals sprang from Cynodonts of some sort. The scapulo-coracoid of Gomphognathus, for example, furnishes the complete key[12] to the derivation of the mammalian from the reptilian type; the humerus also is in every respect transitional between the Permian reptile and the mammalian types. Even the true Anomodonts, far removed as they are from direct relationship with the mammals, show an essentially mammalian manus and pes.[13]

[11] See the discussion in The orders of mammals, pp. 118–119.
[12] Ibid., p. 119.
[13] Ibid., pp. 439–453.

The circle of forms clustering around the true Theriodonts, as Broom has well shown ('07, '10, '11) enable us to pass backward by relatively small steps from the almost mammalian Sesamodon, through Aelurosuchus and Bauria, thence through the Therocephalia to the very lowly order Dromasauria, including small forms with abdominal ribs, a plate-like pelvis and other primitive characters. Thus we are brought within hailing distance of such, in many respects, primitive types as the Pelycosaurs, Poliosaurs, Cotylosaurs, Procolophonia, Rhynchocephalia. This goes far to explain why it is that the mammalian carpus and tarsus, for example, are so clearly foreshadowed in the Permian Varanosaurus (Williston, '11, pls. 8 and 13) and the Pelycosaurs; why the Rhynchocephalia and Squamata have retained certain characters that offer clues for neontological interpretations of the mammalian skull.

In conclusion may be quoted, with entire approbation, the words of Gaupp ('12, pp. 239–240):

> Wir haben gefunden, dass, wenn wir die rezenten Formen vom Standpunct der genannten Forderungen aus betrachten, die Amphibien ganz ausscheiden, und dass unter rezenten Reptilienformen die Rhynchocephalen und Saurier die meisten der gestellten Bedingungen erfüllen. Nicht als ob wir die Saüger unmittelbar von Rhynchocephalen oder Sauerien abzuleiten hätten,—das ist selbstverständlich unmöglich,—das aber können wir wohl sagen, dass die beiden genannten Gruppen unter den lebenden Reptilformen in ihrem Schädelbau die meisten Ähnlichkeiten mit den Säugern darbeiten, und dass wir dadurch einen Fingerzeig erhalten, der bei der ferneren Behandlung des Problemes nicht wird ausser acht gelassen werden dürfen. Und darauf kam es mir hier an. Die endliche Lösung phylogenetischer Fragen bleibt der Palaeontologie überlassen, aber einer Palaeontologie, die sich nicht mit souveräner Nichtachtung über alles hinwegsetzt, was Biologie oder Neontologie, Morphologie der rezenten Formen heisst, sondern die Arbeit auch dieser Forschungsrichtung anerkennt und sich dienstbar macht. Nur aus dem Zusammenwirken von Neontologie und Palaeontologie wird ein gesichertes Ergebnis zu erwarten sein.

POSTSCRIPT

Since the foregoing paper was sent to the editor in June, 1912, several important contributions to the problems discussed above have fallen into my hands. Watson ('11), in his very careful de-

scription of the skulls of Diademodon and other Cynodonts, states that there is no suture between the epipterygoid, or temporal wing of the alisphenoid, and the pterygoid; that the whole pterygoid plus epipterygoid corresponds and is homologous with the mammalian alisphenoid in all its relations to surrounding elements and to nerve exits. The "pterygoid wings of the alisphenoid" in mammals together with the basal portions of the alisphenoids are therefore homologous with the pterygoids of reptiles. The true mammalian pterygoids, which are slips of bone on the inner side of the pterygo-alisphenoids are homologized by Watson with the ectopterygoids of Cynodonts, as first suggested by Seeley.

These conclusions are entirely consistent with the facts set forth in the preceding pages and offer a very satisfactory explanation of the fate of the ectopterygoids and pterygoids in the Cynodont-like ancestors of the mammals.

Dr. R. Broom ('12) in working out a natural brain cast of Dicynodon finds that the fenestra ovalis of the internal ear is filled by the inner end of the rod-like bone which he formerly called tympanic but which he now recognizes as stapes. The outer end of the stapes abuts against the quadrate. The quadrate therefore corresponds in position with the mammalian incus and Broom accordingly accepts the homologies of the Theriodont quadrate and articular which were suggested by the present writer in 1910, when applying Reichert's theory to the Theriodontia.

The writer's application of Reichert's theory to the mammal-like reptiles is contested by Dr. Hugo Fuchs ('12). His most important point, the 'fixed' condition of the quadrate in Cynodonts has been dealt with above (pp. 27, 36). The "caudal displacement of the quadrate" in Monotremes has not removed the quadrate very far behind the glenoid region of the squamosal. His views of the homology and relations of the squamosal and of the epiphysial articular cartilage of the mandible have, it seems, already been answered satisfactorily by Gaupp.

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FURTHER OBSERVATIONS ON THE PARASITES OF SIMULIUM LARVAE[1]

E. HAROLD STRICKLAND

Carnegie Scholar in Economic Entomology

SIX PLATES

CONTENTS

INTRODUCTION

The following paper is based upon observations of the parasites of Simulium larvae during the fall of 1911, in the neighborhood of Boston. Although, prior to the spring of 1911, when I found several distinct parasites in these larvae, there were no

[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 56.

records of their occurrence in North America, they are, at least in the locality above named, extremely abundant and readily visible to the most casual observer. It would seem, therefore, that these parasites, most of which belong to the order Myxosporidia of the Sporozoa, cannot be generally distributed throughout the United States, since there are no previous records of their occurrence, although Simulium larvae have, in many sections, received very careful attention. The only works which have been published in this country dealing with the Myxosporidia are those of Gurley ('93 and '94), who gave very complete accounts of all the forms then known to occur in fishes. Nothing however has been written in connection with the Myxosporidian parasites of insects. For this reason I have deemed it advisable, before describing in detail the species found as parasites of Simulium larvae, to give a brief review of the order Myxosporidia paying especial attention to the suborder Cryptocystes, or Microsporida. From the observations I have made regarding the Protozoa and their effects upon Simulium larvae I have no reason to doubt that their presence is always fatal to their host, and, since in some cases as many as 80 per cent of the larvae are parasitised, it is probable that the inhabitants of this part of New England owe, to a large extent, their comparative freedom from annoyance by these noxious flies to the abundance of their parasites.

I wish to express my sincere thanks to Prof. W. M. Wheeler, under whose directions this paper was prepared, both for his help and advice during its preparation, and for a critical examination of the manuscript.

NOTES ON SIMULIUM LARVAE IN THE VICINITY OF BOSTON

The streams in this locality, though usually small, flow rapidly over rocky beds, and are thus eminently suited to the requirements of the early stages of the various species of Simulium. During the spring of 1911 these streams were found to contain immense numbers of the larvae, the most abundant species being S. hirtipes, which occurred in such vast masses that in certain streams hardly a stone could be found which did not support

one or more colonies. Large numbers of the larvae of this, and those of an undescribed species were parasitised by Microsporidians. I have published a short account ('11) of the appearance of the infected larvae, and of the external structure of the spores of the species which I found. At the time of discovery all the forms had sporulated, so further details could not be given. By the middle of May all the larvae of the generation under observation had pupated and hatched, and during the summer few larvae were to be seen in any of the streams. Only in a few places could I find an isolated specimen of what I have since found to be the hitherto undescribed larva of Simulium bracteatum Coq.

On October 1 I resumed my search for Simuliid larvae, hoping to find the early stages of the parasites. A number of isolated larvae were observed in most of the streams visited, and it was not long before I found evidence of Microsporidian parasites.

The larvae observed in the streams during the fall of 1911 are as follows:

S. bracteatum; most numerous, occurring in all streams in which any larvae were found, though always living a solitary life. S. vittatum; very few specimens found in one stream. S. hirtipes; not found till the beginning of November after which it was common in some localities.

By the middle of November collecting became extremely difficult as the streams were filled with fallen leaves which had to be removed almost one by one, and examined for larvae, as the latter appeared to prefer them to the stones.

DESCRIPTION OF LARVA AND PUPA OF S. BRACTEATUM COQ.

The adult of this species was described by Coquillett in 1898 from specimens taken at Cambridge, Massachusetts, about seven miles from the locality in which I took larvae and pupae which yielded adults agreeing in all details with his description.

Larva: *plate 1, figs. 1 to 7.* The mature larva is 6 mm. long and is of a somewhat brownish color. The cephalic fans have about fifty rays which are provided with very short internal cilia, all of which are of the same length. The antennae (fig.

1) are four-jointed, joints 1 to 3 being sub-equal, the fourth joint small and conical. The labium (figs. 6 and 7) has large swollen lateral teeth, and the central tooth is large and prominent while the intermediate teeth are small. On the ventral surface there are usually three strong setae in a row, and two smaller basal setae. The number and position of the setae, however, as in all species examined, are inconstant characters. The mandibular bristles (fig. 3) are not very pronounced. The maxillary palpus is long and slender (fig. 5). The head capsule is never uniformly dark all over; when freshly moulted it is almost white, with a darker median line and dusky latero-basal areas (fig. 2). These latter fuse with one another till the greater portion of the basal two thirds of the capsule is of a deep brown color (plate 5, fig. 1). The thorax and abdomen are greenish gray to brown. The apex of the abdomen is swollen and of a lighter color ventrally. On each side of the swollen portion is a distinct dorsolateral longitudinal furrow. The gills are simple and trilobed.

Pupa: *plate 1, figs. 8 and 9.* This is 3 mm. long, chestnut brown, turning to black when mature, with four-branched respiratory filaments (fig. 9). The dorsal surface of the posterior margins of the fourth and fifth abdominal segments have eight anterior curved, small, brown hooks. Usually no other segment bears traces of dorsal hooks but the sixth occasionally bears two or three. Ventrally segments 5 to 8 bear at least one pair of obsolete hooks on the posterior margin, and usually there are traces of a second pair of hooks on some of the segments.

Cocoon. This is formed of rather coarse gray silk and does not completely enclose the pupa. It is of the wall-pocket type and is usually found singly, attached to stones or dead leaves.

The pupa of this species is of especial interest, because only two other pupae have been described in which the respiratory filaments are only four branched. One of these is a European and the other a South American species recently described by Lutz ('10).[2]

[2] Since the above was written Forbes ('12) has described the pupa of a new species, S. johannseni, found in the Illinois River, in which the respiratory filaments are four-branched.

STRUCTURAL PECULIARITIES AND HABITS OF SIMULIUM LARVAE BEARING ON THE SUBJECT OF PARASTITISM

Simulium larvae are particularly difficult insects to study in the living condition because it seems to be impossible to keep them alive for any adequate length of time in captivity. There seem to be two reasons for this. It may be due simply to the fact that the respiratory gills are very small and are unable to extract sufficient oxygen from stagnant water, for it will be remembered that the larvae live only in very fast flowing water. When captive larvae are closely watched they are seen to pass faecal matter very frequently and this activity is accompanied by very evident signs of hunger, for the larvae do not remain stationary as before but turn their heads rapidly in all directions and seem to be searching about on the bottom of the receptacle, in which they are placed, for food. I have even seen them turn and re-ingest their own faeces as soon as they had been expelled. If small Chironomid larvae are present in the water, as is frequently the case, the Simulium larvae will rapidly sieze upon, and attempt to devour them, though owing to the peculiar modification of the mouth parts they never appear to succeed in these attempts. It seems from these facts that hunger is very detrimental to the larva, and the probability of this statement is heightened by the fact that larvae which have died in captivity very rarely have any food in the mesenteron. This region of the alimentary tract is then filled with some secretions, probably digestive, which coagulate in the warm fixing fluid. Although life may be prolonged for several days in healthy larvae it is difficult to keep parasitised individuals alive for more than two days at the most. For this reason I have been unable to carry through any successful experiments on reinfection by the parasites or in transferring them to other species of larvae. Such experiments, will, it seems, have to be performed in the field and could probably be more readily accomplished in the spring when the larvae are most abundant.

It is interesting to note, in passing, that the majority of Simulium pupae fail to hatch in captivity. Many of those which I

collected were on leaves which I placed in the water without touching the pupae, so as to prevent any possible damage. Only in three cases, however, in all of which the pupa was almost mature, was development completed, and in all of these the fly failed to reach the surface of the water without wetting its wings.

Mr. A. H. Jennings of the Bureau of Entomology, to whom I mentioned this fact, suggested that it might be due to the inability of the pupa to extract sufficient oxygen from the stagnant water to envelop the contained fly entirely and thus protect it from the water upon its emergence.

a. The cephalic fans

The cephalic fans of Simulium larvae (pl. 5, fig. 1 a) are extremely specialised organs and are wonderfully suited to enable the larvae to obtain food from the water in a vertical position, thus avoiding the necessity of searching for nutriment in the bed of the stream. In the adult larva of Simulium bracteatum they are composed of about fifty curved rakes, which, when the fans are extended, form two very efficient bowl-shaped strainers, capable of collecting a large quantity of small food-particles from the water as it flows through the small spaces between the cilia of the rakes. In very young larvae, however, these organs are far less completely developed. In a larva measuring only some 0.75 mm. in length they are represented by only about ten widely separated rakes instead of the complete number of about fifty. In two very minute larvae not a single rake was present. Whether this is an abnormal condition or not I am unable to state, but in both cases the alimentary tract was filled with food. I have dissected and sectioned many eggs from different masses, but have been unable to find any in sufficiently advanced stages to show whether the fans are normally formed in the embryo or not. It is a significant fact, however, that neither in the work of Kölliker ('42), nor in that of Metschnikow ('66), which represent the only embryological studies of these insects, are the cephalic fans either figured or mentioned. It would seem, therefore, that the youngest larvae have to obtain their food by pick-

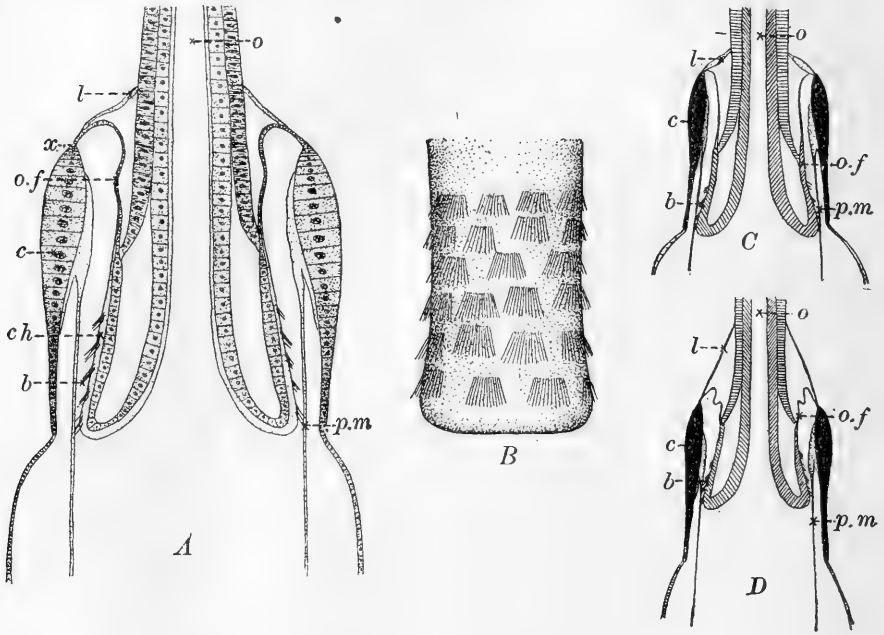
ing it up from the stones and débris of the stream, instead of, as at later stages, by simply ingesting what is strained out by their cephalic fans from the rapidly flowing current.

Sections of the alimentary tract of Simulium larvae are of peculiar interest in connection with the peritrophic membrane, which, owing doubtless to the siliceous nature of the food (Diatoms), is exceptionally thick and well developed. As I am led to believe that the presence of this thick membrane is of importance in connection with parasitism, and since it also shows some unique modifications in development, I will describe its formation and structure in some detail.

b. The peritrophic membrane

Text figure 1 shows diagrammatically at *A* a median longitudinal section through the proventriculus and anterior portion of the mesenteron of a larva. From this it will be seen that at the junction of the stomenteron and the mesenteron the former has been pressed into the lumen of the latter to such an extent that its wall has become everted for a considerable distance, so that it folds back upon itself, thus bringing the internal surface of the reflected portion into contact with the first twenty to thirty cells of the mesenteric epithelium. It is from this band of mesenteric cells, which is termed the cardia, that the peritrophic membrane (*p.m.*) is produced. The cells are much modified in size and shape and are sharply marked off from the more posterior mesenteric epithelium. So great is the modification that there has long been a discussion as to whether the cardia is of stomodeal (ectodermal) or mesenteric (entodermal) origin. Miall and Hammond ('00) have shown, from a study of the embryology of Chironomus that in this fly the cells of the cardia are undoubtedly of entodermal origin, and there is no reason to doubt that this is also the case in the allied family Simuliidae. The junction, then, between the stomenteron and the mesenteron is situated immediately anterior to the cardia, and the reflected portion which faces the cardia represents the extremity of the oesophagus. I shall refer to this portion of the oesophagus in

future as the oesophageal fold (*o.f.*) of which the end attached to the cardia is the apex and that at the flexure the base. The cells of the cardia are columnar in shape, and in stained preparations have a much greater affinity for such stains as haema-



Text fig. 1 Diagrammatic peritrophogen of a Simuliid larva. *A*, Section of peritrophogen in the normal position; *o*, lumen of oesophagus; *o.f.*, oesophageal fold; i.e., reflected portion of the oesophagus; *x*, junction between the oesophagus and the midgut (stomenteron and mesenteron), *c*, cardia composed of enlarged mesenteric cells which secrete the peritrophic membrane *p.m*; *ch*, thick deposit of chitin on the basal half of the oesophageal fold, bearing stout bristles *b*; *l*, ligament holding cardia in position around the oesophagus. *B*, Surface view of reflected portion of the oesophagus (oesophageal fold) showing imbricated tufts of downwardly directed bristles. *C*, Peritrophogen relaxed. *D*, Peritrophogen extended, showing the bristles, *b*, in contact with the freshly secreted peritrophic membrane.

toxylin than the remainder of the mesenteron. As is the case with all the mesenteron cells they are glandular in function, and that they are very active is proved both by the quantity of secretion they produce and by their greatly enlarged nuclei.

In a half-grown larva there are three distinct areas in the cardia (pl. 6, fig. 8). The anterior cells are small and closely crowded together. These secrete very little material. Posteriorly there are a number of cells apparently undergoing degeneration since they are much less regular in shape than the intermediate cells. None of these has any secretion attached to the exposed surface. Between these extremes is situated a series of five or six large cells which are very actively secreting a material of a chitinous nature. This, by a process described below, is drawn out as a film into the mesenteron where it functions as the peritrophic membrane.

In young and half-grown larvae all the anterior cells are small and almost functionless, while in mature individuals they are often enlarged and very actively secreting the material of which the peritrophic membrane consists. This, however, is not always the case for in some mature larvae they remain small. They seem, therefore, to constitute a reserve in case more secretion is required, and this reserve is not always drawn upon. That there is a backward movement of the cells of the cardia is indicated by the fact that those cells farthest behind the secreting portion have every appearance of degeneration, and though they are now, as far as can be seen, non-functional, the nucleus is much enlarged, though showing signs of disintegration. These cells also have the appearance of having been pressed out of shape and in some cases degenerate portions of them appear to be sloughing away. Then again there is no peritrophic membrane in the embryo, and it is not improbable that at this stage all the later functioning cells are undeveloped and are crowded together anteriorly. Unfortunately my preparations of the earliest stages obtained do not show details with sufficient distinctness to confirm this supposition.

That the substance of which the peritrophic membrane is composed is chitinous is proved by the fact that it can be boiled in strong caustic alkalies without dissolving. Vignon ('01) showed that the membrane is produced as a fluid which becomes coagulated soon after secretion. From my preparations it seems that the material remains plastic for some time after secretion, and

that when in this condition it is very ductile. Plate 6, figure 8 shows that the portion of the cardia which secretes the peritrophic membrane is normally distant from the orifice of the proventriculus, and the question arises as to how the secretion passes backward, as a regular film, to the point where it can first come in contact with the food, to form, when hardened, a continuous membrane which will entirely invest it during its backward passage through the alimentary tract. There is, in every species of Simulium which I have as yet examined, a structure peculiarly fitted to accomplish this, and this structure does not appear to have been seen in any other insect. I failed to locate it in Chironomus but have not examined the larvae of other allied families. In the accounts and figures of the cardia and peritrophic membrane of Culicidae by Thompson ('05) and Imms ('07) this structure is neither mentioned nor illustrated, so it is probably not to be found in the larvae of this family. It will be remembered that, as previously stated, the internal secreting surface of the cardia is faced by the internal surface of the oesophageal fold. The latter, being of ectodermal origin, is lined with chitin, and that lining the basal or posterior half of the fold is very much thickened, so as to be quite rigid (text fig. 1, *A*). It is also beset with strong, backwardly projecting, black bristles, placed in radiating tufts, which are arranged in an imbricated manner over the entire surface of this reinforced area (text fig. 1, *B*). The chitin lining the apical or anterior half of the fold is much reduced and hardly discernable in most larvae. The epithelial cells which secrete this small quantity of chitin are themselves also very small. This renders the basal half of the oesophageal fold, which normally lies opposite the functionless portion of the cardia, very rigid, and the apical half, which faces the secreting cells of the cardia, very flexible. The cardia is held in place around the proventriculus by an elastic ligament (*l.*) formed of connective tissue, which is attached to its anterior extremity, and to the external wall of the stomenteron. This ligament is capable of great extension and enables the cardia to be drawn backwards and forwards over the chitinous surface of the oesophageal fold. These movements were actually observed to take place in a liv-

ing larva which was examined in a cell slide under the low power of a microscope. Sections also show the different positions the cardia and proventriculus may assume with respect to each other (text figure 1, *C* and *D*). The result of these movements is that the stout bristles on the basal half of the oesophageal fold are brought forward into contact with the newly formed, plastic secretion of the cardia. Owing to the direction in which these bristles are placed they are able to draw a quantity of this material backward when the proventriculus returns to its normal position. This material soon hardens and in a subsequent forward movement of the proventriculus the bristles are withdrawn from the membrane only to become re-entangled with it at a point nearer to its origin, so that any subsequent retrogressive movement will draw more of the secretion backward till the circular membrane thus formed overhangs the orifice of the proventriculus and entirely surrounds the food, which is being passed from here into the lumen of the mesenteron.

I have not examined in detail the musculature which is involved in these cardiac movements, but believe that the whole mesenteron is contracted by a series of external longitudinal muscle fibres which extend along its entire length (pl. 6, fig. 9). This would also account for the wrinkling up of the peritrophic membrane which is often seen in sections of the midgut.

In Simulium larvae the peritrophic membrane is, as previously stated, exceptionally thick and well developed. This is almost certainly due to the fact that these larvae live to a great extent on diatoms and other siliceous matter which, but for the protection afforded by such a membrane, would be very liable to damage the walls of the mesenteron. In these larvae the membrane also remains, for the greater part, intact in the proctenteron, still closely investing the food till it is voided from the anus. That large quantities are continually being formed is evidenced by the fact that the larvae pass faecal matter very frequently and that the faeces are always enveloped in a plentiful supply of the membrane. The membrane is doubtless impervious to anything but liquids as was emphatically stated by van Gehuchten ('90) who concluded that the digestive fluids and the products

of digestion pass through it by osmosis. Since this is the case, and since, as before stated, the membrane is exceptionally thick and complete from the proventriculus to the anus, it would seem to be very difficult or even impossible for Microsporidian parasites to gain access to the tissues of the larvae after the membrane has been formed.

c. The relation between these structures and parasitism

In describing the structure of the cardia I drew attention to the fact that there is no peritrophic membrane in the embryo, and it does not seem unreasonable to suppose that in the earliest larval stage the membrane does not entirely surround the food. Indeed I have reasons to believe that the first meal or so of the young larva is the most critical of its life if Microsporidian spores are present in the stream in which it hatches. These reasons are three in number. First, in all cases of parasitised individuals there are indications that the parasite gains admission to the body cavity at a very early period in the life of the larva, for I have never succeeded in finding a full or even half grown larva in which the early stages of the parasite were present. Secondly, owing to the minuteness of the spores, which are comparatively heavy and sink in water, and to the smoothness of their shells, they would readily pass through the fans of somewhat developed larvae which have assumed a vertical position in feeding. In the earliest stages, however, as shown above, it is conjectured that food has to be sought out by the larva which picks up what it can find collected in small depressions in the stones over which it moves. In this way it is extremely likely that it would ingest one or more of the innumerable spores which have been liberated in the water and which have, whenever possible, sunk to the bed of the stream. The third reason is that, as inferred at the conclusion of the last paragraph, some of the first food to escape from the proventriculus may not be entirely separated from contact with the mesenteron by the peritrophic membrane. For if one allow that at this time the membrane is already partially formed, it will not be present far beyond the orifice of the proven-

triculus and must remain open at the end all the time the food is carrying it backward through the mesenteron. This would give the young germ, liberated from the spore by the action of the digestive juices, ample time to escape from the open end and thus get into actual contact with the epithelium, from which, soon after, it would be entirely cut off during the remainder of the larval life.

CLASSIFICATION OF THE SPOROZOA

The class Sporozoa consists of essentially parasitic Protozoa, from the attacks of which, in all probability, none of the higher forms of life from the annelids up to the vertebrates is immune. The most salient characters of the class are the following:

1. Nutriment is always of a fluid nature and is absorbed by osmosis.
2. Ingesting and digesting organs are never present.
3. Flagella may be present in certain stages of development; these are used for locomotion or attachment but never for nutrition.
4. Certain stages may be amoeboid, but the pseudopodia are used exclusively for locomotion.
5. All forms are capable of sporulation in order to increase the infected area of their host, or by the spores escaping from it to spread the disease.

The Sporozoa are divided by Schaudin into two subclasses as follows:

Subclass I: Telosporidia. Sporozoa in which spore formation ends the individual life; the entire cell then forms spores. Thus the reproductive phase is distinct from and follows the trophic phase. To this subclass belong three orders: Gregarinida, Coccidiida, and Haemosporidia. I shall have occasion to refer to the first of these three orders, namely, the Gregarinida, later.

Subclass II: Neosporidia. Sporozoa in which reproduction begins during the trophic phase and the entire cell is not at once used up in the production of spores. To this subclass belong two orders: Myxosporidia and Sarcosporidia.

The Myxospiridia, with which we are mainly concerned in this paper, have the following characters:

1. The earliest stage of the trophozoite is amoeboid.
2. Spore formation usually begins at an early period and continues during the growth of the trophozoite.
3. The spores are produced endogenously.
4. Each spore possess one or more polar capsules.

There are two suborders of the Myxosporidia, mainly separable on spore characters. These are:

Suborder I Phaenocystes (Gurley) = Myxosporidia *sens. str.*, with large bilaterally symmetrical spores having two or four polar capsules which are plainly visible in the fresh state. Two spores are formed in each pansporoblast.

Suborder II Cryptocystes (Gurley) = Microsporidia (Balbiani), with minute pyriform or oval spores having one polar capsule, which is visible only after treatment with reagents such as weak HNO_3, and in some cases not even then. One (Nosema), or more than two spores are formed in each pansporoblast.

The Protozoan parasites of Simulium larvae all (with the exception of an undetermined Gregarine) fall into the second of these sub-orders, namely the Microsporidia of Balbiani.

TYPICAL LIFE CYCLE OF A MICROSPORIDIAN

The Microsporidian genera are distinguished entirely by their mode of development, and largely by the final stage, i.e., sporulation. A brief account of the development and means of infection of these parasites will be useful before we pass to the generic classification.

Stage I: The germ. This name is applied to the minute amoeboid, motile body which is liberated in the alimentary canal of its host from a spore which has been taken in with food. At the time of liberation the germ has either one or two nuclei. In the latter case the two nuclei soon fuse. A minute vacuole is also sometimes present (Stempell '09). The germ passes between the epithelial cells of the gut and so reaches the blood sinuses. It is now termed a 'planont.'

Stage II: The planont. This body is also minute and neither this nor the preceding stage have been actually found in the great majority of described Microsporidia. The best account is

that given by Stempell ('09) of Nosema bombycis Nag. Owing to their intercellular life the planonts are readily distinguished from the following stages which are intracellular. They measure (in N. bombycis) from 0.5 to 1.5μ in length and are bullet-shaped, but have sufficient amoeboid movement to enable them to spread over the body. Under favorable conditions the nuclei can be seen. Division occurs in this stage, often quite actively so that masses of these minute organisms are seen.

Stage III: the meront. The planont enters a cell and at once loses its motility, becoming spherical or oval. In this stage division of the nucleus is rapid. In certain genera each division of the nucleus is accompanied by a division of the protoplasm so that *numerous meronts* are formed. In other genera the nucleus divides independently of the protoplasm so that a multinucleate body is formed, which may attain considerable dimensions. This body is usually termed a 'myxosporidium' or 'trophozoite.' The typical myxosporidium consists of a very clear ectoplasm surrounding a granular entoplasm. The former may be capable of throwing out pseudopodium-like processes which are used only as locomotor organs. Most often the myxosporidium is sessile, and in some genera is capable of encystment. The entoplasm is coarsely granular and sometimes slightly yellowish. It contains numerous rapidly dividing nuclei and in addition may possess fat globules, pigments and one or more vacuoles. In the genus Nosema, where the meronts are numerous and uninucleate, each of them matures directly into a 'spore,' but in all the other genera there are intermediate stages between the meronts and spores. During the meront stage the parasite usually breaks down the cell of the host in which it was formed from the planont, and lives for the remainder of its life as an intercellular parasite.

Stage IV: sporont. A small clearly defined sphere of protoplasm collects around each of the nuclei and its peripheral layer condenses to form a delicate envelope. The subsequent development of the sporont varies in different genera and species. Mercier ('08) interpreted the subsequent developmental stages in Thélohania giardi Henn. as follows: The indefinite nucleus

is purified by the rejection of part of the chromatin of which it is constituted. The remaining chromatic matter fuses to form a ring-shaped nucleus which undergoes a division intermediate between mitosis and amitosis. The protoplasm also divides to form two uninucleate bodies. Both of these undergo two subsequent and similar divisions within the membrane of the sporont, so that the latter, which is now termed a 'pansporoblast' contains eight similar bodies or 'sporoblasts,' together with a small quantity of rejected chromatic matter.

Stage V: sporoblast. These eight bodies assume a pyramidoidal shape and their nuclei undergo a somewhat complicated division, at the end of which the sporoblasts contain three nuclei surrounded by a dense cytoplasmic mass. A circular vacuole appears within the cytoplasm and rapidly increases in volume. One of the nuclei is attached to the vacuole. The latter becomes pyriform in shape and the narrowed end comes in contact with the surrounding envelope of the sporoblast. This vacuole is the 'polar capsule' and at the point where it comes in contact with the envelope, a coiled up, evaginable filament is formed within it. In all of the forms I have examined there are two vacuoles, the first of which travels to one end of the somewhat elliptical spore and entirely replaces the cytoplasm of this region to form the vacuole of the spore. The pyriform polar capsule is subsequently formed at the opposite end of the spore, where its location is not very evident on account of its being surrounded by the cytoplasm. It may however increase considerably in size so that it projects far into the vacuole.

Stage VI: spore. At the time when this internal maturation has been accomplished, a very thick, though remarkably transparent shell has been formed around the sporont which is thus converted into a 'spore,' strongly resistent to all external conditions. There is a single minute pore in this shell situated opposite the point of contact with the narrowed end of the polar capsule, and therefore in communication with the base of the filament. The spore thus consists of a very resistant transparent shell containing a single polar capsule, in which is a coiled evaginable thread. Surrounding the base of this capsule is a

collar-like mass of protoplasm containing either two or four nuclei, while the apex projects into a large vacuole occupying almost half of the area of the spore. Both Mercier ('08) and Stempell ('09) find that in T. girardi Henn. and N. bombycis Nag. respectively there are two minute nuclei attached to the shell and one similarly minute nucleus attached to the polar capsule.

By means of these spores the disease is disseminated among new hosts. Where the parasite infects the epithelial cells of the gut and other excretory organs the spores escape into the alimentary tract and are passed out of the body with the faeces. If other organs form the seat of attack the spores are liberated, either by forming a tumor through the bursting of which they escape, or they await the death and subsequent decay of their host. Pasteur ('70) showed, in the case of the pébrine of silk-worms that the spores can pass into the ovary and thus spread the disease by infecting the eggs. If the host is aquatic, as is most often the case, the spores fall to the bottom of the water, and there remain unchanged till taken into the alimentary tract of a new host, while in terrestrial hosts, as in the silk-worm, the spores are scattered in the faeces of infected larvae and thus contaminate the food on which the healthy larvae are feeding.

As soon as the spores reach the foregut of a new host, the digestive juices set up intrasporal pressure either by causing the shell to contract, or by passing through it by osmosis, which contracts the polar capsule and thus ejects the spirally coiled filament through the pore in the spore shell. Stempell ('09) points out that the filament, owing to the manner in which it is ejected, evidently consists of a hollow tube which is everted when it protrudes from the spore. The filament is often very long, that of the spore of [Nosema] Glugea simulii Lutz and Splendore ('08) and of Glugea fibrata sp. nov., which I have recently found in Simulium larvae, being thirty to forty times the length of its parent spore. The function of this filament has been the subject of much discussion. It was first discovered by Balbiani in 1863, when he attributed to it a function similar to that of the antherozoid of the Cryptogams. In 1882 Bütschli

drew attention to the similarity between the polar capsule and its contained filament to the nematocysts of coelenterates. He, however, did not assign to it a similar urticating function, but suggested the now generally accepted theory that the filament serves to attach the spore to new hosts or to their food. It should, however be borne in mind that the filament, so far as known, is not evaginated till the spore has been taken into the gut of a new host, a fact which appears to lessen considerably, if not to entirely nullify the use of this organ, if its function be such as Bütschli surmised. The filament is soon detached from the spore, leaving a small opening in the shell through which the binucleate cytoplasmic contents escape. Stempell ('09) finds that in N. bombycis, where four nuclei are present in the spore, only two of them pass out of the shell with cytoplasm, the other pair remaining behind and degenerating. The small free body thus liberated is the germ, and its nuclei soon fuse to form the single nucleus of the planont.

CLASSIFICATION OF THE MICROSPORIDIA

Stempell '09 has recently revised the classification of the Microsporidia as follows:

Family 1: Nosematidae. The vegetative stage is intracellular and consists of unicellular dividing meronts.

a. Genus Nosema (Nägeli '57). Each meront gives rise to a single spore.

b. Genus Thelohania (Henneguy and Thélohan '92). Each meront gives rise through a sporont to eight spores.

c. Genus Gurleya (Doflein '98). Each meront gives rise through a sporont to four spores.

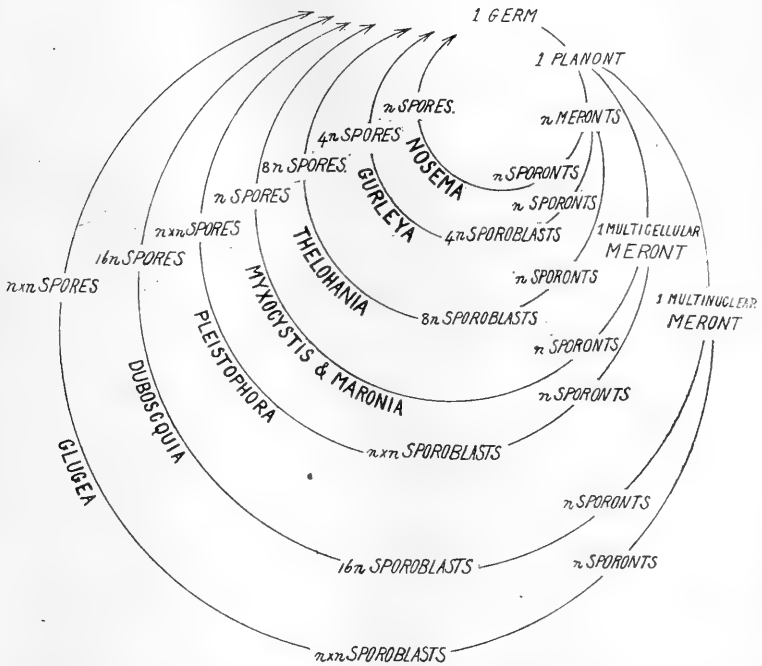
Family 2: Pleistophoridae. The completely mature vegetative stage consists of multicellular, often amoeboid, motile meronts.

a. Genus Pleistophora (Gurley '93). The vegetative stage passes into rounded sporonts from which many spores arise.

b. Genus Maronia (Stempell '09). Spores arise through endogenous budding within the protoplasm of the amoeboid vegetative stage.

c. Genus Myxocystis. (Mrazek '97). Spores arise through endogenous budding within the protoplasm of the vegetative stage whose ectoplasm consists of motionless cilia.

Family 3: Glugeidae. The vegetative stage is multinuclear and motionless, remaining undivided and encysted. Sporonts arise in it by endogenous budding.



Text fig. 2 Diagram showing life cycles of Microsporidian genera

a. Genus Glugea (Thélohan '91). The number of spores formed from a sporont is variable.

b. Genus Duboscquia. (Pérez '08). The number of spores formed from a sporont is sixteen.

For the sake of making clear the life-histories of these various genera of Microsporidia as conceived by Stempell I have constructed the accompanying diagram (fig. 2).

GENERAL HOST RELATIONSHIPS OF THE MICROSPORIDIA

By far the greater number of known cases of Microsporidian infection have been recorded from fish, though infection of crustaceans, particularly of crabs, is not infrequent. In insects about forty cases of infection have been recorded. A great number of the descriptions, however, are very incomplete. All tissues of the host are liable to be the seat of infection, though in fish the muscles are the most favorable, while in insects the fat body usually suffers.

The tissue-infecting forms fall into two categories: (1) *Concentrated*, in which the adjacent tissues form a membrane around the trophozoite, or the trophozoite itself is limited by a definite membrane; (2) *Diffuse*, in which the protoplasm of the parasite and host cells becomes indistinguishably mingled, till the tissue is found to be infiltrated with vast numbers of spores (Nosematidae).

As a general rule, infection by these parasites is not fatal to the host though its vitality is much impaired. When larvae of insects are infected the parasite is often able to pass over into the adult and in the case of pébrine is capable of infecting the ova, so that the disease is transmitted to the next generation.

Insect hosts of Microsporidia

The following list of the insect hosts of Microsporidia is partially adapted from Minchen's work ('03) on Sporozoon hosts, with additions from more recent literature. Doubtful, or incompletely described forms have been in most cases omitted.

DIPTERA

Anopheles maculipennis, larva.....*Thelohania legeri* Hesse ('04)
Chironomus sp., larva.....*Nosema chironomi* L. and S. ('08)
Corethra sp., larva.....*Nosema corethrae* L. and S. ('08)
Limnophila rhombia, larva.....*Thelohania janis* Hesse ('03)
Pachyrhina pratensis, larva.....*Glugea stricta* Monz
Simulium ornatum, larva.....*Glugea varians* Legér ('97)
Simulium venustum, larva.....[*Nosema*] *Glugea simulii* L. and S. ('08)
Simulium ochranum, larva.....[*Nosema*] *Glugea simulii* L. and S. ('08)
Simulium bracteatum, larva } ..... { *Glugea bracteata* sp. nov.
Simulium hirtipes, larva } { *Glugea fibrata* sp. nov.
Simulium vittatum, larva.....*Glugea multispora* sp. nov.
Stegomyia fasciata, larva and adult *Nosema* sp. Simond ('03)
Stegomyia fasciata, adult.....*Nosema stegomyiae* L. and S. ('08)
Tanypus varius, larva.....*Thelohana pinguis* Hesse ('03)

LEPIDOPTERA

Bombyx mori, all instars.....*Nosema bombycis* Näg.
Catopsila eubule, larva.....*Glugea eubulis* L. and S.
Danais erippe, larva.....*Glugea erippe* L. and S.
Danais gelippus, larva.....*Glugea erippe* L. and S.
Diono juno larva.....*Glugea janoris* Los.
Ephialtes angulosa, adult.....*Nosema ephialtis* L. and S. ('08)
Gastrophilus neustria, larva.....*Nosema bombycis* Nag.
Lophocampa flavostica, larva.....*Glugea lophocampae* L. and S.
Mechanitis lysimnia, larva.....*Glugea lysimniae* L. and S.
Scea auriflamma, adult.....*Nosema auriflammae* L. and S. ('08)

COLEOPTERA

Balaninus amaryllis, larva.....*Glugea stempelli* Pérez ('05)
Otiorhynchus fuscipes, larva.....*Mycetosporidium talpi* Legér and Hesse ('05)

ORTHOPTERA

Periplaneta americana,.....*Glugea periplanetae* L. and S.
Termes lucifugus.....*Duboscquia legeri* Pérez ('08)

NEUROPTERA

Ephemerella ignita.....*Gurleya legeri* Hesse ('03)

MATERIAL AND METHODS OF STUDY

Simulium larvae from infested streams were frequently collected and brought to the laboratory, but since parasitised larvae could never be kept alive for much longer than over night, the majority were killed at once, a few being kept for dissection.

Killing for sectioning. The best fluid for this purpose proved to be one recommended by W. Kahle ('08) which is made up as follows: water, 30 parts; 96 per cent alcohol, 15 parts; 40 per cent formalin, 6 parts; glacial acetic acid, 1 part.

A fluid recommended by Stempell ('09) consisting of corrosive sublimate (saturated aqueous solution) 2 parts; 95 per cent alcohol, 1 part; and a trace of acetic acid, also gave good results, but for subsequent staining with iron haematoxylin was inferior to Kahle's fluid. Either fluid was used by being brought to a temperature of about 70° C., when the larva was immersed and allowed to remain in the cooling fluid for about fifteen minutes. To insure penetration the larval skin was punctured with a fine needle as soon after death as possible.

Killing for smear preparations. Kahle's fluid poured over the smear gave good results, but a method recommended by Perrin

'06 for Pleistophora periplanetae proved somewhat superior before staining with an eosin-azure mixture. With this method a smear is made on a cover glass and allowed to dry. It is then immersed in absolute alcohol for ten minutes and again dried. Perrin then stained over night in one of Giemsa's preparations, after which the slide was washed, dipped for a moment in absolute alcohol, rewashed and mounted in cedar oil.

Stains. For general histological purposes Heidenhain's iron haematoxylin in combination with orange-G proved to be the most satisfactory. This combination, however, is almost useless for studying the nuclear matter of the spores, for the cytoplasm takes up the haematoxylin as readily as does the nuclear matter and it cannot be again washed out. The usual stain for this purpose is Giemsa's 'Eosinazur' stain. The blue of this mixture however proved to be too intense. Other Giemsa preparations were not altogether satisfactory and I finally found it preferable to stain separately with azure II and eosin A.G. and to differentiate by overstaining with a 0.8 per cent aqueous solution of azure II, rapidly washing with water and then dehydrating. The absolute alcohol contains a saturated solution of eosin and the slide can be left in it for about five minutes after which it is immersed in xylol and examined in it as a medium. The nuclei of the spores have a strong affinity for eosin which can be readily washed out of the other tissues with water. If the blue be too intense it can be washed out in alcohol. By overstaining with both azure and eosin and bearing in mind that the former can be washed out most readily with alcohol and the latter with water, one can, with a little practice, get both stains nearly counterbalanced.

THE MICROSPORIDIAN AND OTHER PARASITES OF SIMULIUM LARVAE

The earliest account of a Microsporidian parasite of Simulium larvae that I have been able to trace was that of Léger '97. He describes a Glugeid, G.varians, parasitising the larvae of S. ornatum, a species common in France. In 1904 Lutz and Splendore described various forms of Glugeids found in the larvae

of S. venustum and S. ochraceum. In a further discussion of these forms in 1908, they regarded them all as varieties of one species, which they designate as Nosema simulii. The locality in which this presumably South American species was taken is not stated. In a subsequent paper by Lutz ('09) on the Simulidae of Brazil, he mentions the presence of Nosema sp. in the larvae but gives no details. The only other account seems to be the the one which I published in 1911. In that I described the external appearance of the spores of several undetermined species which infest the larvae of S. hirtipes and an undescribed Simulium larva common in the streams in the vicinity of Boston, Massachusetts.

During the fall of 1911 I found three further well-marked forms in the Simulium larvae of the same locality. One of these resembles the form described by Léger. His description, however, is very short and is not accompanied by figures. The filament of the American species is about twice as long as that of G. varians. Another form resembles somewhat Lutz and Splendore's figures of [Nosema] Gluguea simulii but here again the filament of the latter species is about six times the relative length of the American species. Probably, with a fuller account of the development of these exotic species, other differences would be found, especially in the case of G. varians, for it is unlikely that the same species of Microsporidian should occur in both hemispheres parasitising larvae of such specialized and confined habitats as those of Simulium. It is true that this genus of Diptera is cosmopolitan and is recorded by Grünberg ('07) as occurring even in Lapland and Greenland, but since none of the European species has been recorded from America it is very unlikely that their parasites, which also occur in both hemispheres, should be but varieties of the same species.

The Microsporidian species under consideration apparently all fall into the genus Glugea as defined by Thélohan ('91). In 1892 Henneguy erected the genus Thelohania of which I have been unable to obtain the original description. The sporulation of the species I have described as Glugea bracteata is typical of the genus Thelohania according to Gurley's ('94)

interpretation, which, from the same writer's modified definition of Glugea, debars it from this genus. The early stages are however typically Glugeid so that I have provisionally placed the species in this genus. I shall discuss this more fully after describing the species.

During February, 1912, I found a species of Glugea infesting the larvae of Simulium sp. in streams around Port of Spain, Trinidad, B.W.I. The spore formation in this species was polysporic in which respect it resembled S. multispora, of which a description is given further on in this paper. This species was not abundant, although I found some half-dozen infected larvae.

Other parasites which I have found infesting Simulium larvae in the neighborhood of Boston are a nemathelminth belonging to, or near, the genus Mermis, which, during the spring of 1911, parasitised some 25 per cent of the larvae of the streams in which it occurred. As pointed out in my former paper ('11) on this parasite, its presence is fatal to the host in every case. I have since found that the presence of these worms in Simulium larvae was also mentioned by Lutz ('09) in his work on Brazilian species of the genus.

During the fall of 1911 another parasite was found infesting the majority of larvae in certain streams and, as I shall show, its presence is evidently fatal to the host. I sent preparations of this parasite to Professor Calkins, and he informs me that it is probably a species of Gregarine. I have not studied it in detail and shall confine my remarks in the sequel to its general appearance and its effect on its host.

GLUGEA BRACTEATA SP. NOV.: (Plate 2)

Macroscopic appearance. In later stages this parasite is present in the body cavity of its host as a large irregular white mass, sometime measuring as much as 2 mm. in length. This mass may consist of one very large, or several smaller myxosporidia which together only occupy as much space as does the single large myxosporidium. It is usually confined to the posterior portion of the abdomen, which becomes much distended, owing to the large mass of the parasites which is most voluminous latero-ventrally to the alimentary canal (pl. 2, fig. 1).

Microscopic structure. If a portion of one of these milk white masses be dissected and placed in a drop of water under a cover glass it will be seen with the high power of a microscope to consist of many different minute bodies, the most abundant in late stages being small aggregates of eight short-oval spores (pl. 2, fig. 11). If the cover glass be gently pressed these aggregates will open up and take on an appearance like that shown in plate 2, figure 12. By gently rolling these aggregates between the cover glass and the slide they can be broken up and the now separated spores are seen to be nearly uniform in size, short elliptical in shape and to measure about 3μ x $2.5 - 2.7\mu$. If weak iodine solution be added to the water in which they are floating a few of them will eject a short filament measuring about six times the length of the spore (pl. 2, fig. 18).

Life history. In order to trace the developmental stages of this parasite, sections not over 5μ thick must be prepared and stained, preferably with iron haematoxylin and orange-G. The earliest stages, viz., the planont and early meront, are still unknown, but I shall attempt to trace their conjectural development in a later paragraph.

Myxosporidium. At the time of discovery of the parasite the mass of meronts, or, as this is now better termed, the myxosporidium, consisted of a multinucleate mass of protoplasm measuring some 2 to 3 mm. in length, in which no definite ectoplasm and endoplasm could be distinguished. The central, and larger, portion of the myxosporidium had already sporulated and contained ripe spores; surrounding this area were all stages of development up to the as yet almost undifferentiated thin layer of protoplasm which still persisted around the edges of certain parts of the mass and represented all that was left of the true meront stage. In the stained section there is a very definite differentiation between the ripe spores and the early stages, for the former stain very intensely with haematoxylin, whereas the latter are left practically unstained by this, though showing a marked affinity for the orange-G, so that the section takes on the characteristic appearance shown in plate 2, figure 2.

Sporonts. These are typically formed by the condensation of the myxoplasm around the numerous nuclei of the myxosporid-

ium and this results in the formation of small globular bodies, around which the protoplasm hardens to form a fine constricting membrane (pl. 2, figs. 3 and 5). At times, however, they appear to be budded off from the peripheral myxoplasm toward the center of the mass, as shown in plate 2, figure 4. In either case the resulting sporont is the same, consisting of a spherical body mesuring about 5.8μ in diameter. In this and in all subsequent stages up to that of the spore it is extremely difficult to stain the nucleus, for the various nuclear stains tried washed out of the chromatic material almost if not quite as readily as out of the protoplasm. Development, however, seems to progress somewhat along the following lines: The nuclear matter is at first diffuse and may undergo depuration, for in some cases small granules of more deeply staining chromatic matter can be seen near the surrounding cell membrane. The sporont continues to grows till it reaches a diameter of nearly 10μ at which period it becomes evident that the the nucleus has undergone three successive binary divisions, since in medium sized sporonts there are indications of four masses of chromatic matter (pl. 2, fig. 6) while in the largest there are eight readily visible globular bodies (pl. 2, fig. 7). When these ripe sporonts are dissected out, their constricting membrane at first still surrounds the eight contained sporoblasts, for such are the globular bodies within them. Very soon, however, this membrane splits and liberates the small aggregate of sporoblasts (pl. 2, figs. 9 and 10), which now readily stain and show a dense central mass with a strong affinity for haematoxylin. The now detached membrane (fig. 8) is seen to contain a little surplus protoplasm and also often a few grains of chromatic material. Normally, however, this membrane persists till the complete spores are formed, when it dissolves, as I infer from the fact that in none of the sections were there detached membranes among the liberated spores.

Sporoblasts. The mature sporoblast measures about 3μ in diameter. Its nuclear matter could not be differentiated by any of the stains used, but the protoplasm stained deeply with haematoxylin (pl. 2, fig. 10). As the sporoblast matures, a vacuole appears in the center of the protoplasm (fig. 13) and travels

towards the periphery; the protoplasm meanwhile becomes more condensed on the side of the vacuole which is farthest from the periphery of the sporoblast. At the same time the cell assumes a more elliptical form and its superficial layer becomes differentiated into a very transparent thick shell.

Spores. The sporoblasts have now been transformed into spores. These are short elliptical bodies, somewhat truncate at the ends, measuring about 3μ x $2.5 - 2.7\mu$ (pl. 2, figs. 14 to 17 and 21 to 24). They still remain attached to one another in aggregates of eight, although they are not in any way actually united with one another. In size and shape they are extremely uniform, though I have observed two larger spores measuring some 8 x 5μ. As the occurrence of such forms is extremely rare I am inclined to consider that their presence is due to some abormality in development. In a fresh state the spores show no differentiation except a small refractive area near one extremity. This is the vacuole. If weak iodine solution be added to the water in which the spores are floating the inner wall of the very thick shell can usually be seen, while in a very few cases a comparatively stout filament, some six times the length of the spore, is extruded from the end of the spore farthest from the vacuole. Although undoubtedly all the spores are capable of protruding this filament I was able to cause them to do so in considerably less than one out of every thousand cases, though I tried many of the usual reagents for the purpose. From a nuclear study I am inclined to believe that the spores were not quite ripe and that this accounted for my inability to extrude the filament in more cases. As winter approached development evidently ceased, and spores dissected out of larvae and kept in water showed no signs of continued development.

For studying the internal structures of the spores the most successful stain used was that of azure II and eosin A. B. as previously described. In the young spore the nucleus occupies the extremity of the cell opposite to that occupied by the vacuole. At first it is rather diffuse but later becomes more concentrated to form a small globular body (pl. 2, fig. 14). Meanwhile the cytoplasm becomes still further condensed till

it is drawn away from the walls of the spore around the equatorial region as shown in figure 14, etc. With iron haematoxylin all of this stains very deeply and uniformly as though it were all of a chromatic nature, and it would seem that it must have been to some similar appearance in the spore of Glugea varians Léger that Vaney and Conte ('01) referred when they likened the nucleus of this species to a double T. I was unable to find more than two nuclei in these spores. In the spores of Thelohania giardi Mercier ('08) found five nuclei, and in those of Nosema bombycis Stempell ('09) found four. In some of the specimens there were, however, indications of two darker specks one on each side of the vacuole as shown in figure 22. These would correspond to the shell nuclei of Stempell ('09) but I was unable to distinguish any red coloration. As before stated it is not improbable that the spores were immature, and it is quite possible that the two nuclei I was able to demonstrate undergo a further division before maturation. At any rate, this seemed to be the case in the spores of another species studied. At a later stage the cytoplasm is once more pressed back againt the walls of the spore (pl. 2, figs. 16 and 23). This is due to the formation of the polar capsule within this area. As I never found any evidence of this structure projecting into the vacuole I have adapted the diagram of a ripe spore of N. bombycis as given by Stempell ('09) to the form as shown in pl. 2, figure 23. Full details of its growth could not be seen, since it is entirely surrounded by deeply staining protoplasm. A small depression in the protoplasm appears between the two nuclei, at the end of the spore opposite to the vacuole, which increases in depth until it forms a pear-shaped cavity within the protoplasm. In an end to end view of a spore it is seen that the capsule nearly or quite reaches the vacuole, for by focussing up and down one can see right through the spore (fig. 17). The outline of the capsule and its contained filament with the pore in the shell through which it is ejected, were not seen, nor in stained specimens was the filament ever found attached to a spore as depicted in plate 2, figures 23 and 24. These and accompanying figures of the series are purely diagrammatic. The filament is readily detached

from the spore and it is then seen that its base is considerably swollen to a knob-like structure (fig. 25).

The host of Glugea bracteata is the larva of Simulium bracteatum and Simulium hirtipes (?).. It infects about 10 per cent of the larvae. I have found this parasite only in the Arnold Arboretum, at Forest Hills, Massachusetts.

From the short descriptions of [Nosema] Glugea simulii by Lutz and Splendore ('04 and '08) it would seem that Glugea bracteata is closely related to the octosporic varieties of this species. Although it is stated that the size and shape of the spores of this form are very variable, the one figured ('08) is almost identical in shape with those found in G. bracteata, with the exception of the filament which, according to the text, is some six times as long as that of the latter species. In a figure of an octosporic pansporoblast, also, it would appear that the pansporoblast membrane is subpersistent, although no statement to this effect is given in the text. From the descriptions of the polysporic varieties, which were not figured, it is evident that, if these all represent the spores of the same species, it must be extremely polymorphic, for the extremes of variation in this species alone have heretofore been considered to be of generic value. Should further study prove conclusively that this is the case several of our now accepted genera will have to be placed in synonomy.

GLUGEA FIBRATA SP. NOV.: (Plate 3)

Macroscopic appearance. In later stages this parasite is present in the body cavity of its host as several large irregular milk white masses, which, as a rule, spread through the entire body though they are most voluminous in the swollen apex of the abdomen (pl. 3, fig. 1).

Microscopic structure. If a portion of one of these white masses be placed in a drop of water under the high power of a microscope it will be seen to consist of several different bodies, the most abundant of which, in later stages, are small oval spores. These spores are all separate and for the greater part nearly uniform in size, measuring about 5.8 to 6.6μ x 3.5μ. Occasionally however one can find a much larger spore measuring 9 to

7.8μ x 4.7 to 5.1μ. These two types of spores are termed 'microspores' and 'macrospores' respectively. If weak iodine solution be added to the water in which they are floating a greatly attenuated filament which measures about 170 to 220μ, or thirty to forty times the length of the spore, is usually projected (pl. 3, fig. 25.).

Life history. This can be determined only from sections about 5μ thick. The early stages have not been found.

Myxosporidium. The parasite at the time of discovery was in a rather advanced state of sporulation. No differentiation into ectoplasm and entoplasm could be distinguished in the myxosporidium, the more central portions of which had for the greater part sporulated. In stained sections there was no sharp differentiation between the developmental stages and the spores as was the case with Glugea bracteata, since the chromatic matter of the former retained the haematoxylin stain. The secretions, then, have the characteristic appearance shown in plate 3, figures 2 and 3. Around the periphery of the myxosporidium the myxoplasm had not been entirely transformed into sporonts but contained numerous small masses of slightly staining granular chromatin. Many of these apparently free nuclei were in a state of division (fig. 4), which appeared to be typically amitotic. Around these nuclei a slight condensation of the myxoplasm could be detected. These condensed areas became globular and were finally invested with a delicate membrane (figs. 5 and 6) until each became a spherical sporont about 5.5μ in diameter.

Sporonts. The nucleus of the sporonts becomes very irregular (pl. 3, fig. 6) and apparently undergoes a form of purification, for in some cases a few chromatin grains are passed out to the surface of the cell where they disintegrate. The nucleus then assumes a more definite form and the sporont continues to grow until it measures about 12μ in diameter, when the nucleus again becomes active and divides. There seem to be two forms of division at this stage. In one the nucleus becomes ring-shaped and this ring draws out as shown in figures 7 and 8. In the other the globular chromatic mass simply divides by amitosis to form two hemispherical masses as shown in figures 10 and 11. Each of the nuclei thus formed redivides. I saw this division only

twice and in each case the nuclear matter had evidently assumed a ringlike form before division (fig. 12). It is very difficult to trace the developmental stages beyond this point until the sporoblasts are in process of formation, since all the maturation stages are progressing simultaneously in such a small area that they are practically inseparable, and tracing the individual developmental stages is rendered almost impossible. In the next stage that I was able to distinguish with any certainty, there was evidently a third nuclear division for the much enlarged sporont contained eight distinct nuclei (fig. 13).

Sporoblasts. The wall of the sporont now becomes much thickened and indented between the eight regularly spaced nuclei (pl. 3, fig. 14). This indentation progresses between the nuclei which thus become surrounded by a nearly globular mass of cytoplasm as shown in figure 16, which represents a section of one of these bodies. The walls gradually meet around each of these eight sporoblasts and the aggregate is then separated to form eight free spherical sporoblasts (fig. 17) which measure at first about 5.2μ in diameter but later increase somewhat till they measure about 6.2μ.

Spores. The sporoblasts are transformed into spores by a process apparently similar to that described for G. bracteata, except that in this case the spores are entirely free throughout the whole of their development. The spore is oval in shape, measuring 5.8 to 6.6μ x 3.5μ, and is surrounded by a very thick transparent shell. At the broader end there is a very large vacuole, plainly visible in the fresh spore, while at the smaller end is a much smaller vacuole which cannot be seen in untreated preparations. The nucleus does not differentiate so distinctly in the spore of this species as in G. bracteata, though I found indications of it as a minute body which was often dividing or already divided and situated just above the smaller vacuole (figs. 18 and 19). On one occasion each of the two nuclei formed by the division of this primary nucleus was again undergoing division (fig. 20). By staining deeply with haematoxylin the polar capsule is sometimes distinguishable, projecting through the dense protoplasm into the large vacuole (fig 21). The dia-

gram of the spore can be constructed as shown in figure 26, in which the four nuclei, as yet incompletely divided, are indicated, while the polar capsule, projecting far into the vacuole, contains the greatly elongated coiled filament. This filament, when ejected by the action of iodine, attains the comparatively enormous length of thirty to forty times that of the spore (pl. 3, fig. 25). It should be borne in mind that, as Stempell points out, this filament must, from the manner in which it is ejected, consist of a hollow tube which has to be entirely everted during its emergence from the spore! When it first appears it may be in the form of a loose spiral (fig. 23) but this quickly disappears as the filament soon straightens out. This filament is not so readily separated from the spore as is that of G. bracteata, but in the few cases where it was seen to be detached the basal portion was swollen up into a knob (fig. 24) as in that species. In all the spores in which the filament was protruded it was noticed that the spore contents lost their regularity. I was unable to differentiate the parts, but it seemed that the polar capsule shrank and became attached to one side of the spore, while the protoplasm and nuclei settled down into a small area at the narrow end of the spore (figs. 24 and 25).

Macrospores. These were not numerous among the typical microspores, and appeared to be somewhat abnormal, for the shell was much thinner than that of the microspores (pl. 3, fig. 22). When treated with iodine none of them was seen to eject a filament.

This species of Glugea seems to be related to G. varians Léger ('97). The spores, however, are somewhat smaller and the filament is proportionally about twice as long. Léger and Hagenmüller ('08) state that in G. varians the development of the spores is either octosporic or polysporic. This does not appear to be the case with G. fibrata, for though I found on two occasions 16 sporoblasts adhering together, this did not have the appearance of being a normal condition.

The host of G. fibrata is the larva of Simulium bracteatum and Simulium hirtipes (?). In infests about 5 per cent of the larvae. It was found both in the Arnold Arboretum and in Franklin Park, at Forest Hills, Massachusetts.

GLUGEA MULTISPORA SP. NOV.: (Plate 4)

Macroscopic appearance. In its later stages this parasite is present in the body cavity of its host as small rounded white masses often measuring as much as 1 mm. in diameter. There may be but one or many of these masses scattered irregularly throughout the whole body, which is not greatly swollen by their presence (pl. 9, fig. 1). Where there is only one it is not much larger than the more voluminous masses of a multiple infection, and since it does not then distort the body it is not very readily seen, especially as the skin of the host is not very transparent.

Microscopic structure. If a small quantity of one of these masses be placed in a drop of water under a cover glass it is seen, under a high magnification, to consist of many more or less globular bodies, varying in size from 11.5μ to 30μ. The larger bodies consist of aggregates of numerous small sporoblasts or spores. By gently rolling the cover glass the latter aggregates can be readily broken up and will then be seen to consist of somewhat elongate spores measuring about 4μ x 2.5μ.

Life history. Sections of the parasitic masses stained with iron haematoxylin and orange-G show very beautifully the later developmental stages of this parasite, though, owing to the small quantity of material, I have been unable to obtain as complete a series as I could wish. Plate 4, figures 2 and 3, show the characteristic appearance of sections of the parasite in which the later developmental stages are sharply separated.

Myxosporidium. The parasite at the time of discovery was in a rather advanced state of sporulation. Around the myxosporidium was a very definite membrane, though at this stage of development it was impossible to determine whether this was formed by the parasite or the host. The earliest stages were the sporonts, which occurred round the edges of the mass.

Sporonts. These are rounded bodies measuring some 9μ in diameter. The chromatic substance (fig. 4) is very diffuse and spreads in a network throughout the cell. I did not observe the primary division of the sporont, but it is evident that in later stages the nucleus assumes a more regular form at division, after

which it becomes once more irregular. The nucleus divides many times and at each division the protoplasm condenses between the newly formed nuclei to form a fine membrane separating them (pl. 4, figs. 5 to 9). Usually the sporont retains its globular form throughout its entire life but in some instances small irregular masses of dividing cells (fig. 6) were formed, which were too small to be primary sporonts and must therefore have represented a stage in some subsequent division. The numerous cells are so closely packed together as division advances that the exposed surface of each assumes a polygonal, usually hexagonal, form (fig. 9). A section through one of these masses shows that the septa dividing the nuclei do not in all cases completely separate the nuclei with their surrounding protoplasm from each other, since toward the center of the sporont they become less pronounced and finally disappear entirely (fig. 10). It is also seen that in the center there are a few nuclei surrounded by protoplasm, around the edges of which fine membranes are in process of formation. The nuclei are still somewhat irregular though much less so than in the earlier stages of division.

Sporoblasts. When a number of nuclei, varying from about 30 to 60, have been thus formed and surrounded by a membrane, the whole sporont swells and each of the numerous uninuceate sporoblasts into which it has now been transformed assumes a more globular shape. At this period of development neither the haematoxylin nor the orange-G stain is retained, but numerous bodies of very irregular size and form, having the appearance shown in plate 4, figure 11, make their appearance in the myxosporidium. Each sporoblast gradually retains more and more of the haematoxylin till by the time it is transformed into a spore its protoplasm stains very deeply.

Spore. Plate 4, figure 4, represents a section through one of the masses of newly formed spores. There does not appear to be any membrane around this aggregate, but the spores are held together by a quantity of surplus protoplasm which does not stain with haematoxylin as does that of the spore. As will be observed, the spores are not symmetrically arranged but are scattered irregularly throughout the protoplasm which forms the

basis of the mass. This irregularity is apparently produced by the swelling of the sporoblasts, for it is at this period of development that the sporont usually loses its globular shape. The elliptical spore measures about 4μ x 2.5μ and is less pronouncedly ovoid than that of Glugea fibrata. On treatment with iodine a filament is ejected. Unfortunately I have no preparations showing the evaginated filament, but in a free-hand sketch of an unstained specimen I drew it about ten to fifteen times the length of the spore and this is roughly its length. My preparations were also stained with haematoxylin so I was unable to see the nuclei, but in figure 14 I have, by analogy with G. fibrata indicated their probable position. I observed in unstained specimens that when the filament was ejected by iodine the spore contents shrank in a similar manner to that described for G. fibrata, giving the spore the appearance of figure 13.

It is seen from this that the ejection of the filament in both of these species is accompanied by a decided enlargement of the vacuole, which suggests that its fluid contents are swollen by the iodine entering through the shell by osmosis, and that the as yet undiscovered function of the vacuole may have some connection with the ejection of the filament.

The host of G. multispora is the larva of Simulium vittatum and Simulium bracteatum. It was found in one out of four larvae taken from a part of the stream in the Arnold Arboretum, where larvae were very scarce. Three other infested specimens were taken at Hyde Park and Franklin Park in the neighborhood of Boston.

NOTES ON THE GENERIC POSITION OF THE SPECIES DESCRIBED

Although I have placed all three species above described in the Genus Glugea of Thélohan, the first two, especially G. bracteata, according to Gurley's interpretation of the genus ('94), cannot be included, for his definition of the genus is as follows. "Glugeidae possessing a myxosporidium, and in which the pansporoblast produces *an inconstant but large number (always more than 8) of spores, pansporoblast membrane not subpersistent.*"[3] In

[3] Italics mine.

G. bracteata and G. fibrata the pansporoblasts produce regularly eight spores and in the former case the pansporoblast membrane is subpersistent. This latter type of sporulation is characteristic of the genus Thelohania of Henneguy, but in this genus the meronts remain separate, are uninucleate, and each gives rise to one pansporoblast. G. bracteata therefore cannot be placed in this genus, neither can it be included in Pleistophora of Gurley, in which also the pansporoblast is multisporulate (always more than octosporulate), although its membrane is "subpersistent as a polysporophorus vesicle." This species must therefore either be placed in the genus Glugea, in which case Gurley's definition must be modified, or a new genus will have to be made to include it. The peculiar shape of the spores and the subpersistence of the membrane might justify the latter course, but since Lutz and Splendore ('04 and '08) have pointed out that in the same species the pansporoblasts may be octo- or polysporic, and since according to the interpretation of Minchen ('03) and Stempell ('09), this species can be included in the genus Glugea, I am placing it provisionally there.

I am convinced that the species above described are closely related to the South American microsporidian described by Lutz and Splendore ('08) as Nosema simulii. These authors ('04) also place the species varians of Léger in the same genus. The reason for transferring this species is based mainly upon the fact that, as these authors show, the number of spores formed in a pansporoblast (i.e., whether octo- or polysporic) is an unreliable generic character. Nosema, however, has no myxosporidium and maturation takes place by its numerous uninucleate and separate meronts giving rise directly to spores, thus omitting a sporont and sporoblast stage. The absence of a myxosporidium places this genus in the family Nosematidae. That simulii does not belong to this family is shown by the fact that the myxosporidium is present as "rounded cysts, with fine membranes, in which are contained thin walled secondary cysts" (pansporoblasts). This character places this species, as well as varians, and those above described, with either the Pleistophoridae or the Glugeidae, which are separated by having multicellular, or multinuclear unicellular

myxosporidia respectively during the period of growth. This character disappears when sporulation begins, for in both families the sporonts are surrounded with a fine membrane, and the myxosporidium therefore at this stage is multicellular in both families. As before stated, all the material I have examined was in rather an advanced stage of sporulation, so that this character was poorly defined. As the earlier stages were not described by Léger ('97) or by Lutz and Splendore ('04), I conclude that their material was in about the same stage of maturation as that which I examined. In Glugea fibrata, however, I saw (pl. 3, figs. 4 and 5) what appeared to me to be free nuclei in the protoplasmic mass between the already formed sporonts. Some of these seemed to be still undergoing division. In G. bracteata also, sporonts appeared to be budded off from a multinucleate mass of protoplasm (pl. 2, fig. 4). If my interpretation be correct, both of these species certainly, and probably the other three also, belong to the family Glugeidae, and must therefore, as before stated, be provisionally placed in the genus Glugea. If this is not the case and the myxosporidium is multicellular during its entire development, these species will have to be placed in the Pleistophoridae, and, since Lutz and Splendore ('08) show that octo- and polysporic development is not of generic value, all can be included in a modified conception of the genus Pleistophora Gurley, though that author limited the genus to polysporic, i.e., more than octosporic, species.

THE EARLY STAGES OF INFECTION

As stated in the detailed account of the various species given above, the early stages of infection and development were not found, and if, as I infer, they are present only in the youngest Simulium larvae, it is unlikely that these incipient stages will be readily discovered. From analogy and from observations upon the later stages it is, however, possible to trace the probable means of infection and subsequent development.

As in all other Myxosoporidia, it may be assumed that infection is effected by way of the alimentary tract, and it is almost safe to say that this infection can take place only in the mesenteron,

for the stomenteron and proctenteron are rather heavily lined with chitin, which would repel any attacks of the unarmed germ which escapes from the spore. In order that the filament may be expelled previous to the escape of the germ it is necessary that the spore be acted upon by some dehydrating, or similar reagent, such as is found in the digestive juices of its host. Bütschli ('82) kept spores for a long time in water and noticed that there was no change in them. This is also the case with the spores from Simulium larvae which I kept for two months in water, at the end of which period they had undergone no change. Rare instances of spores dehiscing while still in the body of the original host have been described by Lieberkühn ('54) and Simond ('03), but these are exceptional.

The germ, then, is liberated in the mesenteron of a young larva. If, according to the theory explained above, the peritrophic membrane has not been completely formed at this time, it is able to come in direct contact with the mesenteric epithelium and to work its way in amoeboid fashion between the cells till it escapes into the body cavity of its host. If, on the other hand, the peritrophic membrane already completely lines the entire mesenteron and proctenteron it seems that the minute germ must pass straight through the intestines and be voided with the faeces. In this way I would explain, as before stated, the absence of any indications of parasitisation being effected in any but the earliest stages of larval life.

The young germ, after entering the body cavity, lives freely in the blood plasma, but does not, probably, multiply by division as does that of N. bombycis Näg. (Stempell '09), and where several myxosporidia are present in one host these must, it would seem, be considered as separate infections, except in the case of Glugea multispora in which so many myxosporidia sometimes occur that it would seem to indicate some such multiplication. The germ attacks a cell of the fat body. Evidence of this is shown in many cases where the walls of these cells are seen still surrounding small irregularities of the myxosporidium (pl. 2, fig. 3). Here the germ loses its motility and a constant (binary?) division of the nucleus begins, which continues throughout the

entire meront period, at the end of which the myxosporidium has grown to a very great size and consists of a multinucleate protoplasmic mass measuring up to 2 to 3 mm. in length. Each nucleus in the center of the myxosporidium now collects around itself a small mass of protoplasm, which becomes somewhat denser and is finally enveloped by a membrane, thus forming a globular unincleate sporont as previously described. This action spreads from the center outwards until the whole myxosporidium has been converted into sporonts, although by this time those in the center have been already transformed into spores.

When several myxosporidia are present in one host they all remain small so that they together occupy only as much space as a single fully developed myxosporidium. The reduction in size does not, however, interfere with development, for the small trophozoites begin to sporulate at the same time as do those which are fully grown. It seems, moreover, that the life history of the parasite closely coincides with that of its host, for in every case where sporulation was nearly complete, its host was full grown and the surrounding larvae from the same batch of eggs were pupating. In northern latitudes Simuliidae pass the winter in the larval stage and at about the end of October development practically ceases. It appears that the parasites in such larvae likewise cease to develop, for in all infected larvae collected during November and December the parasites were in precisely the same condition of development. This cessation of sporulation during the winter was noticed also by Cohn ('96) in a species of Myxidium.

THE EFFECTS OF THE PARASITE ON THE HOST

I was not able to find that any organs except the fat body are attacked by these parasites. The musculature, spinning glands, and epithelial cells of the alimentary tract showed no signs of infection. I have not, however, been able to find the reproductive organs in any parasitised larvae. These are always small, but can as a rule be found in a good series of sections from a healthy larva. When the parasite, however, is present no trace of these organs can be seen.

Pérez ('06) found, that in the case of crabs, the presence of Thelohania sp. in the ovary caused the parent to reabsorb its eggs. There are no indications in Simuliid larvae that the reproductive organs are actually infected. Since they are absent when but one myxosporidium is present, which may be situated ventrally to the alimentary tract, it seems more probable that, as in the case of Trichonympha spp. in Termites, the presence of the parasites causes suppression in the development of the reproductive organs without actually coming into contact with them.

In no preparations was there any noticeable hypertrophy of the alimentary tract, as seen by Léger ('97) in S. ornatum when parasitised by G. varians Léger or by myself ('11) in S. hirtipes when parasitized by various Myxosporidians. There was, however, a varying effect upon the histoblasts, from an almost complete atrophy to hardly any noticeable reduction in size (pl. 6, fig. 6). For a fuller account of the histoblasts, and the effects of the parasites upon them, I would refer the reader to my former paper ('11).

From numerous observations, I feel convinced that parasitised larvae never pass through the pupal to the adult stage. Unfortunately, owing to the difficulty of keeping these larvae alive under artificial conditions, I am unable to make this statement with absolute certainty, especially as evidence goes to show that in the infection of allied insect larvae by Glugeid parasites the host does not always suffer. In all of the latter cases, however, the parasite belongs to the family Nosematidae. The following is a brief account of two interesting species of this family.

In 1903 Simond described a Nosema sp. parasite of the larva of the yellow fever mosquito, Stegomyia fasciata Theob. from Rio de Janeiro, in which he found two distinct types of spores. The most numerous spore was unicolored and measured 3 to 5μ x 2 to 3μ. These, he states, dehisced within the host and thus caused auto-infection. This is the only case in which regular auto-infection has been described. Less frequently he found brown spores, which were less symmetrical in form and gave rise to an attenuated brown filament, which was occasionally

branched. This attained a length of 20 to 30μ, when it assumed a necklace-like form, after which it disintegrated without apparent further development or function. The infected larvae pupated and the parasite was present in the adult. In 1908 Lutz and Splendore also described briefly a species from the adults of Stegomyia fasciata which they named Nosema stegomyiae. The size of the spore was approximately that given by Simond and it is quite possible that this is the same species.

Hesse ('04) described a species, Thelohania legeri, parasitic in the larvae of the malarial mosquito, Anopheles maculipennis Meig. The fat body of these larvae was the seat of infection, as in the case of Simulium. There were two types of spores present, viz., microspores, 8 x 4μ, and macrospores, 12 x 5μ. The filament was readily evaginated by the use of iodine. Although he did not find the parasites present in any adults he states that it is doubtless to be found in this stage, for the host does not suffer in any way from the effects of the parasite.

In these two cases the parasites were not, as far as the observations show, in any way detrimental to their host. It must, however, be borne in mind that neither Nosema nor Thelohania, which are both genera of the family Nosematidae, form such large masses of parasitic material as Glugea, and that in the descriptions of these two cases there is no mention of the parasite causing the body of its host to be in any way distended. This fact would surely not have been omitted had the effect been as marked as in the case of the Simulium larvae.

In the parts of the stream where the parasites were most abundant I collected all the pupae I could find. These were sectioned, or dissected, but in no case could signs of any stage of the parasite be detected. When healthy and parasitised larvae were brought together into the laboratory the healthy larvae always lived for a much longer time than the parasitised individuals, and in several of the latter death was apparently caused by the skin rupturing, when the mass of parasitic material protruded through the rent thus made.

Mature larvae turn brown a little before pupation and the histoblasts of the pupal respiratory filaments blacken (pl. 6, fig.

5, *r.f.*). In no cases of parasitised larvae was this stage of maturation observed, and it is probably seldom, or never, reached.

In comparing sections of healthy and parasitised larvae, it is seen that in the latter the quantity of fat-body stored up for the formation of adult tissues is greatly reduced, and is probably entirely inadequate for the requirements of a full-sized fly. It is also evident that, owing to the large quantity of parasitic material, which is of a somewhat firm consistency, a large rent in the skin of the host would be necessary, in order to liberate it, and this would certainly cause the death of the host. Such was observed to be the case in larvae which died in this way when in captivity. On the other hand, should the parasite pass over into the adult, it is inconceivable how the fly, already weakened by the diminution of its fat body, could ever get out of the water when handicapped with a solid mass of spores which would swell its abdomen to quite three times its normal size. Taking into account all these facts, which in brief are the absence of mature larvae containing parasites, the absence of parasites in the pupae, the suppression of the reproductive organs, and often of the histoblasts, the voluminous proportions of the parasite and the resulting restriction of fat-body, I feel justified in stating that in almost if not all instances, the presence of Glugeid parasites prevents the maturation of their larval host.

A GREGARINE PARASITE OF SIMULIUM LARVAE: (Plate 5)

During early October and later it was found that, in two of the streams inhabited by Simulium bracteatum, a number of the larvae were somewhat enlarged and had generally a lighter color than the average individuals. Closer inspection showed that they were heavily parasitised by innumerable small cysts measuring up to 0.25 mm. (pl. 5, fig. 1). A number of these larvae were collected, killed and sectioned, when it was seen that these cysts affected various tissues of the body. The greater number of them were already free, floating in the blood plasma, but those which were still retained at the point where they began growth, were situated in the epithelial cells of the integument (pl. 5, fig. 2), in the cells of the fat body (fig. 3) and in the pigment cells

which cover the nervous system (fig. 4). The sexual organs were never found and it is probable that they had been parasitised and destroyed. I saw no signs of the epithelial cells of the intestine, spinning glands or Malpighian tubules suffering, neither did it appear that the muscles were ever affected. Under a higher magnification the cysts were seen to consist of a grandular cytoplasm containing small, irregularly distributed masses of chromatic material (fig. 5). In some young cysts there were vacuoles, but these were only detected in living specimens. In other fresh material there seemed to be a distinct ectosarc layer of a perfectly clear fluid. Otherwise the contents of the cyst seemed to be quite homogeneous and to consist of a granular protoplasm. On treating with osmic acid this turned a deep brown, indicating the presence of fat. By the end of November I noticed that the protoplasm was beginning to collect around the chromatic masses and the cell contents were divided up into many multinucleate irregular bodies (pl. 5, fig. 6). During December these bodies split into uninuclear, nearly globular bodies (fig. 7). If a cyst was then dissected and allowed to float in a drop of water it soon burst, liberating countless numbers of these minute globules. After they had been liberated about a quarter of an hour they began to move independently. Soon they became very active, though their power of locomotion was very slight, for they did not move their relative position appreciably but darted back and forth over a limited area. Each was provided with a flagellum. The actual movement could not be accurately observed as all motion ceased as soon as the specimens were placed under a cover glass in a cell slide. I sent prepared specimens of these parasites to Professor Calkins who very kindly replied that they probably belonged to the order Gregarinida. I have not, however, sufficient stages to be certain of this, but if this be the case there are one or two characters which are not quite in accordance with those usually connected with Gregarinida. In the first place it is evident that this parasite increases by schizogeny in its early stages, for in every host in which it was found the number of cysts present was estimated to be between 500 and 1000 or in some cases more, while surrounding larvae

of the same generation were free from attack. Secondly, the nucleus is not single from the earliest observed stages, but seems to occur for the greater part of the organism's life in the form of diffusely scattered chromatic masses. There is, again, usually no sharply defined layer of ectoplasm surrounding the endoplasm, while in the latter vacuoles are present. Another feature is that the presence of this parasite has a marked pathological effect, for parasitised larvae have the development of their histoblasts arrested (pl. 6, fig. 7) so that they must die at, if not before, pupation. Since in the streams in which this parasite is present it must kill well over 50 per cent of the somewhat scarce larvae, it would seem that it must be an important agent in the reduction of Simulium larvae in the neighborhoods where it occurs, and for this reason, apart from its scientific interest it would be of advantage to ascertain in detail its life history and distribution.

The host of this Gregarine is the larva Simulium bracteatum. It was found infecting about half of the larvae in streams in Franklin Park and at Hyde Park near Boston, Massachusetts, from October to December. The streams where it occurred had not been examined earlier in the year and contained no species except S. bracteatum.

THE ECONOMIC VALUE OF PARASITES OF SIMULIIDAE

It will be seen from the foregoing notes that there are in the neighborhood of Boston three distinct classes of parasites infecting, and in each case killing, Simulium species in their larval stages. Summarizing my observations for the single year, 1911, there are the following:

I. Parasites of the spring brood of Simulium.

a. Various Myxosporidia *sens. lat.*, up to 80 per cent mortality

c. Mermis sp., up to 25 per cent mortality

II. Parasites of the fall brood of Simulium.

a. Glugea bracteata, about 10 per cent mortality

a. Glugea fibrata, about 5 per cent mortality

a. Glugea multispora, rare

b. Gregarine species, up to 50 per cent mortality.

From no other locality in North America have these, or similar parasites, been recorded. As mentioned earlier in this paper, Simulium larvae have been carefully and extensively studied in several sections of the country, notably at Ithaca, New York, and in Maine, but in none of these places has a single case of parasitism been recorded. It is evident, as will be seen from the illustrations of parasitised larvae, that such individuals could hardly escape the eye of the observer, especially as, in streams in which infection is heavy, the greatly distended and whitened larvae can be readily seen from a distance of several yards from the water. We thus have evidence, though by no means conclusive, that these parasites are not widely distributed throughout the United States. Other evidence is found in the fact that in this neighborhood the black flies are not a serious pest, although the nature of the country is eminently suited to the requirements of their larval life. In surrounding states, in every direction, these flies are not only a great nuisance, but also a dangerous pest. The southern states, especially, suffer from these blood-thirsty flies. In the interesting report of Professor Lugger, of the University of Minnesota ('96), it is stated that in the State of Tennessee alone, these flies were responsible, in the year 1892, for the loss of $500,000, through their attacks on cattle. But their ravages are not confined to the southern states, for in the northern state of Maine, and in New Brunswick, one hears of the death of animals due to the attacks of these flies, while in the same localities it is impossible at certain seasons of the year to enter forests, unprotected, in the neighborhood of streams. It would therefore seem that we owe our deliverance largely, if not entirely, to the fact that a large percentage of these flies is annually killed off before maturity.

I have made no experiments upon the transference of these parasites from one Simulium species to another, but, as far as can be seen, there should be no difficulty in accomplishing this, for in all cases observed *the parasites infected all species of larvae, present at that time, in the stream where the former occurred.* There is, however, a seasonal variation of parasitism, for the species found in the spring were not retaken in the fall, and vice versa,

so that it is probable that only those species of Simulium, whose life history coincides with parastised species, could be infected with the parasites of the latter. Since, as previously stated, I am led to believe that infection is possible only during the early stages of larval life, it would probably be necessary to collect a number of infected larvae, and place them in streams where the other species is always abundant, some little while before the eggs of the latter hatch. If the parasite is present in the larvae of the following generation it will be readily detected during the later stages of larval development.

It will be seen from the list of Myxosporidia, and the seasons in which they were found, that those occurring in the spring brood parasitise a much higher percentage of larvae than those occurring in the fall brood. This is due, it seems, to the much greater numbers of larvae present in the streams during the former season, for at this time they are living a gregarious life, and all stages of development are present together. This results in the newly hatched larvae always being in close proximity to some recently dead larva, from which innumerable spores have escaped. In the fall, however, larvae are comparatively rare, and are more solitary in their habits, so that the infection of young larvae is less certain.

The Gregarine species, however, which occurred in two streams, not examined in the spring, was found heavily to parasitise larvae which were not by any means abundant in the streams, and this is probably due to the motility of the bodies liberated by the host upon its death. It would thus seem that, were the parasites liberated in streams where Simulium larvae are very numerous, the spread of infection would be rapid, and the disease should soon be firmly established.

It is not easy to understand how it is that the parasites do not slowly travel down-stream, from year to year, so that in time the stream becomes clear of infection. That this is not the case, is indicated by the fact that where the source of a stream has been found as a spring, Simulium larvae occurring near this region were found to be parasitised. As before stated, I do not believe that the adults are capable of spreading the

infection through the ova. Krassilstschik ('96) found that the spores of N. bombycis (pébrine) could pass through the intestines of birds and be still capable of infecting silkworms. This may also be the case with these spores, since many must be ingested by birds which drink at the streams. Other insects, frogs, etc., may also carry the spores upstream. Perhaps also, Simulium larvae themselves move about more than is generally believed, and as stated in my previous paper ('11), parasitised larvae are more active than healthy specimens. It may be, therefore, that such larvae occasionally travel a considerable distance up stream before death, and thus prevent the disease from being washed out of the stream.

I found no definite signs of Mermis during the fall of 1911 although, in one or two dissections of S. bracteatum, I found minute worms, measuring about 20μ among the masses of G. bracteata contained in these larvae. This Mermis should also prove of good economic value, since there can be little doubt that it is a general parasite which could be readily transferred from one species to another.

SUMMARY

Although the country in the vicinity of Boston is eminently adapted to the breeding requirements of black flies (Simuliidae), they do not occur in sufficient abundance in this neighborhood to constitute a dangerous pest, or even a serious annoyance. Black flies or 'buffalo gnats,' are most destructive in the southern states, as an example of which Tennessee may be cited, where in one year the cattle-raisers suffered a loss of $500,000 through the attacks of these insects. Their ravages are not, however, confined to the southern states, for in the more northern states, as in Maine and Wisconsin, these flies, at certain seasons of the year, are extremely abundant, and are very injurious to stock. It would thus seem that in the neighborhood above named the comparative freedom from annoyance by these small, though vicious, flies must be due to some other cause than climatic conditions. An examination of the larvae in the small streams, which occur so frequently in eastern Massachusetts throws some

light on this phenomenon, for it is seen that, in many cases, a large percentage of the insects contain parasites that are in every case fatal to their host. The Simuliidae occurring in this neighborhood are most abundant in the spring, and it is at this season that parasitism is most effective. The most common species is Simulium hirtipes. This is found to be heavily parasitized with species of Myxosporidia (*sens. lat.*). Sometimes as many as 80 per cent of the larvae are found to be greatly distended with masses of this white parasite, which in every case spells death to its host. Another spring parasite is a Nemathelmith belonging to, or near, the genus Mermis. The presence of this, also, is fatal to the Simulium larva in which it lives, and by this means some 25 per cent of the larvae in certain streams were destroyed during the spring of 1911. Very few Simuliidae are to be found in the streams throughout the summer, but in the fall the early stages of S. bracteatum are common. The hitherto undescribed pupa of this species is of interest in that the respiratory filaments are only four-branched, a condition previously not recorded from North American species, and only twice found elsewhere. Together with these larvae occur a few of those of S. vittatum, and later in the season S. hirtipes is once more in evidence. All of these suffer from Microsporidian parasites, which do not confine their attacks to any one species of Simulium larva, but freely parasitise all species present in the streams during the season of their occurrence. Three Microsporidian species have been found, and their later developmental stages have been traced with sufficient detail to determine their probable taxonomic position.

Since no accounts of Microsporidia, and their relation to insects have been made in this country, it was deemed advisable to give a somewhat detailed account of this suborder of the Sporozoa.

The three species described from Simulium larvae are:

I. *Glugea bracteata sp. nov.* Pansporoblast octo-sporulate. Pansporoblast membrane subpersistent. Spores about 3μ x 2.5μ, short elliptical, filament about six times the length of the spore.

II. *Glugea fibrata sp. nov.* Pansporoblast octosporulate. Pansporoblast membrane not subpersistent. Spores about 6μ x 3.5μ

ovoid, filament about thirty to forty times the length of the spore.

III. *Glugea multispora sp. nov.* Pansporoblast polysporulate. Pansporoblast membrane not subpersistent. Spores about 4μ x 2.5μ.

The earliest stages were never seen. It is conjectured, however, that the parasite, which undoubtedly enters the body cavity through the alimentary tract, is able to parasitise only young larvae. This is thought to be due to the postembryonic development of the cephalic fans and peritrophic membrane, both of which are highly specialized organs and in Simulium larvae show unique and interesting modifications. The parasite was found attacking only the cells of the fat-body. These are soon ruptured and the parasite subsequently leads a free life in the body cavity of its host, where it grows to comparatively enormous volume, consisting of a multinucleate mass of protoplasm which greatly distends the body of its host. As the latter matures, the myxosporidium, as the parasite is now termed, is converted into innumerable thick-shelled spores. These escape from the larva, which has succumbed to the attack, and by entering the alimentary tract of fresh larvae spread the disease.

Since these parasites are to be found in all species of Simulium larvae present in the streams where they occur, it is probable that they could be transferred to fresh streams and thus cause the infection of species of Simulium larvae in which as yet no parasites have been found. The parasite rarely, if ever, passes over into the adult, so that the natural spread of the disease from stream to stream is slow. That these Microsporidians are not widely distributed throughout the United States is indicated by the fact that though Simulium larvae have been carefully studied in several localities no cases of their presence have been recorded. When infected larvae are at all abundant in streams, their greatly distended and whitened abdomens are very conspicuous, and are readily seen at some distance from the water, under conditions in which normal healthy larvae are almost or quite invisible.

A further parasite in the fall generation, probably a Gregarine, has been found, infecting in some streams as many as half of

the larvae of S. bracteatum, which was the only species present in those parts of the streams where it occurred. Its life history has not received careful attention, but it is evident that it is fatal to its host, and it produces if anything more 'spores' than do the Microsporidia. This parasite, therefore, deserves careful study, for though it was found only in one species of Simulium, this was probably due to the fact that no other species was present where it occurred.

It is hoped that economic entomologists will, in future, look for the presence of these various parasites. If it is then found that those districts in which Simulium is not a serious pest, owe their deliverance to the presence of these parasites, experiments can be undertaken to prove whether the latter can be artificially propagated. If this be feasible, and the writer sees no reason to doubt it, there is a prospect that in those districts where black flies are most numerous, the diseases would spread with great rapidity and considerably reduce the devastations and annoyance caused by these little Diptera.

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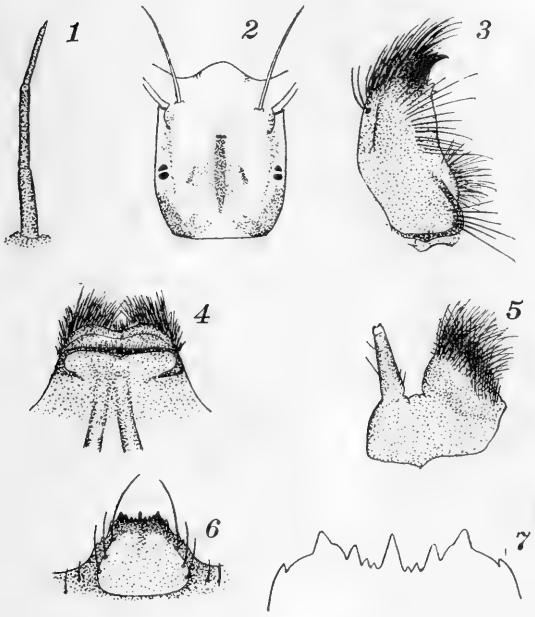
PLATE I

EXPLANATION OF FIGURES

Larva and pupa of Simulium bracteatum Coq.

1 Larval antenna × 260
2 Head capsule of recently moulted larva to show the characteristic markings, × 32.
3 Mandible of larva, × 260.
4 Hypopharynx of larva, × 260.
5 Maxilla of larva, × 260.
6 Labium of larva, × 260.
7 Labial teeth, × 1040.
8 Pupa in cocoon, × 20.
9 Characteristic branching of respiratory tubes × 40.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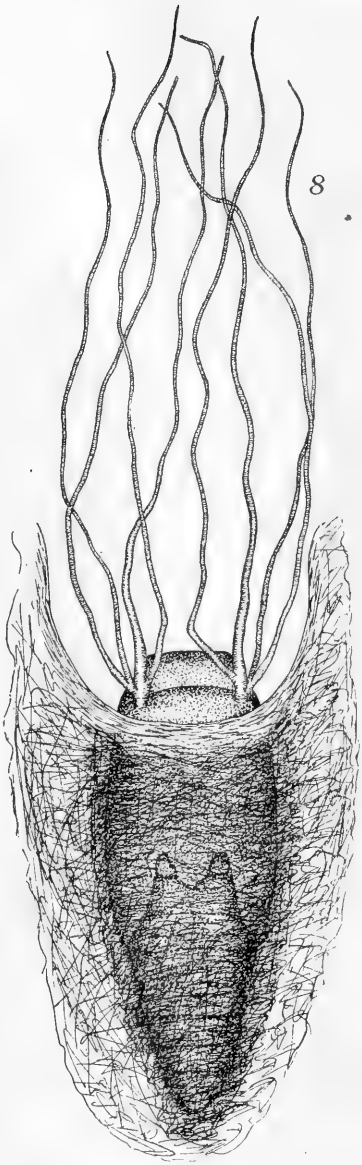
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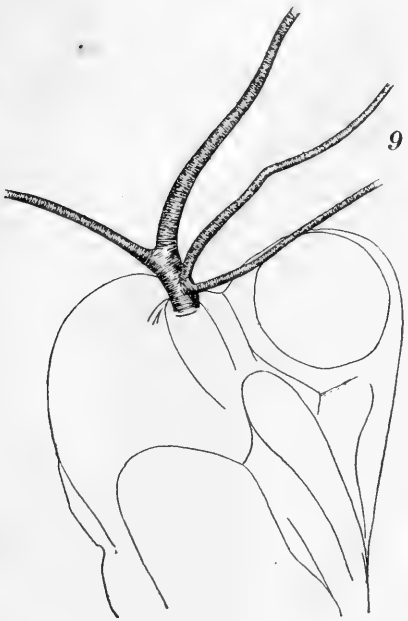
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PLATE 2

EXPLANATION OF FIGURES

Glugea braceata sp. nov.

1 Parasite in situ, × 12.

2 Section through abdomen containing parasites, × 12.

3 Section through sporulating Myxosporidium, × 470; *f.*, fat body tissues still attached.

4 Portion of Myxosporidium showing budding of sporonts, × 470.

5 A newly formed sporont, × 1400.

6 Sporoblasts in process of formation, within sporont, × 1400.

7 Pansporoblast = Sporont containing 8 complete sporoblasts, × 1400.

8 Empty shell of pansporoblast with a little chromatic material and surplus cytoplasm still adhering, × 1400.

9 and 10 Aggregate of sporoblasts, stained with haematoxylin and orange-G, × 1400.

11 and 12 Aggregates of spores unstained × 1400.

13 to 16 Maturation of the spore, stained with azure and eosin, × 1400.

17 End to end view of a mature spore, × 1400.

18 Mature spore, unstained, with filament ejected, × 1400.

19 and 20 Diagrammatic representation of maturing sporoblast to show the formation of the vacuole, × 7000.

21 and 22 Diagram of maturation of the spore, nucleus dividing and cytoplasm becoming more dense, × 7000.

23 Diagram of situation of polar capsule, with enclosed filament, × 7000.

24 Diagram of spore with filament ejected, × 7000.

25 The swollen knob at the base of the filament.

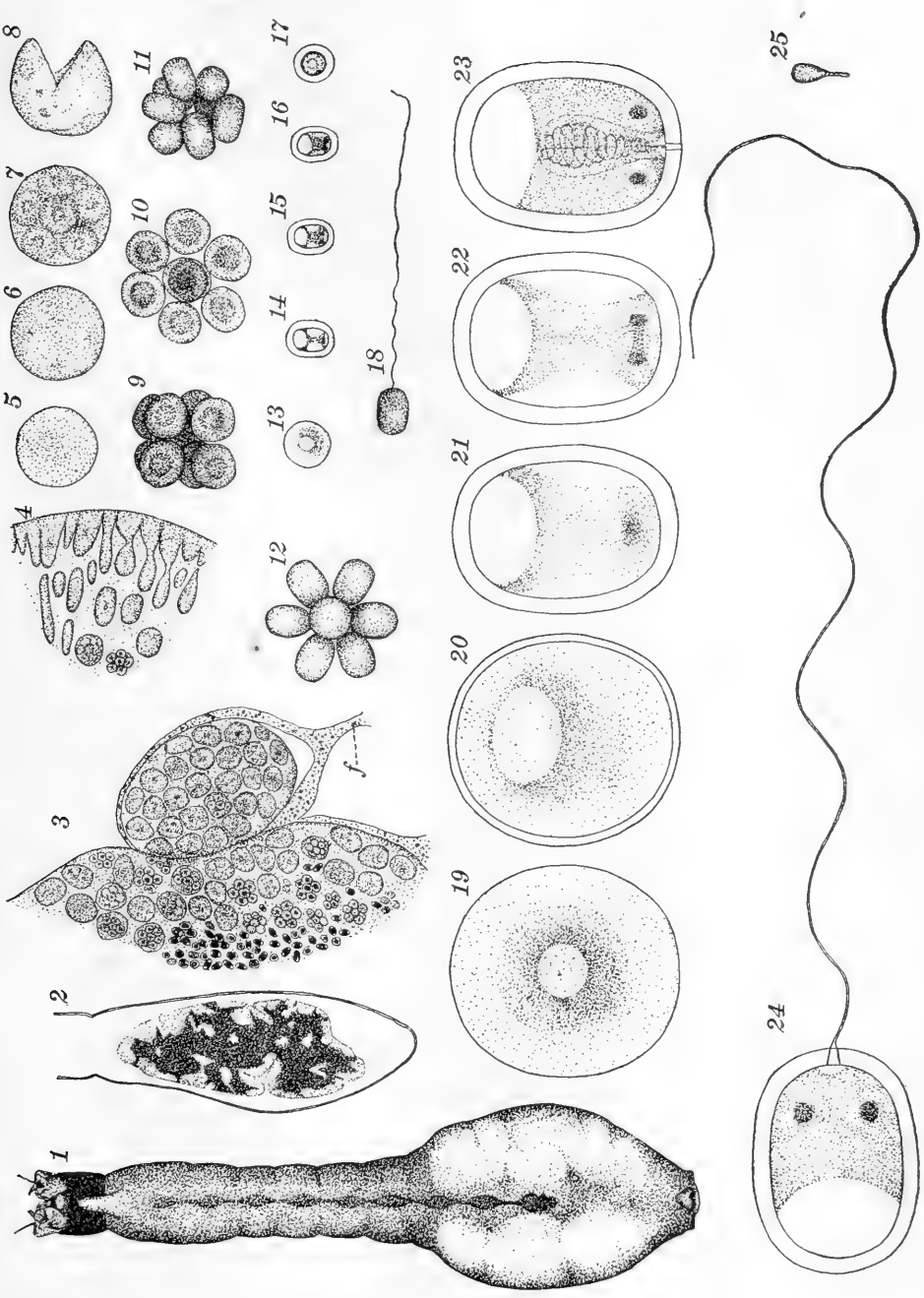
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PLATE 3

EXPLANATION OF FIGURES

Glugea fibrata sp. nov.

1 Parsites in situ, × 12.
2 Section through sporulating Myxosporidium, × 470.
3 Section through abdomen containing parasitic mass, × 12.
4 and 5 Dividing nuclei of meronts, × 1400.
6 Newly formed sporont with scattered chromatic matter, × 1400.
7 to 11 Division figures of sporont nuclei, × 1400.
12 Second division of nuclei in sporont, × 1400.
13 Sporont containing 8 nuclei, × 1400.
14 Protoplasm collecting round each nucleus to form 8 sporoblasts, × 1400.
15 Sporoblasts nearly complete, × 1400.
16 Section through 15, × 1400.
17 Free sporoblast, × 1400.
18 to 20 Maturation of the spore, stained with azure and eosin, × 1600.
21 Spore deeply stained with haematoxylin to show the polar capsule, × 1600.
22 A macrospore, × 1600.
23 Spore with filament partially ejected, × 1600.
24 Spore with filament detached, × 1600.
25 Spore with fully extended filament, up to 40 times its length, × 1600.
26 Diagram of mature spore, adapted from W. Stempell, × about 5700.

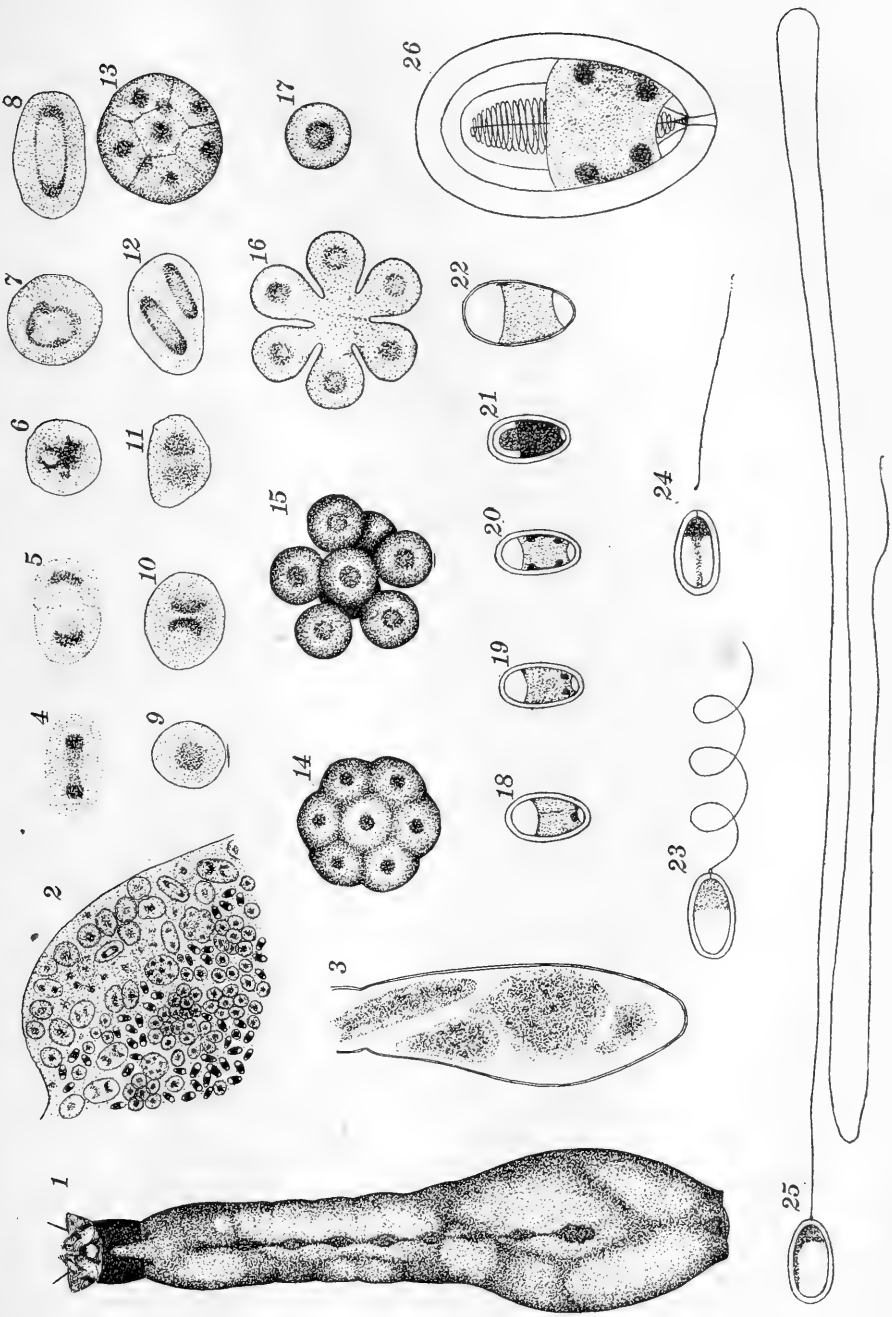
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PLATE 4

EXPLANATION OF FIGURES

Glugea multispora sp. nov.

1 Parasites in situ, × 12.
2 Section through sporulating myxosporidium, × 470.
3 Section through abdomen containing parasite, × 12.
4 A young sporont, × 1400.
5 to 9 Division of sporont into sporoblasts, stained with iron haematoxylin and orange G, × 1400.
10 A section through 9, × 1400.
11 Pansporoblast unstained, × 1400.
12 Section through mass of spores, × 1400.
13 Spore with filament ejected, × 1400.
14 A diagram of a spore, × 7000.

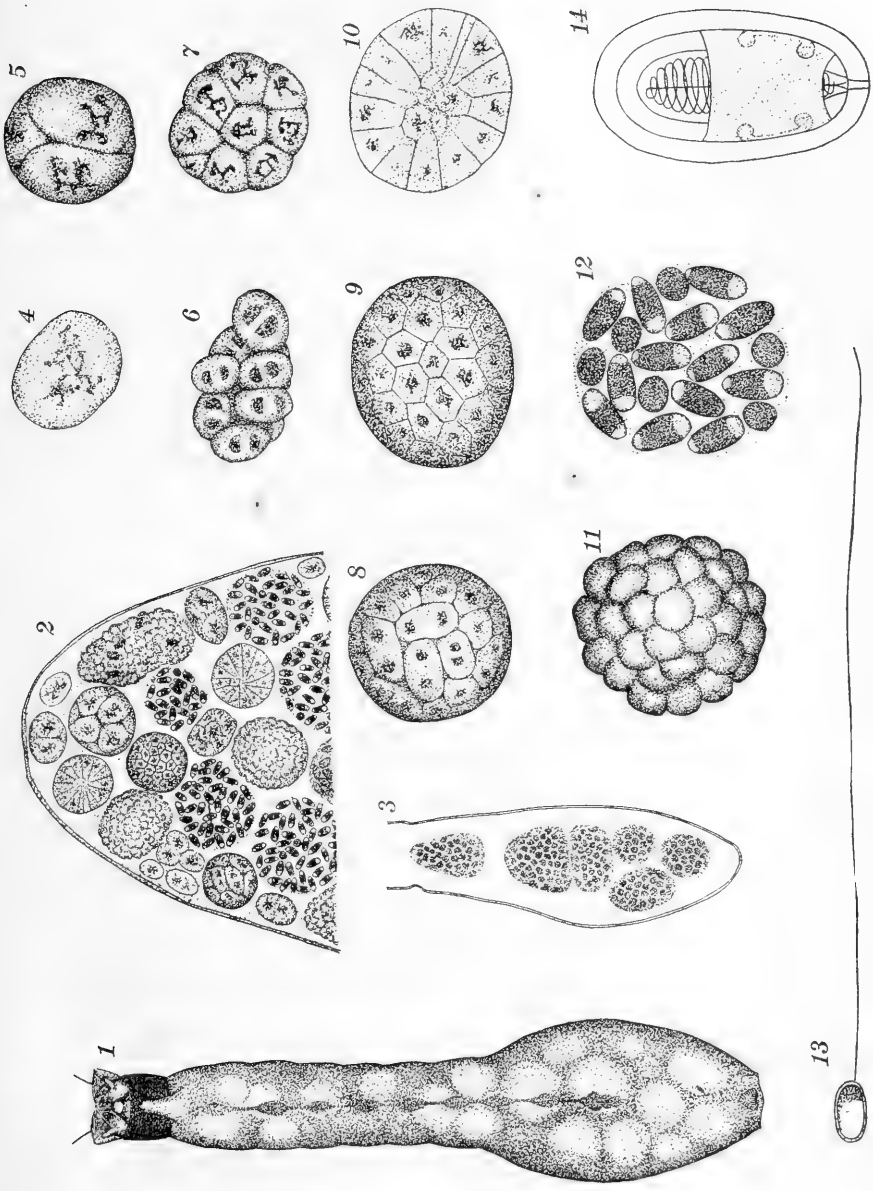
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PLATE 5

EXPLANATION OF FIGURES

Gregarine parasite of Simulium bracteaum

1 Parasites in situ, × 20.
2 Cysts from integumental epithelium, × 170.
3 Cysts in cells of the fat-body, × 170.
4 Cysts from pigment cells covering the nerve ganglia, × 170.
5 Portion of a cyst at an early stage, × 1400.
6 'Sporulating' cyst, × 1400.
7 'Spores' in a cyst, almost mature, × 1400.
8 Flagellate 'body' from a cyst, × 1400.

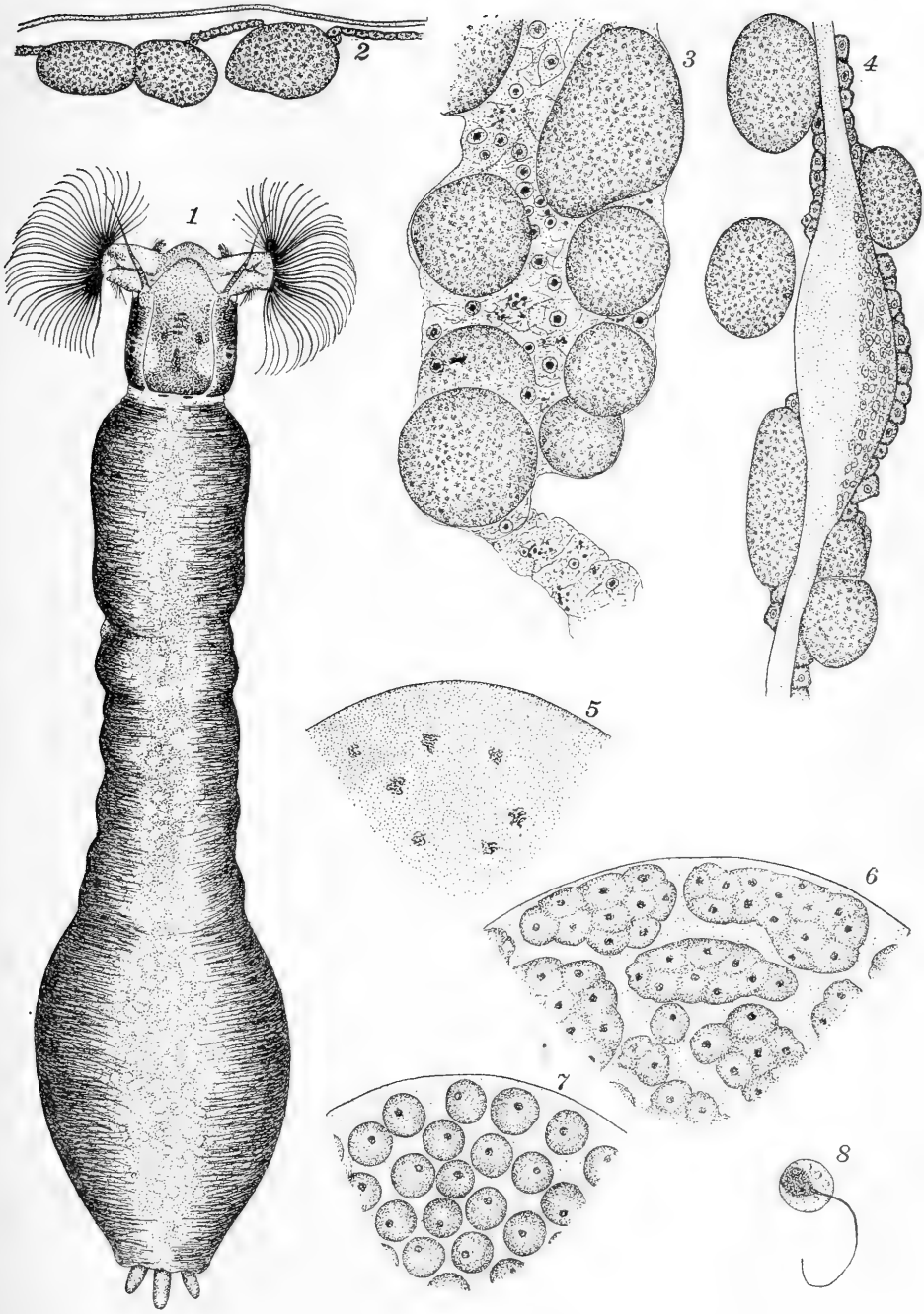
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PLATE 6

EXPLANATION OF FIGURES

1, 2, and 3 Types of flagellate bodies from Gregarine cyst, × 1400.

4 Most common type of flagellate body, × 2200.

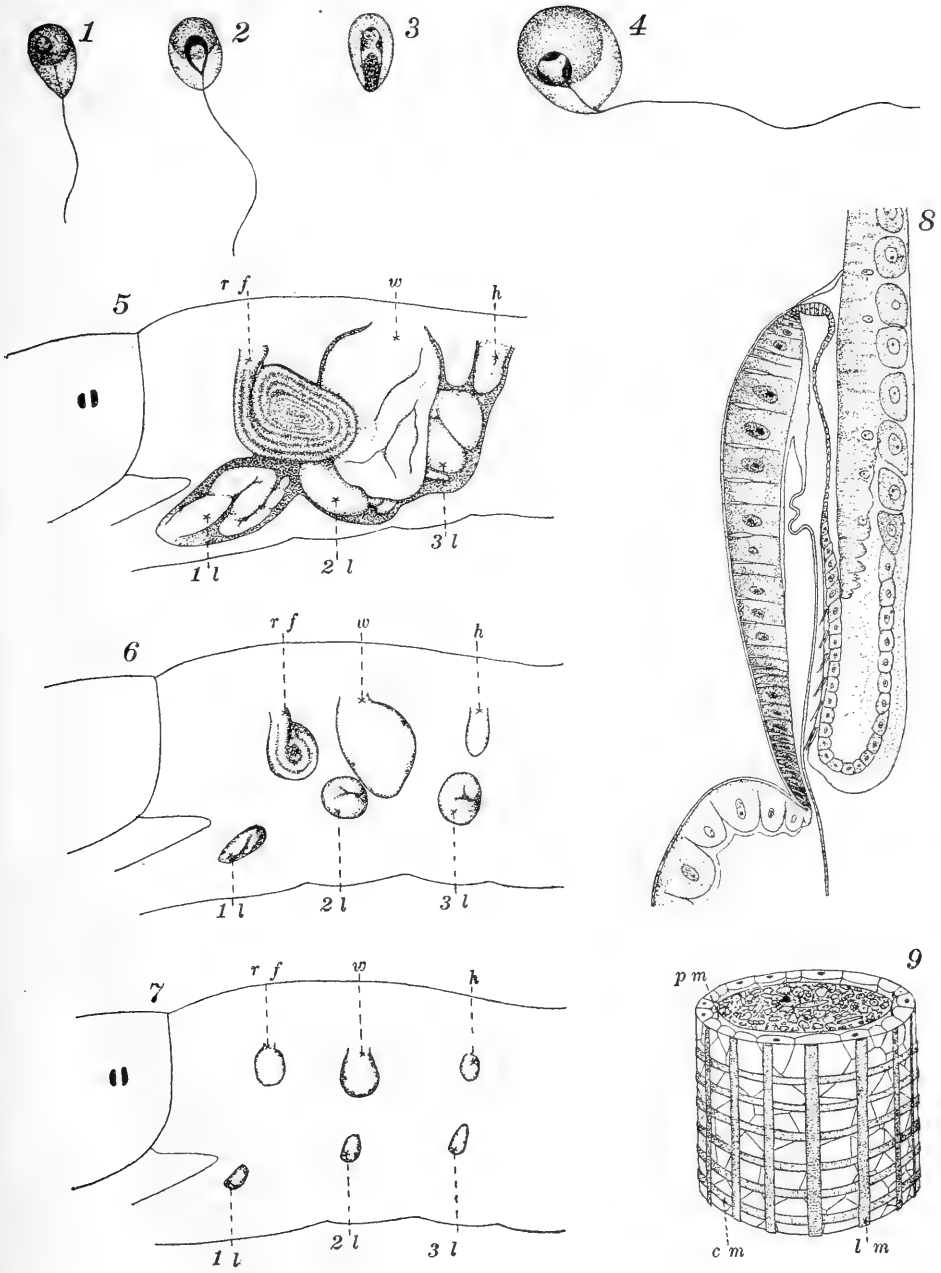
5 Diagram of histoblasts of mature healthy Simulium larva; *r.f.*, respiratory filament (pupal) histoblast; *w*, wing histoblast; *h*, halter histoblast; 1b, 2b, 3b, leg histoblasts.

6 Diagram of histoblasts of full grown Simulium larva containing Microsporidia, lettering as in fig. 5.

7 Diagram of histoblasts of full grown Simulium larva containing 'Gregarine;' lettering as in fig. 5.

8 Longitudinal section through one half of the proventriculus and cardia (see text figure, page 00, for explanation).

9 Diagram of a section of the mesenteron. *p.m.*, peritrophic membrane; *c.m.*, circular muscles; *l.m.*, longitudinal muscles.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THE MYOLOGY OF POLYODON

C. H. DANFORTH

From the Anatomical Laboratory of Washington University

TEN FIGURES

I. INTRODUCTION

Besides rather numerous incidental references to Polyodon, there are to be found in the literature a few papers that deal more or less specifically with the anatomy of this fish. Bridge ('78, '96, '97) has worked on different aspects of the skeletal system, and the visceral skeleton and nerves have been studied to some extent by van Wijhe ('82). More recently work on the vascular system has been done by Allen ('07), Allis ('11) and Danforth ('12). A few more special papers might also be mentioned. The myology, as a whole however, has apparently been left untouched until now.

The present paper aims to supply a brief account of the musculature of Polyodon which may be available for comparative purposes or for future developmental studies. It is based chiefly on dissections of adult fish, about a meter in length, which had been preserved in formalin. The blood vessels of a part of them were injected. A few smaller individuals, three to four decimeters long, were studied and use was also made of serial sections of a 74 mm. specimen.

For purposes of description the muscles are grouped under the following heads: Eye muscles, Muscles of the mandibular and hyoid arches, Muscles of the branchial arches, Hypoglossal muscles, Muscles of the trunk, Muscles of the median fins, Muscles of the pectoral arch, Muscles of the pelvic fin.

The terminology of muscles in fishes has not yet become uniform. In the case of cranial muscles I have followed the designations of Vetter ('78) as applied to Acipenser wherever the

homologies seemed clear. Elsewhere I have endeavored to select terms which seemed least likely to admit of ambiguity. In the following account muscles are described as taking origin from that attachment which would seem ordinarily to be the less movable, and as being inserted on the more movable one. For this reason, in a few cases, the descriptions here are not quite parallel with those of Vetter for Acipenser. The *action* of the several muscles has been determined only by inference and may very frequently be inadequately, or even somewhat inaccurately, stated, since in contracting every muscle works with or against a number of ill-defined forces which tend to modify its proper action, often to a marked degree.

The statements regarding nerve supply are based on dissections and study of serial sections. The nerves are referred to by the same names as are employed by van Wijhe ('82) whenever the nerve in question is described by that writer.

In describing the blood supply the papers of Allis ('11) and the present writer ('12) are followed in so far as the arteries are concerned. Since no adequate account of the veins has yet been published, references to these vessels are necessarily less complete.

II. EYE MUSCLES

The muscles of the eye and the arrangement of structures in the orbit conform essentially to the ganoid type worked out by Allis ('97) in his Amia paper.

The two oblique muscles arise at the anterior extremity of the orbit in the angle between the cranial wall and the olfactory capsule. Their points of origin, however, are so widely separated that the two muscles are practically parallel throughout their whole course. The superior oblique arises high up and runs diagonally back through the orbit in a nearly horizontal plane to its insertion near the median level of the eye and dorsal to all the other muscles. The inferior oblique, arising below and anterior to the foregoing, crosses the floor of the orbit and is inserted on the ventral side of the eye capsule. The four rectus muscles are fused proximally in a common short tendon of origin

which arises from the cranial wall behind the foramen of the optic nerve and in front of the ganglion of the trigeminus. There is no canal for this part of the muscles to pass through. As they diverge from one another, the external and superior rectus are the more dorsal and at the same time the external and inferior are the more lateral. The superior rectus is inserted immediately under and in part posterior to the insertion of the superior oblique. The inferior rectus, which is usually the largest of them all, is inserted ventrally at a point somewhat posterior to the insertion of the inferior oblique. The external rectus has its insertion near the posterior corner of the eye about midway between the insertions of the two foregoing. The internal rectus is more central than any of the others. It is also the smallest of the eye muscles. It passes forward ventrally to the optic nerve and is inserted slightly anterior to its point of emergence from the eye.

Innervation. The muscles are innervated by the usual nerves. The trochlear nerve emerges from the cranium through a small foramen above and somewhat anterior to that of the optic nerve. It runs forward some distance closely appressed between the cranial wall and the protractor hyomandibularis muscle. Anteriorly it comes out across the ventral face of the muscle and crosses into the orbit to supply the superior oblique. The oculomotor nerve also passes through a foramen of its own in leaving the cranium. Medial to the trigeminus it comes into intimate relation with the abducens. An anastomosis between the two may take place but it could not be demonstrated. As it enters the orbit a dorsal branch is supplied to the superior rectus while the main portion of the nerve continues outward along the anteromesial margin of the inferior rectus. It supplies numerous twigs to this muscle and gives rise to a rather complicated plexus in the floor of the orbit from which branches rise to supply the internal rectus and inferior oblique. The abducens nerve leaves the cranium beneath the posterior part of the trigeminal ganglion. It runs forward median to the ganglion and nerve and comes into relation with the oculomotor as described above. It is distributed to the external rectus.

Blood supply. The ophthalmic branch of the external carotid supplies the rectus muscles and more anterior branches of the same artery supply the two obliques. The veins of the orbit are tributaries of the jugular.

Rather an unexpected tendency to variation was met with in connection with these muscles. The internal rectus, referred to above as the smallest of the group, was twice—once in a medium sized individual, once in a small one—found to be double throughout most of its extent. Both parts were tendinous near their insertion. In a third specimen, a large fish, careful dissection failed to reveal any trace of it on the left side although present and normal on the right. Such variability may be indicative of a retrograde tendency on the part of this element. An intensive study of the eye muscles of a large number of specimens might yield interesting results.

III. MUSCLES OF THE MANDIBULAR AND HYOID ARCHES

M. geniohyoideus: figures 1 and 2, m.gha., m.ghb.

In Polyodon the primitive superficial muscles of the head are represented ventrally by a thin lamina, the geniohyoideus, crossing the space bounded laterally on either side by the ramus of the mandible and the branchiostegal ray and posteriorly by the margin of the opercular flap (figs. 1 and 2). As indicated in the figures, the muscle does not extend forward quite to the symphysis of the jaw. The fibers arise laterally and with the exception of the most posterior, are all inserted in a median aponeurotic thickening. As is frequently the case, this muscle is in two parts, anterior and posterior. The anterior part arises (a) from Meckel's cartilage, beginning at a point a little behind the symphysis and extending back nearly to its posterior end, and (b) from the overlying dentary bone of the same region. In a 74 mm. specimen, apparently all the fibers arise directly from the cartilage. In reaching their insertion the anterior fiber bundles run somewhat obliquely backward and inward, the intermediate ones are transverse, and the posterior run obliquely forward and inward as indicated in figure 1. These last are very nearly parallel

with, and not clearly separated from, the fibers of the posterior part of the muscle which they slightly overlap. The posterior part of the geniohyoid takes its origin (a) from the ceratohyal between the groove for the facial nerve and the posterior angle of the cartilage, (b) from the ventral margin of the interhyal, and (c) from a line along the ventral margin of the branchiostegal ray. The fibers do not arise directly from the ray, but especially in younger individuals, from the skin beneath (medial to) it. The anterior muscle bundles are directed obliquely forward to the median line, the posterior become more and more transverse in their direction. Some of the latter seemingly cross to the opposite side without any median insertion.

Innervation. The anterior part is supplied by the end twigs of the ramus maxillaris inferior trigemini, and the posterior by several branches of the ramus hyoideus facialis. In addition to these nerves the ramus mandibularis facialis externus courses over the surface of the muscle immediately superficial to the main trigeminal branch. In Amia these two nerves unite (McMurrich '85) but in Polyodon I could not detect any anastomosis between them, and apparently the former is distributed entirely to the lateral line organs as suggested by van Wijhe ('84).

Blood supply. Terminal branches of the facial artery in front and of the hyo-opercular behind ramify over the ventral surface of the muscle. The corresponding veins lead away from it. The anterior part also receives a dorsal supply through small arteries originating in anastomoses between the end of the facial artery and descending branches of the lateral hypobranchial. The posterior part may get a little of this supply. The veins of the dorsal side drain into the inferior jugular.

Action. Contraction of the muscle tenses the pouch-like fold beneath the jaw and between the opercular flaps. Action of this muscle must tend to prevent spreading of the rami of the mandible and of the hyoid and also assist in drawing the opercular fold against the body, thus performing an accessory function in inspiration and deglutition.

As to the homologies of this muscle, it seems to me that it is to be regarded simply as geniohyoid. The anterior part might

ABBREVIATIONS

a.an, innominate artery
a.br.a. (*1*, *2*), afferent branchial artery
a.br.e. (*1*, *2*, *3*, *4*), efferent branchial artery
a.cc., common carotid artery
a.ce., external carotid artery
a.fa., branches of the facial artery
a.hy., afferent hyoidean artery
a.hyo., hyo-opercular artery
a.lhb., posterior end of lateral hypobranchial artery
a.nu., artery to adductor branchialis
a.pa., branch of parietal artery
a.ph., pharyngeal branch of second efferent branchial artery
a.seg., segmental artery
a.va., ventral aorta
br., branchiostegal ray
c.cer. (*2*, *4*), ceratobranchial cartilage
c.ep. (*1*, *2*, *3*, *4*), epibranchial cartilage
c.hy., hyoid cartilages
c.hyb. (*1*, *2*, *3*, *4*), hypobranchial cartilages
c.hyo., hyomandibular
c.ih., interhyal
c.mc., Meckel's cartilage
c.pec., cartilages of pectoral arch
c.phbr., first pharyngobranchial cartilage
c.pq., appendage of palatoquadrate
li.al., linea alba
m.abp., abductor of pectoral fin
m.adb. (*2*, *4*), adductor branchialis
m.adm., *adm.'*, the two parts of m. adductor mandibulae
m.adp., adductor of pectoral fin
m.bmd., branchiomandibularis muscle
m.bmd.', fibers from m. branchiomandibularis
m.coar., coraco-arcualis muscle
m.con., dorsal conical portion of myomere
m.gha., anterior part of geniohyoid
m.ghp., posterior part of geniohyoid
m.lat., lateral musculature
m.lev. (*1*, *2*, *3*, *4*), levator arcuus branchialis
m.oes., musculature of oesophagus
m.phg. (*1*, *2*, *3*), pharyngoclavicularis
m.pro., protractor hyomandibularis
m.ret., retractor hyomandibularis
m.sthy., sternohyoideus muscle
m.tr.d., m. transversus dorsalis
m. tr.v., m. transversus ventralis
m.trp., trapezius muscle
m.ven., ventral body musculature
myoc., myocomma
myot., muscle segment
n.ad., nerves of adductor branchialis
n.am., branches of inferior maxillary nerve.
n.h., branches of ramus hyoideus facialis
n.ix., glossopharyngeal nerve
n.int., ramus praetrematicus internus (of vagus)
n.x.po., posttrematic rami of vagus
n.x.pr., praetrematic rami of vagus
no., notochord
oper., operculum
op.f., opercular flap
os.cl., clavicle
os.den., dentary bone
os.fr., frontal bone
os.inf., infraclavicle
os.mx., maxillary bone
os.spr., supraclavicle
pec., pectoral fin
per., wall of body cavity
sep., median dorsal septum
thy., thyreoid gland
v.ad., vein from m. adductor branchialis
v.dc., duct of Cuvier
v.fa., facial vein
v.h., hyomandibular vein
v.sup., superficial lateral vein
ven., ventral fin, abductor muscle

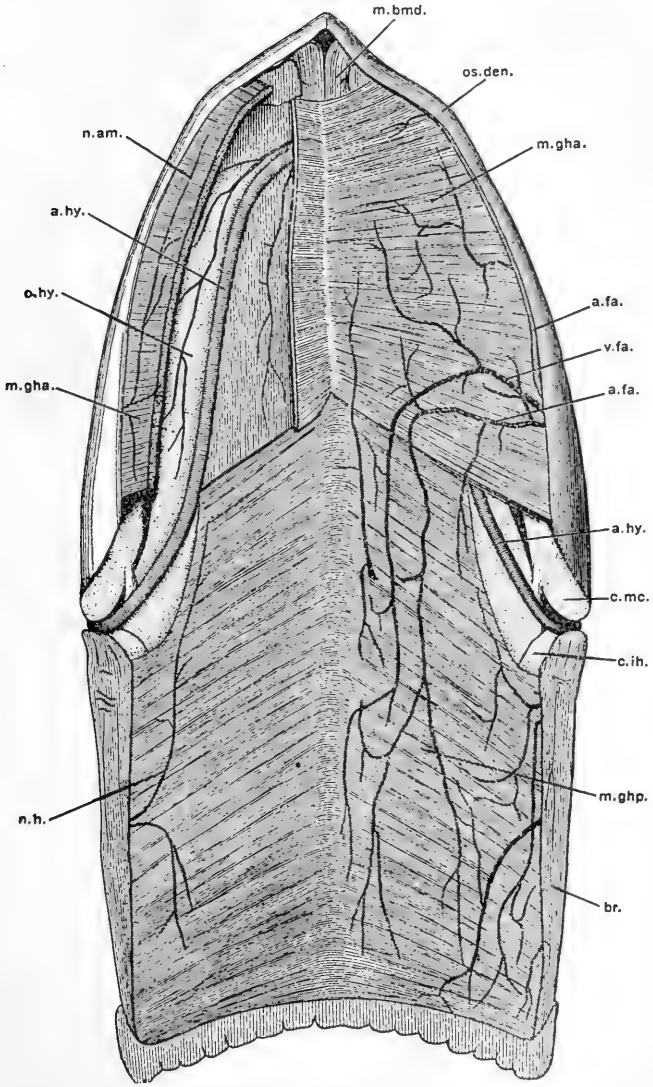


Fig. 1 Dissection of region beneath the lower jaw

at first suggest an intermandibularis, but in teleosts and Amia, where such a muscle is recognized, it is found to consist of fibers which lie close to the symphysis and pass from one ramus of the mandible to the other with no median interruption. Both of these characters indicate that the muscle in question is not a true intermandibular. Vetter ('78) quotes Stannius to the effect that the geniohyoid of Acipenser is supplied in part by the trigeminal nerve, which is in accord with conditions found here. The anterior part of the muscle seems to correspond with the ventral constrictor of Heptanchus which Vetter ('74) designates as CSV_2. The posterior part represents the superficial fibers immediately posterior to this. Such an interpretation seems the more probable when it is recalled that with the great development of the opercular folds which occurs in Polyodon the web of tissue connecting the two flaps ventrally is extended backward to a very marked degree (fig. 3). There is in consequence this extensive development of skin musculature representing a transverse band which was primitively very much narrower.

M. adductor mandibularis: figure 2, m.adm., m.adm.'

The M. adductor mandibularis is in two parts, a long rounded superficial portion (*m.adm.*), and a short flat mesial part (*m.adm.'*). These two elements are somewhat distinct but become confluent where in contact and especially towards their insertion. The superficial division arises on the dorsal surface of the palatoquadrate from the median line in front back to the middle of the cartilage. Anteriorly it is horizontal in position and occupies the space between the M. protractor hyomandibularis above and the palatoquadrate cartilage and maxillary bone below. Near the angle of the mouth it passes under a strong triangular fascia and turns abruptly downward to be inserted (a) in the anterior part of a broad shallow groove in Meckel's cartilage, and (b) on the median aspect of the overlying dentary bone. The deep division of the muscle, which extends somewhat further caudad than the other, arises laterally from the posterior third of the palatoquadrate, but not from its lateral projection which over-

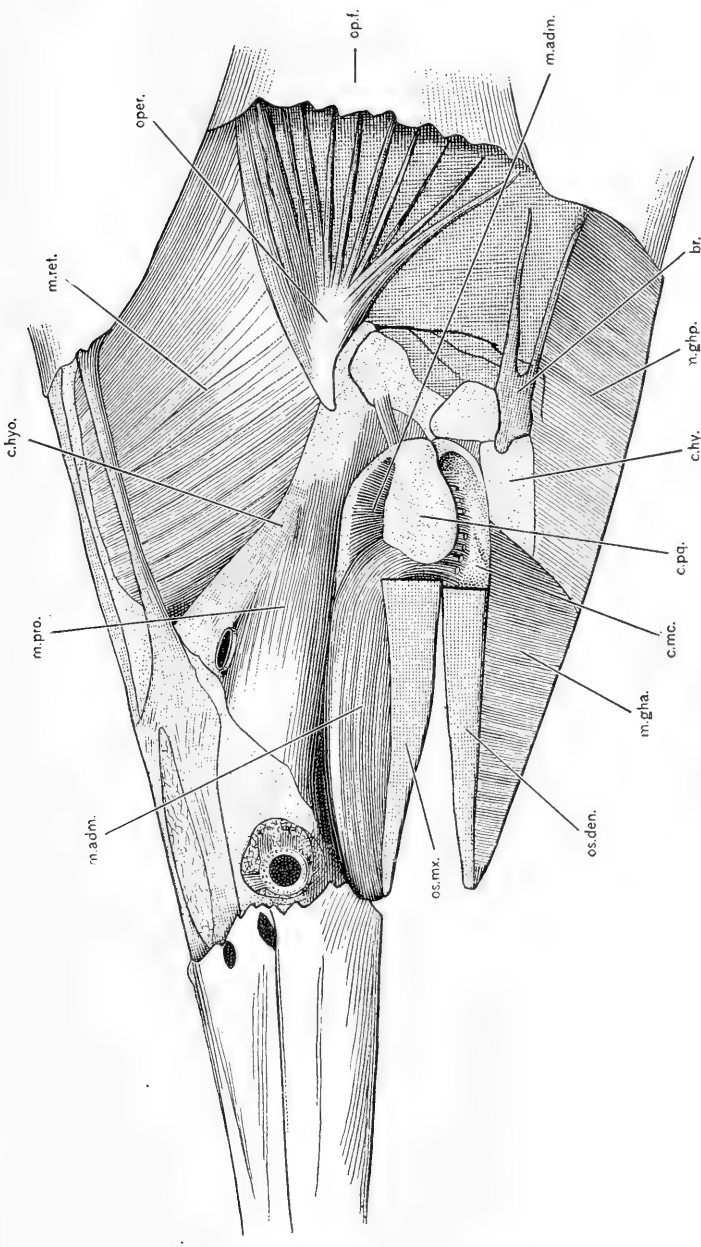


Fig. 2 Dissection of side of the head

hangs the muscle. Its fibers are very nearly vertical in position. The anterior are inserted in the groove in Meckel's cartilage, medial to the insertion of the superficial division of the muscle. The posterior fibers are inserted (a) into the posterior part of the groove and (b) into the dentary bone.

Innervation. Both divisions are supplied by the inferior maxillary branch of the trigeminus which runs along the dorso-mesial side of the superficial part and crosses the lateral face of the deep part. The main branch to the former is given off near the middle of the muscle and is directed anteriorly.

Blood supply. The facial artery and facial vein supply both divisions.

Action. The anterior portion, besides helping to close the mouth, must also tend to protract the mandible, since its pull is somewhat diagonally forward and upward. The deep part may tend in a measure to oppose its action as a protractor. Working together or separately they would close the mouth. In both origin and insertion this muscle corresponds fairly well with the adductor mandibularis of Acipenser. There, however, according to Vetter's description, the muscle is a weak flat element which becomes tendinous towards its insertion. Some of the fibers are inserted on the mandible as in Polyodon. In Acipenser there is, in addition to the adductor mandibulae, a strong constrictor muscle (*Cs.*, of Vetter) which overlies it. The latter arises from the antorbital process and extends around the lower jaw. The anterior part of the adductor in Polyodon has a superficial resemblance to this muscle, but none of its fibers arise from any part of the cranium proper and I have been unable to find any indication that they ever pass over into the ventral constrictor below the jaw. Consequently, from adult material alone, it can not be stated with any certainty that the anterior adductor of Polyodon finds a homologue in the constrictor of Acipenser, although there is a possibility that such is the case. If not, then the constrictor is unrepresented in Polyodon and the adductor is somewhat more specialized. In comparison with the adductor muscles of Amia and the teleosts that of Polyodon is remarkably simple.

M. protractor hyomandibularis: figure 2, m.pro.

Of the two muscles in connection with the hyomandibular apparatus the anterior resembles very closely the muscle in Acipenser designated by Vetter ('78) and Gegenbaur ('98) as the protractor hyomandibularis. It is a large muscle, which in Polyodon arises in two separate parts, which soon unite. The smaller portion, whose fibers constitute the ventro-median part of the muscle, arise laterally on the cartilaginous base of the skull from a small area lying medial to the anterior opening of the facial canal, close to the roof of the mouth and immediately in front

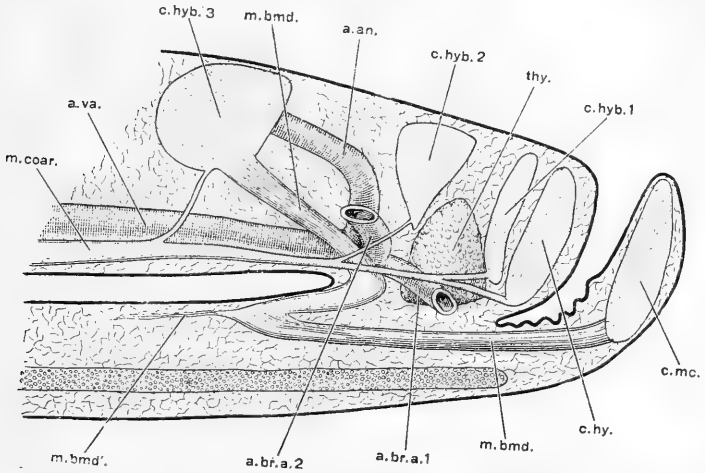


Fig. 3 Schema to show relationships of the branchiomandibular and sternohyoid muscles

of the spiracular cleft. From below, its origin is concealed by the parasphenoid bone and the overlying cartilage. The second and much larger portion of the muscle arises from the postorbital process, from the side of the chondrocranium and from the overhanging supraorbital cartilage, nearly as far forward as the olfactory capsule. The most anterior fibers, which are somewhat tendinous at their origin, are dorsal and medial to the eye. This muscle fills the angle between the hyomandibular and skull and presses against the membrane stretching across the

spiracular canal in front. With the preceding muscle it fills out the side of the face. It is inserted on the anterior aspect of the hyomandibular, from the lateral margin of the spiracular canal throughout the middle third of the cartilage. Toward their insertion the fiber bundles tend to become grouped and tendinous.

Innervation. In a dissection of an adult the rather large nerve of supply clearly comes from the inferior maxillary division of the trigeminus. Serial sections seem to show it arising from the undivided main stem as Vetter suggests it may do in Acipenser. Perhaps its exact point of origin is subject to some variation.

Blood supply. It is supplied by small twigs from the hyoopercular artery, which pass over the hyomandibular cartilage and from branches of the external carotid anterior to the facial canal. The venous supply apparently is by branches of the jugular.

Action. Contraction of this muscle tends to rotate the hyomandibular on its median articulation, swinging its distal end and the attached operculum forward and outward.

The partial division of this muscle is of some interest, since the homologies of the levator arcuus palatini and dilator operculi in teleosts are rather uncertain. This question has been taken up from several points of view by Vetter ('78), McMurrich ('85) and Allis (97). It does not seem profitable to discuss it here beyond calling attention to this one point. Apparently the hyomandibularis of Acipenser, which Vetter homologizes with the above-mentioned teleostean muscles, is a simple muscle, and it appears from his account that the trigeminal nerve passes through it. In Polyodon the parts on either side of the exit of the nerve are separated at their origin. If this separation should extend to the insertion there would result two muscles, one deep, the other superficial, and the independent action of each would be somewhat different from their combined action in the form of a protractor hyomandibularis. This observation merely indicates a possibility, or it may be, shows a tendency in the phylogeny of this muscle.

MM. retractor hyomandibularis et opercularis: figure 2, m.ret.

The retractor hyomandibularis and the opercularis are practically identical with similar muscles in Acipenser (Vetter '78). In Polyodon, however, they are confluent at their contiguous margins. Nevertheless the line of union is probably indicated by differences in size of the muscle bundles, those of the retractor portion being much the larger. The combined muscle arises from a rather large groove on the side of the cranium, extending from near the articulation of the hyo-opercular back about to the level of the dorsal corner of the opercular cleft (fig. 4, *m.ret.*). Some of the posterior fibers may arise cutaneously from the dorsal margin of the opercular flap. Few, if any, arise from the overlying frontal bone as they do in Acipenser. From its origin the muscle spreads out fan-like and descends to its insertion on the hyomandibular, to which it is attached from the medial articulation to the distal end. Beyond the end of the hyomandibular the fibers, probably all belonging to the opercularis proper, are inserted along the upper edge of the operculum, or more strictly, in the skin immediately beneath it. The most posterior fibers reach about as far caudad as the end of the dorsal spicule of the opercular bone. These fibers have a relation to the operculum which is identical with that which the posterior geniohyoid fibers bear to the similar branchiostegal ray below. They probably represent the two ends of the same primitive superficial constrictor.

Innervation. They are supplied by the hyomandibular branch of the facial nerve which runs along the cartilage and sends superficial branches over the muscles. The ramus oticus trigemini passes through the occipital cartilage and runs over the surface of the muscle. It sends twigs to the muscle and also passes through it to anastomose with a branch of the vagus. Whether either of these nerves supply motor fibers to the muscles cannot be stated.

Blood supply. The blood supply is through the hyo-opercular artery and the accompanying vein. There are also twigs from the trunk of the second efferent branchial artery.

Action. The combined muscle acts as an opponent to the protractor and also raises the hyomandibular and dependent structures. The posterior fibers tend to constrict the opercular aperture. Obviously 'levator' would be quite as appropriate a term for this muscle, but since both Vetter and Gegenbaur designate the same muscle in Acipenser as 'retractor,' I have retained their nomenclature.

IV. MUSCLES OF THE BRANCHIAL ARCHES

Within the membranous septum between each pair of demibranchs there is developed a lamina of striated muscle, M. interbranchialis, the fibers of which are grouped in irregular bundles which extend chiefly from the cartilage on the (morphologically) posterior edge of the groove for the efferent branchial artery diagonally across the septum to its anterior lateral margin. Very few fibers take the other diagonal course so as to cross these. The innervation was not definitely determined but obviously the supply comes from the neighboring fused pre- and post-trematic rami which are the only nerve fibers in the vicinity. It is difficult to state their function definitely. Pulling on the septum, they probably tense the filaments which are borne on it and throw them into a position favorable for the circulation of water among them.

Besides this musculature, which would perhaps be more appropriately described as the intrinsic musculature of the organs of respiration, there are for each branchial arch three additional muscles, one at the median end of the dorsal half of the arch, one between the two moieties and one at the ventro-median end. Posteriorly there is a single transverse muscle above and one below. These muscles will now be described in the order named. Muscles relating the branchial arches to the shoulder girdle will be discussed in another section.

MM. levatores arcuum branchialium: figure 4, m.lev.

The four levator muscles of the gills arise as a continuous sheet from a broad line on the back of the chondrocranium beneath the area of origin for the retractor hyomandibularis. The anterior fibers are the most ventral and have their origin

immediately behind the posterior opening of the facial canal. The posterior fibers arise more and more dorsally and medially as shown in the figure 4. The anterior fibers are shortest, the posterior longest. Somewhat beyond its origin the muscle mass becomes indistinctly separated into its four parts, the anterior of which tends to overlap the next posterior. These are inserted into the dorsal margins of the four epibranchial cartilages. Some of the fibers also may have a cutaneous insertion in the tough skin at the dorsal angles of the gill slits.

Innervation. Each muscle is supplied by the appropriate ramus posttrematicus which crosses its anterior surface a little above the insertion. In the case of the first gill this is a branch of the glossopharyngeal nerve, all the others are from the vagus. The next posterior ramus praetrematicus also crosses each of these muscles, passing over its median side, but I could find no fibers being given off from it to the muscle. There is also still another branch of the vagus which passes up under the origin of the muscle to anastomose with the ramus oticus trigemini. No muscular branches of this nerve, however, were detected.

Blood supply. Blood is supplied by the pharyngeal branch (*aph.*) of the second efferent artery, which sends twigs into the medial side of the muscle, and from smaller arteries arising from the efferent branchials within each gill.

Action. These muscles serve to raise the gill arches and draw them slightly toward the median line.

In the nature of their origin, insertion, innervation, and to a certain degree their blood supply these muscles compare very closely with the retractor hyomandibularis. Their function is also similar. The fact that they do not appear on the side of the head is due simply to the pressure of an overhanging opercular apparatus. They are none the less superficial muscles, and quite homologous with the foregoing. The tendency for the first to overlap the one behind is parallel to the tendency on the part of the retractor hyomandibularis to overlap the muscles posterior to it. The confluence of these muscles at their origin is possibly not a primitive condition. From a consideration of Polyodon it is not easy to understand Vetter's uncertainty regarding the apparently homologous muscles of Acipenser.

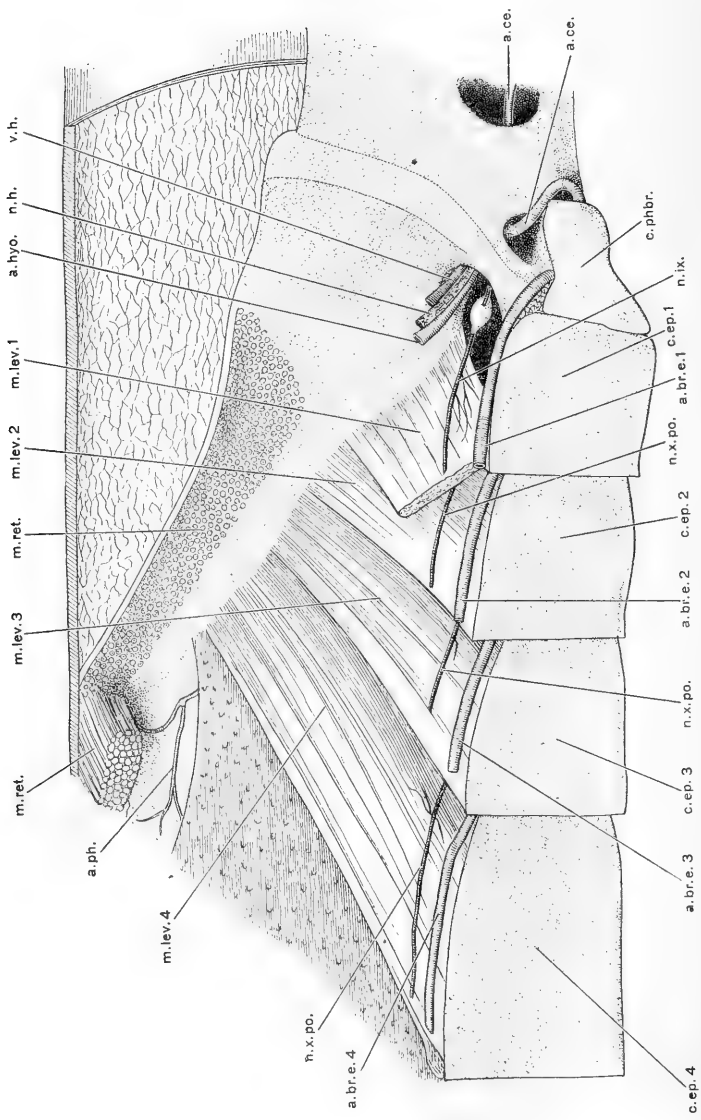


Fig. 4 The levator muscles of the gills

Adductores arcuum branchialium: figure 5, m.adb. (2, 4)

Within the branchial apparatus there are four pairs of well developed adductors. As has frequently been stated, the branchial cartilages of Polyodon, instead of having the usual somewhat rounded form, are flattened into very broad thin plates as shown in figure 5. From the flat posterior surface of each epibranchial there arises the corresponding adductor muscle. The area from which fibers take origin covers the middle portion of the cartilage and does not approach the margin at any point. The length of the area may be more than a third of the length of the whole element. The muscle is covered by a tough apo-

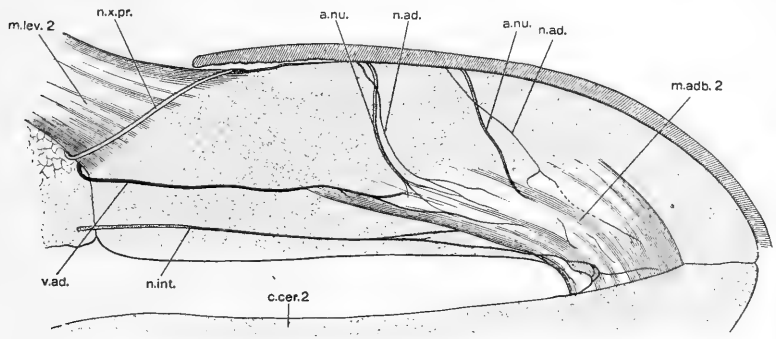


Fig. 5 A dissection of part of the second gill viewed from behind

neurotic sheet which binds it to the cartilage and also serves as a secondary basis of origin. The somewhat converging fibers run obliquely downward and outward and at the ventral margin of the epibranchial, cross to the anterior side of the ceratobranchial of the same gill, where they are inserted in a shallow excavation. Many of the fibers become tendinous toward their end and the tendons blend more or less with another very tough oponeurosis which covers this part of the muscle.

Innervation. The rami prae- and post-trematicus, in entering the gill above, cross the anterior and posterior faces of the levator muscle of the same gill. At its lateral margin they either join completely or else anastomose to such an extent that it is no

longer possible by ordinary methods to determine the real source of subsequent branches. From the thus-formed nerve or plexus there are two branches which pass down the posterior side of the cartilage and enter the muscle. It is possible that in some cases there are more than two of these branches. Sewertzoff ('11) has recently described a new nerve which, in some elasmobranchs and ganoids, enters the gill internal to the branches heretofore recognized. This nerve, which he designates as Ramus praetrematicus internus, I find to be present also in Polyodon (fig. 5, *n.int.*). It enters the gill posteriorly and follows the margin next to the mouth cavity well around toward the median line below. In crossing from the epi- to the cerato-branchial region it traverses a part of the adductor muscle and sends fibers over its surface and down into it. Whether or not these are actually motor in their nature I cannot state. All of these internal pretrematic nerves to the gills are branches of the vagus.

Blood supply. The intra-branchial branches of the corresponding efferent arteries supply these muscles. Usually there is one branch (*a.nu.*), arising toward the dorso-medial end of the gill and accompanying one of the nerves that is especially well developed. A large vein (*v.ad.*) on the posterior side crosses the cartilage lengthwise to reach the jugular.

Action. These muscles approximate the roof and floor of the mouth and act as opponents to the dorsal and ventral musculature. Speaking in general terms the adductors within the gills represent a group of disappearing organs. They are present and apparently uniformly developed in the elasmobranchs. In Chimaera also there are five of them (Vetter) but not strongly developed. In Acipenser their number is reduced to three. In Amia (Allis) there are two and in Ameiurus only one. Quite often they are altogether lacking in teleosts. Polyodon is thus shown to be exceptional in retaining a full complement (as many as there are gills) all of which are strongly developed. The matter of origin and insertion appears at first sight to present a problem, for each muscle arises on the posterior side of an arch and is inserted on its anterior side, whereas in other fish the origin and insertion both appear from published descriptions to

be on the anterior side except in a very few cases, as for example in Amia, where the muscle, already at the point of disappearing may be slightly altered in its relations. The several descriptions show, however, that the origin and insertions are often very nearly or quite on the inner margins of the cartilages concerned. If, therefore, the muscles of Polyodon were already differentiated and in this condition before the cartilages became flattened, they might, it would seem, migrate indifferently to either the posterior or anterior sides of the modified branchial cartilages, and the condition observed in Polyodon established. If this really represents the phylogenetic development of the muscle, then there seems to be no objection to following Vetter in comparing these muscles with the adductor muscle of the jaw, the origin of which has moved to the anterior face of its cartilage as the branchial adductors have tended to do in many other forms.

As explained above there is some uncertainty as to the nerve supply of these muscles. It is easily shown that the nerve to each reaches it by crossing the posterior face of the cartilage. And so they do in Amia according to Allis ('97). Vetter on the other hand, while sometimes difficult to interpret, nevertheless seems to imply the existence of other conditions in the fishes that he describes. He states ('78, p. 449) that in Chimaera the adductor muscles are supplied by the 'R. post.' of the corresponding inter-branchial branch of the vagus. If 'R. post.' means ramus posttrematicus vagi, as seems most probable, then it is the anterior nerve of each gill that supplies the muscular branch, and he leaves the first adductor muscle of Chimaera entirely unaccounted for, probably a mere oversight. If this interpretation of his meaning be correct, it brings this statement into accord with what he says elsewhere ('74, p. 445) regarding the elasmobranchs, where the glossopharyngeal nerve is mentioned as supplying its appropriate adductor muscle, for, it will be recalled, the glossopharyngeal supplies the ramus posttrematicus of the front gill. His comment on Acipenser throws no light on the subject. Now if Vetter's observations are accurate and his statements correctly interpreted it appears that the adductor muscles belong with the posttrematic nerve of their respective gills. If

such be the case it is difficult to see how, on the one hand, in Polyodon and apparently Amia the innervation of the muscle has been changed, or on the other hand, if the same fibers still supply it, how they have crossed over the cartilage so as to approach the muscle from the other side. A further comparative study on this group of muscles seems very desirable.

MM. interarcuales ventrales

The four ventral interarcuate muscles seem to be identical in all respects with the similar muscles in Acipenser. In the following brief account of each, I have described them in the reverse order, as compared with Vetter's account of Acipenser, i.e., from a physiological rather than a morphological standpoint.

1. The most anterior arises by a short tendon of origin from the hypohyal, a little lateral to the insertion of the coraco-arcualis tendon. It is inserted on the cerato-branchial I for a short distance along its ventral margin and on its anterior face close to the margin. It differs from the following muscles in that it extends between two different arches, the hyoid and the first branchial.

2. The second is a short muscle filling the small triangle between the hypo- and cerato-branchial cartilages of the second arch, ventral to their articulation with each other.

3. The third has relationships in the third arch similar to those of the second in its arch. Some of its fibers, however, may be inserted on the posterior as well as the anterior face of the cerato-branchial near its ventro-mesial margin. It is in this respect somewhat transitional between the fourth and those anterior to it.

4. The most posterior is the only one of these muscles that arises from a basibranchial cartilage. It has its origin from the third basibranchial cartilage, close to the articulation of the fourth branchial cartilage, and is inserted on the ventro-medial margin and posterior face of the latter.

Innervation. Each of these muscles is supplied by the same nerve plexus that supplies the corresponding adductor arcuus branchialis.

Blood supply. They receive arteries from the recurrent branchials of their own gills. Their veins are tributaries of the inferior jugular.

Action. Working in conjunction with the levator muscles they slightly increase the cavity of the pharynx. They also apparently tend to separate the gills from each other, especially in the case of the first and the fourth.

MM. transversus dorsalis et transversus ventralis: figsures 6 and 7, m.tr.d., m.tr.v.

There remain to be considered in this group two muscles, the transversi, of whose homologies I do not feel entirely certain. One is dorsal, the other ventral. Although entirely distinct from each other anteriorly, they become confluent posteriorly at their margins and form the proximal musculature of the oesophagus. Possibly we see here the phylogenetic origin of the upper striated muscle in the oesophagus of higher vertebrates.

The transversus dorsalis (fig. 6, *m.tr.d.*), which is apparently unrepresented in Acipenser, is a thin flat muscle whose fibers extend transversely between the fourth epibranchial cartilages of the two sides. The muscle is attached to the inner side of each cartilage forward nearly to its anterior end. Posteriorly, where the line of their insertion is interrupted by the presence of the adductor IV, they are inserted in a strong membrane covering the latter.

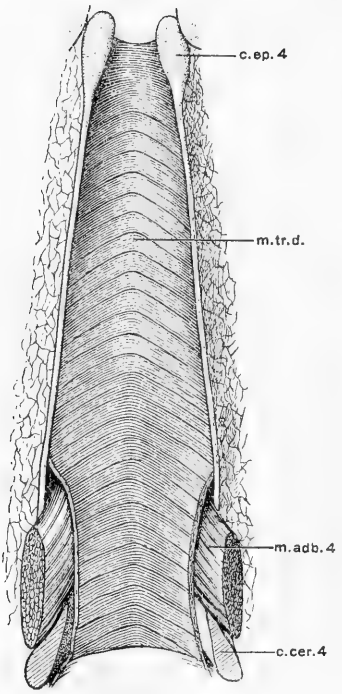
The individual fibers of the ventralis (fig. 7, *m.tr.v.*) do not cross the middle line but arise from a strong median aponeurosis and are inserted on the fifth ceratobranchial cartilages toward their posterior (distal) ends. The line of insertion does not extend forward beyond the posterior third of the cartilage.

Innervation. Both muscles are supplied by small branches of the vagus.

Blood supply. The dorsal muscle is supplied by the pharyngeal arteries, the ventral by the A. coraco-cardica.

Action. They are decidedly constrictor in function, approximating the cartilages with which they are connected and narrowing the cavity of the posterior part of the pharynx.

The transverse muscles reach their maximum development in Amia and teleosts. They are generally considered in the same category as the oblique muscles (interarcuales), and Vetter classes the single ventral transverse muscle in Acipenser as the inter-



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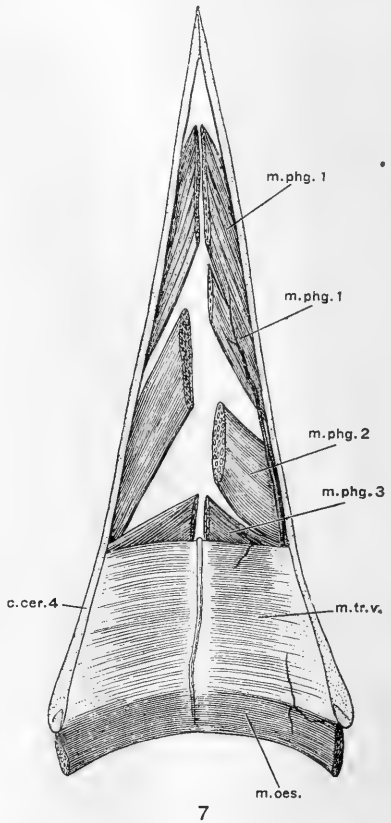

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Fig. 6 The dorsal transverse muscle of the pharynx
Fig. 7 Pharyngoclaviculares and ventral muscle of the pharynx

arcualis ventralis V. If it be such, both its origin and insertion have migrated back to a marked degree. Good evidence on this point is apparently not to be sought in the adult Polyodon. Possibly the embryology may throw some light on the question.

V. HYPOGLOSSAL MUSCLES

That portion of the ventral body musculature which lies anterior to the shoulder girdle is in intimate relation with the mandibular, hyoid and branchial arches. In fishes generally this musculature is supplied by the first spinal nerve in anastomosis with one or more postvagal roots (the 'hypoglossal nerve'), or by the first and second spinal nerves. Postvagal roots probably occur in all the ganoids. In Amia there are two. Van Wijhe (l.c.) states that he was unable to detect any in Polyodon, but the present writer finds a very delicate strand which leaves the medulla close to the root of the vagus and emerges from the skull through a minute occipital foramen. It ultimately joins with the first spinal nerve, i.e., the first postvagal nerve which has both ventral and dorsal roots. The conjoined nerve supplies, so far as could be determined, the whole hypoglossal musculature.

This musculature in Selachians consists of the coraco-arcuales (Vetter) which in the simpler forms, such as Heptanchus, extends forward from the coracoid, giving off slips to each branchial arch, to the hyoid and to the mandible. In the higher forms these elements are variously modified and reduced. The question of the relation to the trunk musculature cannot be fully considered in this connection. For a full discussion of this musculature in lower forms, the reader is referred especially to the work of H. V. Neal ('97).

The elements of the original coraco-arcuales that remain in Polyodon are the following:

M. branchiomandibularis: figure 3, m.bmd.

The branchiomandibular muscle is bilateral at its origin and insertion, but single and medial throughout most of its length. Its fibers arise on either side directly from the hypobranchial cartilage of the third arch just medial and anterior to the insertion of the tendon of the coraco-arcualis (hyopectoralis) to that arch. The muscle slip on either side passes forward, downward, and inward, medial to the main tendon of the coraco-arcualis, to meet its fellow of the opposite side at a point ventral to the

aorta and at about the level of the second gill. The single median muscle that results turns downward and backward in the opercular fold (fig. 3). In this region it is largely tendinous. After coursing posteriorly for a few millimeters it again turns forward, thus giving the muscle when at rest a *Z*-form. At the point where it turns forward it is joined by a few fibers (*m.bmd.'*) from the opercular folds. Anteriorly this median muscle again divides into lateral halves which are inserted on the rami of the mandible very near to the median line.

Innervation. Repeated dissections failed to disclose the nerve of supply. In those fish where it is described (e.g., Amia, Allis) it is a branch of the first spinal nerve.

Blood supply. Arterial blood is supplied by the hypobranchial arteries and the terminal twigs of the facial with which they anastomose anteriorly. The veins are tributaries of the inferior jugular.

Action. No amount of stretching suffices to pull the muscle into a straight line, so the anterior and posterior parts doubtless act separately, both serving as depressors. Their action, however, must be feeble, for the muscle is very small.

McMurrich (l.c.) calls attention to the fact that this muscle diminishes as we ascend the scale and points to Amia as probably the last piscine form to show it. In that fish it is in relation with the second, instead of the third arch, as in Polyodon and also Acipenser. The few fibers which run into the opercular fold and presumably tense the median fascia into which the geniohyoid is inserted may be the last remnant of a primitive connection of this muscle with the system of superficial constrictors.

M. coraco-arcualis: figures 3 and 8, m.coar.

This is the hyoclavicularis or sterno-hyoid muscle. It takes origin chiefly from the anterior third of the cartilaginous part of the pectoral arch (fig. 8, *m.coar.*). The fibers arise directly from a long crescentic area located medially and toward the ventral side of the cartilage. A conspicuous portion of the mus-

cle, however, is directly continuous with the great ventral musculature (fig. 9) of which it appears to be simply an anterior prolongation. Several deep intermuscular septa, in serial continuity with those of the body musculature, cross the belly of this muscle obliquely. The muscles of the two sides meet in front of the pericardium and unite, but the two halves are separated by a median aponeurotic septum into which fibers are inserted. The median muscle now grows rapidly smaller anteriorly and passes through a longitudinal canal formed between the two infraclavicles. In front of them the two halves again separate and are produced forward as long slender superficial tendons (fig. 3). The main continuation of each tendon is inserted on the hypohyal cartilage. Dorsal slips are also given off from the tendon to the first, second and third basibranchial cartilages.

Innervation. The muscle is supplied by the combined first spinal and post-vagal nerve. Its branch arises from a large trunk near the origin of the pharyngoclaviculares and may even pass through them to reach its destination. Within the muscle it can be traced a considerable distance and probably successful dissection would carry its terminal ramifications forward to the branchiomandibularis.

Blood supply. The arteries which reach this muscle are: in front, the large posterior branch of the posterior commissure of the hypobranchial system; behind, the infra-pericardial branch of the coronary.

Action. It depresses the whole hyoid and branchial apparatus.

This muscle agrees very well with the coraco-arcualis anterior of Acipenser, at least in so far as its insertion is concerned. In Acipenser there is also a coraco-arcualis posterior which is inserted on the fourth hypobranchial cartilage. This is lacking in Polyodon. The above described (anterior) coraco-arcualis of Polyodon is especially interesting since here we find it *actually* continuous with the longitudinal body musculature, a condition which apparently does not often obtain in animals with a pectoral arch. In this respect it recalls Petromyzon (Neal, '97).

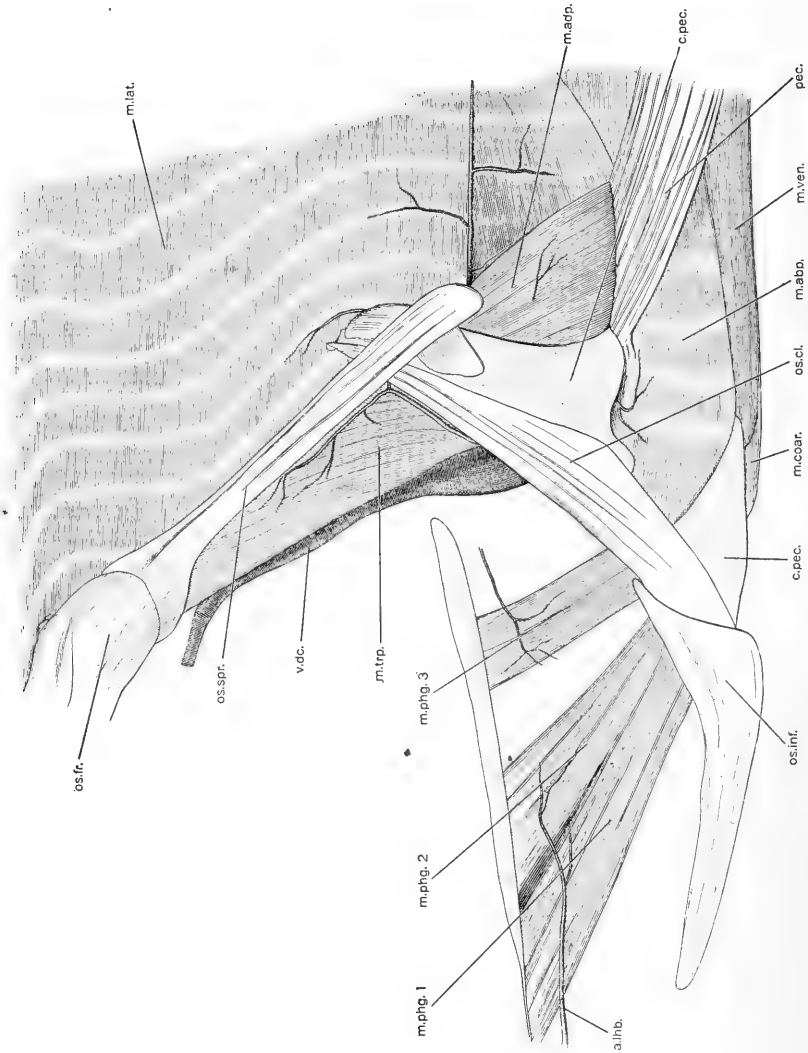

Fig. 8 Muscles of the pectoral arch

MM. pharyngoclaviculares: figure 8, m.phg. (1, 2, 3)

The remaining muscles of this group might be considered as one, two or three pairs, depending on the tendency of the writer. They all arise in one mass, as in Amia. The fibers destined to form the anterior and intermediate muscles take origin from a crescentic area just dorsal to the site of origin for the coraco-arcualis, with which they are practically continuous. The remaining fibers arise still higher up from the cartilage and from the membranous septum that covers the large fontanelle in the cartilage. The anterior division is inserted on the anterior part of the slender basipharyngeal cartilage and into connective tissue, between or actually upon the fifth ceratobranchials. The intermediate portion is inserted on the basipharyngeal behind the foregoing and the posterior part has a similar insertion still further back. The latter, however, especially in young fish, is not connected closely with the cartilage, but rather with a median aponeurosis, the same from which the transversus ventralis takes origin.

Innervation. These muscles are supplied at their common origin by branches of the first spinal nerve beyond its anastomosis with the postvagal nerve. I could trace no branches of the vagus into their upper ends.

Blood supply. Dorsally they are supplied by the a. coraco-cardiaca and the posterior dorsal branch of the fourth recurrent branchial artery which may show an end to end anastomosis with the former. Ventrally the artery of supply is the infra-pericardial branch of the coronary.

Action. They serve to depress the posterior part of the floor of the mouth and assist in the first act in swallowing (?).

These muscles seem to be entirely lacking in Acipenser sturio since Vetter makes no mention of them. They are present in Amia, however, and, as McMurrich hints, probably represent remains of coraco-arcuales of the fifth, sixth, and possibly seventh arch of some ancestral form (cf. Heptanchus). Usually there are two of them, the ones referred to above as anterior and intermediate being called the 'external,' the other the 'internal.'

Their tendency to remain distinct may indicate their primitively separate nature.

VI. MUSCLES OF THE TRUNK

Lateral trunk muscle

The musculature of the trunk is in two separate parts, the lateral and the ventral. The former, which can very well be considered as a single muscle, arises from the occipital part of the skull and from the strong fascia back of the branchial region and is inserted in the caudal fin, both above and below the level of the notochord. It is crossed by about fifty-eight tendinous septa, the myocommata, which divide it into its myomeres and attach it firmly to each segment of the axial skeleton. The fibers, which run in a longitudinal direction, arise from one myocomma and are inserted on the next posterior. The muscles of the two sides are in contact with each other dorsally throughout the whole length, except where separated by the dorsal and caudal fins, and ventrally between the posterior end of the anal fin and anterior margin of the caudal. They approach but do not actually meet along the line between the pelvic fins and vent. Toward the insertion, above the notochord and within the caudal fin, the dorsal (epaxial) part of the muscle becomes decidedly tendinous. This is also true, but to a much less degree of the hypaxial part.

The several longitudinal body muscles recognized in teleosts are not differentiated in Polyodon, although homologous regions may be more or less clearly indicated by the foldings of the myocommata and muscle segments. The so-called dermal musculature is clearly evident both in dissections and in transverse section, where it can be distinguished by the smaller fibers which are more loosely arranged. It is, however, segmented and each segment is directly continuous with the subjacent myomere.

Since the septa do not cross the muscle in a strictly transverse plane but proceed from axis to periphery in a zigzag fashion, the separate myomeres are somewhat complicated in form. Fig. 9 represents a dissection of the tenth one. Roughly speaking, it is in the form of a cone, the medial half of which has been cut away. Its apex lies just above the notochord and points forward.

From the base of this half cone, which opens caudally, there are two wings, one extending ventrally the other dorsally. They separate from each other at the level of the lateral line. The ventral wing runs obliquely backward and downward to meet the ventral musculature. The lateral margin, being more posterior than the medial, is overlapped by the preceding, and itself overlaps the following segment. The dorsal wing of the myomere at first runs diagonally back and up and then folds on itself so as to run forward and up, terminating as far anterior as the point from which it originated. Where this wing of the myomere bends forward a pocket is produced with its opening directed forward. This results in the formation of a true cone, the apex of which reaches further caudad than any other part of the myomere. It fits into the posterior cones and is filled by those in front of it like a nest of beakers. Above the lateral line all the myomeres are essentially similar, but below, their form changes somewhat both anteriorly and posteriorly. In front, from the fifth myomere on, the ventral wing bends forward below, and its margin is also rolled outward somewhat, thus producing a kind of incomplete cone-like formation. Behind the ventral fins the succeeding myomeres end relatively further and further forward and this consequently results in the formation of caudally pointed cones and a duplication of the upper half of the musculature so that the epaxial and hypaxial portions come to be similar to each other.

Innervation. The postvagal nerve root joins the first spinal nerve at some distance from its point of emergence from the skull, and lower than the level at which the branches to the lateral muscle are usually given off. Nevertheless it is possible that a few of its fibers do reach the first segment, although it is much more probable that they are all destined to the hypoglossal musculature. The fifty-eight spinal nerves each give rise to dorsal, lateral, and ventral branches. The large lateral branch is very short and enters the corresponding myomere directly. A small dorsal branch follows the edge of the myomere upward and the main ventral branch follows the lower wing of the myomere downward.

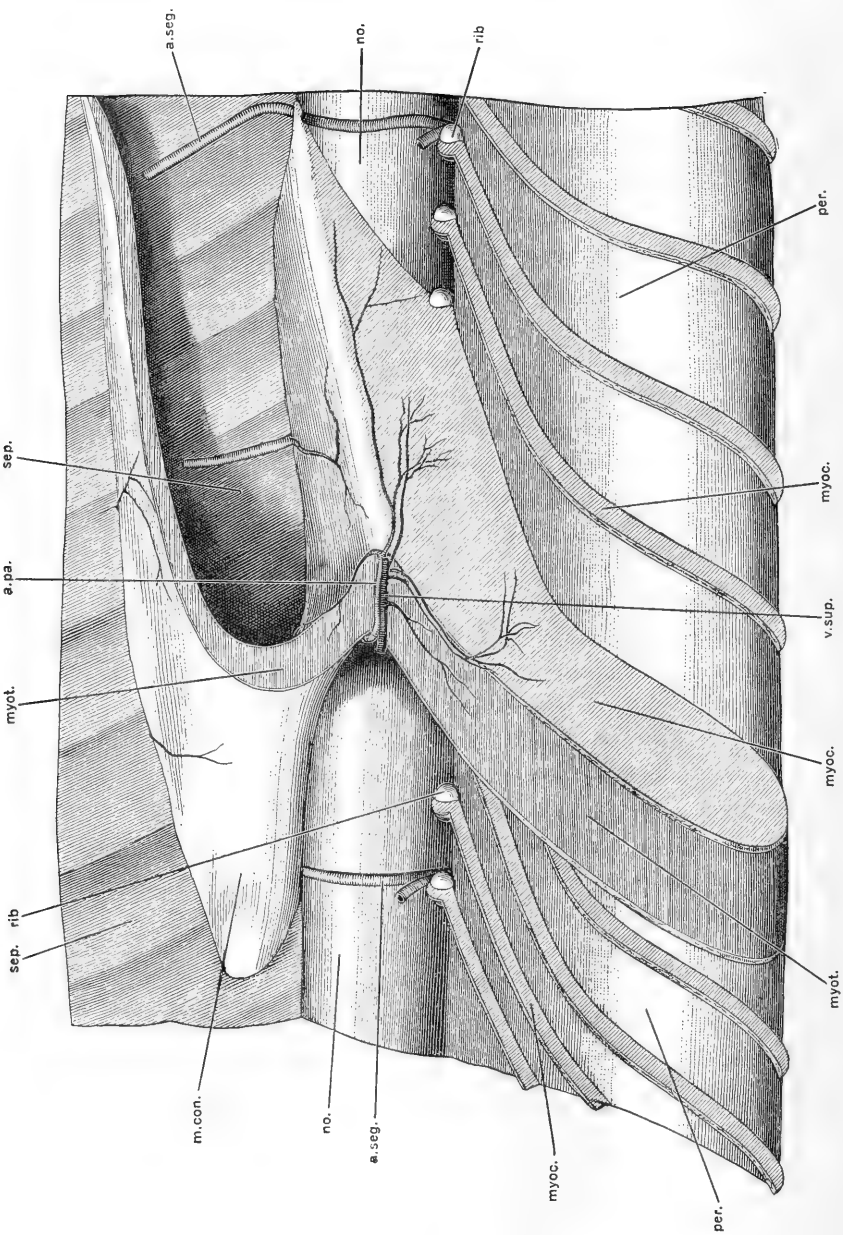

Fig. 9 Stereogram of the tenth muscle segment and associated structures

Blood supply. The myomeres are supplied throughout by the segmental arteries. Some of the anterior are also supplied in part by the a. thoracico-dorsalis. They are drained by the segmental and lateral abdominal veins.

Action. This is the muscle of locomotion. Its contractions bring the head and tail toward each other, bending the body laterally. Through the attachment of the myocommata the action is not alone on the tail but on all the segments back of the skull. The corresponding lateral muscle of the opposite side is its opponent.

The great ventral muscle: figure 10

Embryological studies on a number of lower vertebrates have shown that the ventral musculature arises from a series of buds that form one at the end of each segment of the lateral musculature. Consequently the ventral muscle is segmented in the same manner as the lateral. In the adult Polyodon the fifth segment of the lateral muscle is directly in contact below with one of the segments of the ventral muscle; each succeeding segment back to about the twenty-fifth is similarly related to a corresponding segment of the ventral muscle. Anterior to the fifth segment of the lateral muscle the segments of the ventral part, owing perhaps to the intervention of the pectoral fin, are no longer in contact with those above. Some of the anterior part is extended forward as an element of the coraco-arcualis upon which there are transverse inscriptions, but these are sufficiently numerous to preclude the possibility of their representing remains of primitive myocommata—unless there are a number of otherwise aborted postoccipital segments. The ventral muscle does not reach the median line, being separated from its fellow of the opposite side by a wide, tough, linea alba. It tapers posteriorly and ends in front of the vent almost as a point. Anteriorly its medial superficial fibers are slightly oblique, being directed forward and inward. Maurer ('12, p. 38) designates these fibers as the musculus obliquus inferior. He further subdivides the fibers of the remaining ventral musculature into mm. obliquus superior and obliquus medius. The latter is composed of fibers parallel to the former but covered by the obliquus inferior.

Innervation. The segments are supplied by the ventral branches of corresponding nerves.

Blood supply. The Aa. thoracico-dorsalis et thoracico-ventralis and the terminal ends of the segmental arteries supply blood to these muscles. It is carried away by the lateral abdominal vein.

Action. This musculature is accessory to the lateral muscle and also tenses the body wall between pectoral and ventral fins and tends to increase the intra-abdominal pressure.

VII. MUSCLES OF THE MEDIAN FINS

Muscles of the dorsal fin

As has been stated by Bridge ('96) there are twenty-one radial cartilages in the dorsal fin. Corresponding with each of these cartilages there are developed two muscles on each side. The two muscles on the same side of a ray however are only indistinctly separated from each other. The more superficial arises on the aponeurosis of the myomere beneath from its dorsal and to some extent its mesial aspect. There are two of these muscles for each myomere. The first ones arise in connection with the twenty-fourth. They are all inserted beneath the horny rays of the fin. The deep muscles correspond throughout with the superficial. They arise from the fascia near the median line and from the basalia and radialia of the fin. They are inserted on the same structures as the superficial muscles and possibly also upon the pterygiophores.

Innervation. These muscles are supplied by the nerves of the myomeres with which they are associated, the nerve from each passing in between the two muscles of a pair and giving off its fibers anteriorly and posteriorly. There is also developed, at least behind, a small longitudinal trunk derived from anastomoses of the several nerves.

Blood supply. Segmental vessels.

Action. Contraction of these muscles pulls the fin towards the side. The posterior muscles, being more oblique and at the same time the hind part of the fin being more or less free, there is more motion permitted behind than in front.

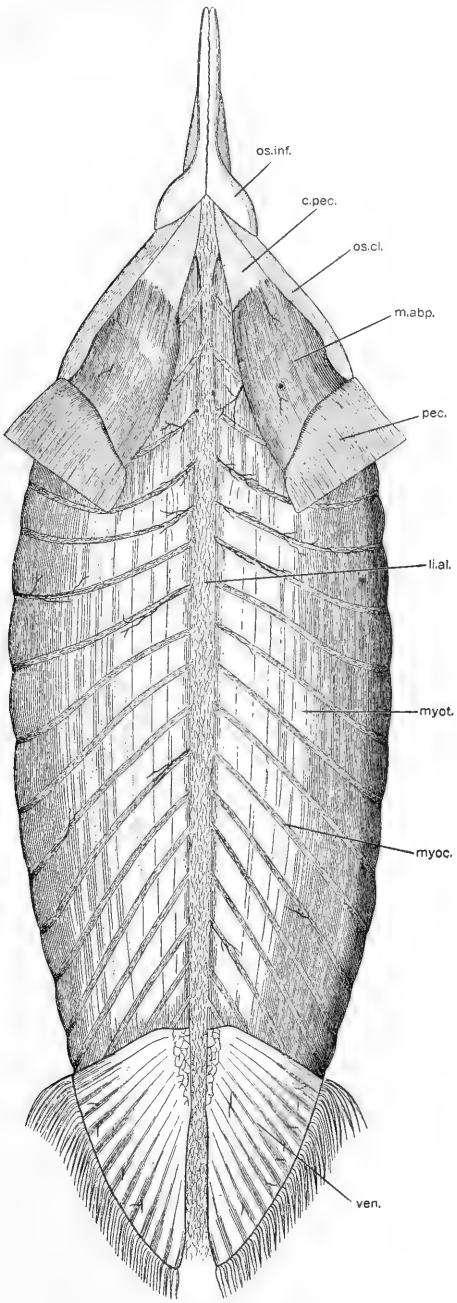

Fig. 10 The ventral body musculature

Muscles of the anal fin

The muscles of the anal fin correspond exactly with those of the dorsal fin except in number. Here there are only eighteen radialia and the pairs of muscles on each side are correspondingly reduced.

Innervation. The innervation corresponds with that of the dorsal fin except that the branches are here derived from the ventral rather than from the dorsal divisions of the nerves involved. The most anterior nerve to the anal fin is number thirty-four.

Action. The action of these muscles is also comparable to that of the dorsal fin.

Muscles of the caudal fin

As already stated, the lateral muscle of the body is the main muscle of the tail, and in its action it corresponds more nearly with that of the muscles of the fins already described. There is, however, in the caudal fin, an incomplete double set of oblique muscles extending between the cartilaginous rays. The superficial muscles run backward and toward the mid-horizontal plane, the deep muscles cross these obliquely. In the upper part of the dorsal lobe of the tail, which is encased in a bony covering, these muscles are nearly or quite lacking.

Innervation. The nerve supply is from two longitudinal trunks that result from the union of branches of a number of the most posterior spinal nerves.

Action. These little muscles seem to be divaricators of the rays.

VIII. MUSCLES OF THE PECTORAL ARCH

The muscles of the pectoral arch are described as abductors adductors and trapezius. A few fibers of the great ventral muscle are also attached to the arch but they are not sufficiently differentiated to warrant a separate description. In keeping with the simple condition which obtains in this fish, deep and superficial abductors and adductors are not separated from each other. These deep and superficial muscles, in forms where they

occur, presumably represent the deep and superficial muscles of the median fins, and so perhaps Polyodon is 'simplified' rather than 'primitive' in this respect.

M. trapezius: figure 8, m.trp.

The trapezius muscle arises (a) to a slight extent from the skull, (b) from the upper two-thirds of the supraclavicle, and (c) from the adjacent aponeurosis over the lateral muscle of the body. Ventrally the body of the muscle turns slightly anteriorly and is inserted chiefly into the upper angle of the cartilage of the arch. Towards its insertion, however, it is somewhat tendinous and this tendinous portion is more or less merged in the neighboring sheets of fascia.

Innervation. This muscle is supplied by a long branch of the vagus nerve which enters it dorsally near the upper end of its insertion by crossing to it from the jugular vein which it has followed back. This innervation is rather unexpected, since in some forms, e.g., Ameiurus (McMurrich '84), apparently the same muscle is supplied by the first spinal nerve. The innervation found here suggests its homodydamy with the levator muscles of the gills and hyoid arch.

Blood supply. The arteries of supply come from the coronary and the subclavian arteries, chiefly the latter.

Action. Its action is to raise the pectoral girdle.

Adductor of the pectoral fin: figure 8, m.adp.

The fibers of this muscle have a rather extensive field of origin, but no intermuscular septa or other noticeable demarcations warrant its being considered as more than one unit. The posterior and superficial fibers arise (a) from the anterior two-thirds of the posterior ramus of the coracoid cartilage along a groove on its ventral margin, and (b) from an aponeurotic sheet investing the body of the muscle and binding it to the cartilage both laterally and medially. Anteriorly, some of these fibers are somewhat tendinous, both at their origin and at their insertion. The fibers in the center of the muscle take origin (a) from all sides of

an oblique canal through the cartilage, (b) from its dorsal expansion above the canal, (c) from the inner side of the clavicle, and (d) from the dense tissue around the corocoid fossa medially. Fibers of this group mostly become tendinous toward their insertion. Finally, the deepest fibers arise from the basalia and radialia of the fin itself. As stated above these various fibers are not separated into groups and the areas over which they arise form one continuous field. The muscle bundles are inserted directly or by small tendons into the curved ventromedial ends of the bony rays of the dorsal moiety of the fin.

Innervation. The chief supply is a large nerve which results from a plexus of the second, third and fourth spinal nerves. There is also a plexus formed from the fifth and sixth spinal nerves and twigs from this also reach the muscle by running along the ventral side of the basal cartilage.

Blood supply. The muscle is supplied by the ultimate branches of the subclavian in anastomosis with the coronary artery.

Action. It adducts the fin drawing it up and toward the body.

Adductor of the pectoral fin: figure 8, m.abp.

This muscle arises (a) from a small area on the ventral side of the clavicle towards its anterior end, (b) from a membrane bridging the fontanelle between this part of the clavicle and the pectoral cartilage, (c) from a furrow on the lateral aspect of the cartilage, and (d) from the ventral side of the basale and radialia of the fin. It is inserted on the base of the ventral moiety of the dermal rays. The most dorsal fibers, arising from the overshelving lateral expansion of the cartilage, are inserted on the anterior ray by a small separate tendon.

Innervation. Nerves reach the muscle in three ways. The largest supply is a nerve derived from the above-mentioned plexus of the second, third and fourth spinal nerves which reaches the muscle by coming in back of the clavicle, across the adductor and through a large foramen in the cartilage. The second is a small branch of the same plexus which reaches the muscle by passing medial to the cartilage. The third is derived from a

plexus of the fifth and sixth spinal nerves. It follows the basal cartilage and sends twigs up into the muscle.

Blood supply. The muscle is supplied by ventral branches of the subclavian artery.

Action. This muscle pulls the fin downward and inward. It also tends to rotate the anterior part of the fin and in this function the fibers inserted on the first ray are of especial importance.

IX. MUSCLES OF THE PELVIC FIN

The pelvic fin is the most complicated in structure of all the fins. Von Davidoff ('79) in his paper on the hind limbs of fishes includes Polyodon among the ganoids studied. He gives figures of the skeleton of the ventral fin and a brief description of the musculature. The innervation was not fully worked out for Polyodon. So far as the present work goes it is in accord with the results obtained by him.

Following the analogy of the pectoral fin, the muscles here might also be classed as a dorsal adductor and a ventral abductor. The dorsal musculature is in two parts, superficial and deep, like the musculature of a median fin. The two parts are separated by an incomplete tendinous septum. The fibers of this part arise from the aponeurotic covering of the lateral musculature and are inserted beneath the rays of the fin. The deep part is peculiar in that its most superficial fibers seem to be in direct continuity with the lateral body musculature. The deeper fibers arise (a) from the upper surface of the basalia, (b) from the dorsal lateral margin of the crest of the same, and (c) from the radialia. The muscle is divided by the crests of the basalia and by thin connective tissue sheaths into as many subdivisions as there are radialia, thirteen or sometimes fourteen.

The ventral muscle, which is an abductor in function, is relatively simple. It arises (a) from the median ventral aponeurosis, (b) from the basalia, and (c) from the radialia. It is inserted on the ventral side of the fin in the same manner as is the adductor muscle on the dorsal side.

Innervation. The branches of several spinal nerves, apparently four or five, of which number eighteen is probably the most

anterior, anastomose with each other through a longitudinal cord, and then continue into the dorsal side of the fin where they branch profusely, supplying the muscle and anastomosing with one another. Some of the branches pass through the foramina in the basalia and between the different basalia to the ventral side where a longitudinal trunk is developed from which the abductor muscle is supplied.

Blood supply. The arteries to the fin are several of the splanchnic divisions of the segmental vessel.

Action. As stated above, the upper muscle is an adductor, drawing the fin up against the body. The ventral muscle is its opponent, pulling the fin outward and downward.

X. CONCLUSION

The musculature of Polyodon has proved to be rather simple in character. How to interpret such simplicity is not always evident. On the whole, the resemblance to Acipenser is rather close but there are a few points which contrast it very strikingly with that form. The system of superficial constrictors, so strongly developed in Acipenser, is here reduced to a minimum. The condition is even simpler than that found among the elasmobranchs. In this case we are probably justified in regarding the simplicity as the result of reduction. Simplification in the same direction is also characteristic of higher forms. In the case of the protractor hyomandibularis we apparently see another advance over Acipenser. With the transverse muscles of the pharynx and the pharyngo-claviculares the case is different. To be sure, in the possession of these elements Polyodon approaches the teleosts more nearly than it does Acipenser, but if one regard the muscles as morphological units which phylogenetically can only arise from preëxisting muscles, then we are forced to consider Polyodon more primitive than Acipenser in this particular, since the muscles in question have apparently been lost in the latter form. The question of the ventral fin is also of interest in this connection. The numerous radialia are interpreted by von Davidoff as the result of division of an element which in most forms is a single plate and on this ground, chiefly, he would

place Polyodon at the end of a line leading back through Acipenser and Scaphirhynchus to a type more primitive than the Selachians. The other view that the fin of Polyodon is in reality very primitive and that the radialia found here are comparable to those of an unpaired fin seems to the present writer quite as plausible. The musculature of the fin seems to present nothing against this latter view.

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THE GERM-CELLS AND THE PROCESS OF FERTILIZATION IN THE CRAB, MENIPPE MERCENARIA[1]

RAYMOND BINFORD

Guilford College, North Carolina

NINE PLATES

CONTENTS

[1] The study of the life-history of Menippe mercenaria, which led to the discoveries presented in this paper, was undertaken at the suggestion of Prof. E.

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1. INTRODUCTION

In spite of the extensive researches into the spermatogenesis of the Decapods, the use of the peculiar structures found in the spermatozoa of these animals is still an unsolved problem. This is due to the fact that the entrance of the spermatozoon into the egg has never been reported. While studying the habits and structure of Menippe mercenaria, a large edible crab found along the southern part of the Atlantic coast of the United States, I had the good fortune to obtain material which shows the essential features of this process. In order to show which parts of the seminal cell are involved in the process of fertilization, the genesis of the spermatozoa and the formation of the pronucleus in the fertilization of the egg, as well as the entrance of the spermatozoon, are here described. The history of the male cell from its origin in the epithelium of the wall of a tubule of the testis to its association with the female nucleus in the center of the egg, is here presented.

2. SPERMATOGENESIS

The testis

The testis of Menippe is a large paired organ lying just underneath the dorsal wall of the carapace. The inner ends of the right and left portions lie close together, just anterior to the heart and from here diverge anteriorly and laterally to the outer edge of the carapace. It is composed of relatively long and complexly folded tubules which vary in diameter from 0.14 to 0.33 mm. The deferent duct, one on each side, leads from the

A. Andrews, and at every step in the progress of this work I have received his kind advice and helpful criticism. I am also greatly indebted to the Hon. Geo. M. Bowers, United States Commissioner of Fish and Fisheries, for the privilege of working in the Marine Biological Laboratory at Beaufort, North Carolina, and for the liberal help extended to me in carrying on my researches there. My thanks are also due to Mr. H. D. Aller, Director of the Laboratory, for his ready cooperation in placing at my disposal the conveniences necessary for carrying forward my work.

testis to the base of the last thoracic leg. It is extensively convoluted so as to form two large masses, one lateral to the posterior part of the testis and the other beneath the posterior part of the heart. The deferent duct is lined with a layer of columnar epithelium which secretes the substance that forms the walls of the spermatophores.

Methods

Pieces of the testis, obtained by cutting across the organ, were fixed in Worcester's fluid. This is a saturated solution of sublimate in 10 per cent formalin. Other fixing fluids were used but did not give as satisfactory results. The sections were cut 7 μ to 10 μ thick. The stains used were thionin and eosin, safranin and Lichtgrün, iron-hematoxylin, and Delafield's hematoxylin.

The testicular tubules

The walls of the tubules of the testis are thin and contain flattened nuclei. Figures 1, 2, 3 and 4 are drawings of transverse sections of the tubules and show different stages in the development of the seminal elements. In figure 4 the tubule is seen to be made up of three regions: The smallest one which borders the more sharply curved side at the bottom of the drawing contains mature spermatozoa. Next to this, and filling the central region, is a space filled with spermatozoa nearly mature. The third region, which forms the crescent shaped portion on the upper side, contains spermatocytes in the early prophase of the first maturation division. There are more or less definite layers of epithelial cells between the different regions of the tubule. The outer wall and sometimes these inner partitions which border the regions containing mature spermatozoa, become thick and columnar in structure (figs. 3 and 4).

Not only do the seminal elements in these separate parallel cavities of the tubule differ in the stages of their development but in the same cavity the elements at one end of the tubule

are further along in their development than those at the other end. Thus in one end of a cavity the cells may be in the early prophase of the division of the spermatocytes of the first order while in the other end they have reached the spermatid stage. All the stages in the transformation of a spermatid into a spermatozoon may be found in passing from one end of a tubule to the other.

At the center of the upper border of figure 4, *p.s.*, there is one cell with a large nucleus. This is one of a single row of cells along the side of the tubule which may be called the primary spermatogonial cells since they, by division, give rise to a new lot of spermatogonia. Near the top of figure 3, *p.s.*, we find a similar cell. The cells forming the crescent-shaped region are in this case not so far advanced as in figure 4. Figure 1 represents a tubule, the largest portion of which is filled with spermatids which have already entered upon their transformation into spermatozoa. In the upper portion of the drawing we have an early stage in the formation of a new batch of spermatogonia. There are four large spermatogonial nuclei surrounded by many epithelial nuclei and a considerable amount of cytoplasm. Delicate cell walls cutting out the cytoplasm which belongs to each spermatogonial cell can sometimes be made out at this stage. A later stage in the multiplication of these cells is shown in figure 2. A large nucleus, *p.s.*, near the middle of the convex border of the spermatogonial mass, doubtless marks the position of the row of primary spermatogonial cells which will persist unmodified to form, at a later period, another lot of spermatogonia. The largest cavity of this tubule contains spermatids well advanced in their transformation into spermatozoa. In figure 3 the mass of spermatogonial nuclei is still further enlarged. Indeed most of them have probably reached the spermatocyte stage. The spermatocytes in the early prophase of the first maturation division are shown in figure 4. By putting these observations together we may determine the approximate order of events in the genesis of the spermatozoa.

A general statement of spermatogenesis

There persists along one side of the tubule a single row of cells with large nuclei, the division of which give rise to the spermatogonia. The latter multiply irregularly to form a large mass which, in transverse sections, has the shape of a crescent. At first cell walls can be made out, but later the nuclei seem to lie in an undivided mass of cytoplasm. Gradually the division of these nuclei ceases and a spireme is formed within each of them. The division up to this time has taken place without the formation of any spireme structure. The appearance of the latter is the first indication that the cells have reached the spermatocyte stage. After the spireme has been formed the nuclei pass into synapsis which lasts for a comparatively long time, so that all the nuclei in a considerable portion of a tubule will be found in this stage at the same time. After synapsis, cell walls are formed in the cytoplasm which persist up to the anaphase of the first maturation division. In the nucleus the chromosomes are formed and the maturation divisions follow one another in quick succession. They begin at one end of the tubule and pass along it like a wave, so that the spindle-figures are found in only a small section of the tubule at any given time. While these events are taking place within the tubule the cells in the wall of the latter multiply so that the wall becomes considerably thickened. The primary spermatogonial cells also divide to start a new group of spermatogonia. Between these cells and the spermatocytes there is always a layer of epithelial cells which persist to form the partitions between the two successive batches of seminal elements. The mass of spermatogonial nuclei remains small until the spermatids are well advanced in their transformation into spermatozoa.

As the mass of spermatogonia increases, the developing spermatozoa are crowded more and more to one side of the tubule. These spermatozoa reach their mature state before the second batch enter synapsis. The epithelial cells surrounding the mature spermatozoa, we may suppose, secrete a fluid which, together with the increasing mass of spermatogonia, press the mature

spermatozoa out of the tubule. This process is not completed, however, before a third batch is formed or even a fourth started.

Spermatogonial mitoses

In the resting spermatogonial nucleus the chromatin is arranged in a loose net-work with enlargements at various places (fig. 5). This net, for the most part, lies just inside the nuclear membrane, the central part of the nucleus containing almost no staining material. The behavior of the chromatin during the prophase of mitosis is as follows: the knots of chromatin become enlarged and more regular in outline while the connecting threads become smaller and disappear. The chromatin finally assumes the form of a large number of paired spheres (fig. 6). An effort was made to count these spheres and numbers were obtained as follows: 51, 55, 57, 58, 62, 62, 68 and 80. One may not, however, place very much dependence in these numbers for some of the spheres are always somewhat aggregated in one or two places so that they can not be definitely distinguished. These chromosomes at first lie in the outer part of the nucleus just inside the nuclear membrane, but are later massed in the center, from which condition they move to their positions in the equatorial plate. Figure 7 is an optical section of the nucleus showing the peripheral arrangement of the chromosomes. In the metaphase and anaphase of the mitosis, the members of each pair are separated from each other and pass to opposite poles of the spindle (fig. 8). These divisions of the spermatogonial nuclei do not occur simultaneously throughout the mass, but singly here and there among the nuclei. The spermatogonia become smaller as they become more numerous.

Maturation mitoses

Finally the spermatogonial divisions cease and the nuclei prepare for the reduction divisions. The quantity of chromatin seems to increase and the spireme makes its appearance. At first it is very long and slender and complexly folded all through the nucleus. The iron-hematoxylin stain can be controlled so

that the spireme has the appearance of a brown thread with granules distributed irregularly along it (fig. 9). The diameter of the granules is slightly greater than that of the thread between the granules. The spireme now becomes shorter and thicker and is finally massed at one side of the nucleus in the condition of synapsis (fig. 10). This stage persists for a comparatively long period. The spermatocytes enter synapsis irregularly, in a sort of one-at-a-time fashion but they tarry here until all of the cells in the greater part of the tubule have reached this stage; then the nuclei of a given portion all proceed to the open spireme stage, shown in figures 11 and 12. These figures show only the chromatin which lies on the side from which the nucleus was observed. The chromatic material is again arranged in the peripheral portion of the nucleus and is segregated into the chromosomes which become somewhat massed in the center of the nucleus. The spindle next makes its appearance (fig. 13) and the chromosomes are drawn into the equatorial plate (fig. 14).

The mitotic figure represented in figure 15 shows the possibility of a tri-polar division. Such a condition may have been brought about by the formation of one of the spindles of the second division before that of the first division was completed. There is a small portion of the chromatin of this nucleus which is not involved in the mitotic figure. This portion is shown at *e.*, in figure 15 a, which is a drawing of what was seen at a lower level than that shown in figure 15.

The chromosomes in these nuclei are so small and so closely crowded together it is very difficult to determine their structure or their number. In one preparation, however, I obtained a ring-shaped appearance of the chromosomes (fig. 16). These forms were seen in the equatorial plate and also before the chromosomes had been arranged in the plate. In most of the preparations the chromosomes appear as mere granules. It may be that the ring-shaped forms were produced by the fixing reagent, which may have caused a swelling of the chromosomes. This result was not always obtained, however, by the same reagent.

When destaining is carried so far as to remove all the stain from the cytoplasm and the achromatic figure, the equatorial

plate may be shown to have a structure like that represented in figure 16 a. This was drawn from a section cut from the edge of the plate. Here it appears that the chromosomes are stretched as they are pulled apart. Strands of chromatin pulled out between the separating groups of chromosomes may be seen in the later stages of the anaphase. By more extensive destaining we may obtain what appear to be only the cores of the chromosomes as shown in figure 16 b.

In figures 17 to 22 various stages in the anaphase are represented. The interzonal fibers and the mid-body are very distinct in figures 19 to 21.

The second miotic division follows very soon after the first. The chromosomes become somewhat separated and are then drawn together again into the equatorial plate ready for the second division (figs. 23 to 25). Figure 26 shows the beginning and figure 27 the end of the anaphase. Here again the interzonal fibers and the mid-body are distinctly seen and a portion of the cytoplasm is definitely associated with each daughter nucleus. The nucleus of the spermatid is now organized and persists in a sort of resting condition for a comparatively long time. The centrosome may also be distinguished for a considerable time, but later I was unable to recognize it (figs. 28 to 32). A clear space surrounding the nucleus is also seen in these figures. The spermatid as it appears in figure 32 rests for a considerable period before any change towards the formation of the spermatozoon is observed. The boundaries between the cytoplasm of the different cells disappear and the nuclei come to lie in a sort of plasmodium.

The transformation of the spermatid into the spermatozoon

In serial sections of a single tubule we may trace every stage in the transformation of the spermatid into the spermatozoon, and since the two ends of the series are in opposite ends of the tubule and the intermediate stages lie in serial order between these ends, we may use the position of a seminal element in the tubule as a criterion for determining its relative stage in the

course of development. The first evident step in the transformation of the spermatid is the appearance of vacuoles in the cytoplasm next to the nucleus. These are small at first but by coalescing they soon form a large, clear vacuole on one side of the nucleus (figs. 33 to 38). Sometimes it appears that the vacuole may have arisen by the nucleus settling to one side of the clear space surrounding it as in figure 31. The nuclei, each with its accompanying vacuole, now lie in a common mass of cytoplasm. In the further development of these cells there are three parts which must be constantly borne in mind, namely, the nucleus, the vacuole (hereafter called the capsule) and the cytoplasm. We will take up certain stages in the differentiation of these three parts, and consider their relation to each other.

In figures 37 to 41 the shape of the nucleus may be somewhat modified by strains in the cytoplasm or by the crowding of the elements in the tubule. In these drawings there is no evidence of a granular or reticular structure, although such structure was made out in some preparations which were destained to a greater degree. In figure 37 it may be observed that the outer layer of the nucleus stains more densely than the inner portion. The nucleus in figure 38 contains a vacuole which does not take the stain. The cytoplasm surrounding the nucleus and capsule in figures 37 to 39 is nearly uniform in appearance, with probably a tendency to be a little more deeply stained near the nucleus. In figures 40 and 41 there is a concentration of a portion of the cytoplasm on one side of the capsule and bordering the nucleus. This is finely alveolar and stains more deeply than the rest of the cytoplasm. It may be that this patch of cytoplasm is seen in an earlier stage in figure 36 *c*, *a*. This portion of the cytoplasm crowds in between the nucleus and the capsule (fig. 42). About this time the capsule begins to take a brownish color when stained with iron-hematoxylin.

The origin and development of this portion of cytoplasm which appears on the side of the capsule and nucleus and wedges in between them is a striking feature in the development of the spermatozoon. Its behavior is well brought out in figures 45 to 48. In figure 45 we see this substance slipped in like a wedge

between the nucleus and the capsule, with a clear space between it and the nucleus. If the spermatid shown in this figure were rotated to the right through 90° so as to bring the outer surface of the wedge of cytoplasm on the side toward the observer, we would have the appearance presented in figure 46. If we should turn this through 180° so as to throw the wedge on the opposite side from the observer, the spermatid would appear as in figure 47 where just the tips of the crescent shaped wedge are seen. The tips of this crescent progress around the capsule along the boundary line between the nucleus and the capsule. At the same time the thick side of the wedge is reduced and the material is distributed equally around this border-line to form a complete ring, which viewed from *any* lateral direction, has the appearance shown in figure 48. At first the substance of the wedge is finely alveolar in appearance but by the time the ring is completed it seems to be uniform throughout and is stained black with iron-hematoxylin. It seems to be identical with the mitochondrial substance described by Koltzoff ('06).

After the mitochondrial ring is completed, the nucleus becomes widely separated from it and the capsule (figs. 50 to 52). This however is not always the case. In two preparations from which figures 33 to 35 and 37 to 43 were drawn, the nucleus remained fitted closely down on the capsule as shown in figure 43. As the two different conditions were obtained with the same fixing fluid it is hardly probable that the difference was caused by the fixing. The nucleus at this time loses the last trace of any granular or reticular structure and becomes uniform in its staining reactions, and somewhat reduced in size.

About the time the mitochondrial mass begins to slip in between the nucleus and the capsule, one or two deeply staining granules appear on the border line between the nucleus and the capsule (figs. 44 to 48). Koltzoff ('06) in his researches on the spermatogenesis in Galathea squamifera, has identified these granules with the centrosome. In my preparations of Menippe mercenaria I am able to distinguish the centrosome for some time after the second maturation division (figs. 28 to 32) but, in the later resting period of the spermatid and in the stages during

the origin of the capsule, I am unable to distinguish any granule which can with any certainty be identified with the centrosome. I shall call the structure developed from this granule, the 'central body.' I am unable to follow the development of two distinct granules although two could sometimes be clearly distinguished as shown in figure 44. Probably only the outer one is concerned in the development which is here presented.

This outer granule elongates (fig. 47) and becomes tubular (figs. 50 to 56). There soon appears at the outer end a vesicle which increases in size as the central body elongates. While the vesicle is still small there appears in its outer wall a flattened granule which is usually seen to be connected with the end of the central body by means of a fine strand as though it might have been derived from this body. As the central body increases in length and the vesicle enlarges, its outer wall approximates the outer wall of the capsule. The deeply staining substance in the outer wall of the vesicle now becomes connected with the wall of the capsule (figs. 56 to 58). A second vesicle now forms (fig. 59). These two vesicles become transformed into a tubule containing the central body. This tubule will hereafter be spoken of as the 'inner tubule.' At its outer end a ring of darkly staining substance is found (fig. 60). This seems to have been derived from the central body. At least a study of figures 54 to 60 may well suggest such an interpretation. The central body finally becomes reduced in diameter and appears to be a solid rod. It is not stained by thionin nor by safranin, but is readily stained with iron-hematoxylin. The inner tubule is stained green with safranin counter-stained with Lichtgrün; blue with thionin counter-stained with eosin; and black with iron-hematoxylin.

During this whole period the content of the capsule shows an increasing affinity for chromatin stains. It is colored brown with iron-hematoxylin. In some series a sort of ring-shaped cloud appears in the capsular contents. At first it is near the outer wall but gradually it contracts towards the vesicle at the end of the central body and finally settles in the wall of the tubule when that structure takes its final form. With Delafield's hematoxylin the contents of the capsule is readily stained, and with

safranin it takes a dull red color. In the early stages of development the content of the capsule is stained green when the preparation is treated with the safranin and Lichtgrün combination, but in the later stages the green is masked by the red. In stages represented in figures 53 to 55 a sort of foam or alveolar structure can sometimes be observed in this substance.

While the capsule and the structures within it are assuming their mature form, the nucleus has become less densely stained and settles down upon the capsule like a cap (figs. 52 to 59). It becomes thin in the center so that its final shape is that of a cup with a rounded, thin bottom and a thickened rim. This thickened border fits upon the mitochondrial ring so that in the mature spermatozoon it is not possible to distinguish it from that ring.

Protoplasmic rays or pseudopodia develop from the rim of the cup. I have been unable to determine whether they arise from the mitochondrial substance or from the nucleus.

The spermatozoon

We may now consider the structure of the mature spermatozoon. Figure 61 is a drawing of a spermatozoon taken from the seminal receptacle of the female and killed in the vapor of osmic acid, then stained with gold chloride after treatment with formic acid. We observe the nuclear cup (*n.c.*) from which the pseudopodia (*ps.*) arise. Inside the cup is the spherical capsule (*c.*) within which there is the capsular cavity (*c.c.*); and the inner tubule (*i.t.*) with its cavity divided into the inner tubular cavity (*i.t.c.*); and the outer tubular cavity (*o.c.*). Running through the inner tubular cavity and through the wall of the inner end of the tubule to the bottom of the capsule we see the central body (*c.b.*). Figure 62 was drawn from a live spermatozoon in 4 per cent KNO_3, and figures 63 to 65 are from spermatozoa mounted in the serum of the crab's blood. Movements of the blood have bent the pseudopodia of these spermatozoa. Otherwise they have more nearly the natural shape and propor-

tions than those shown in figures 61 and 62. The diameter of the capsule of these spermatozoa is about 3.8 μ and the pseudopodia are sometimes as much as 7 μ long.

Spermatozoa in the deferent duct

The mature spermatozoa pass from the tubules of the testis into the deferent duct. The latter is a long, extensively folded, tube lined with glandular epithelium. The spermatozoa form a common mass when they enter this tube, but the secretion formed by its lining flows in among them and separates them into groups. The secretion surrounding each group then hardens and so forms a membrane, so that finally there are an immense number of capsules containing the spermatozoa. These capsules are known as spermatophores. In this condition the spermatozoa are transferred to the seminal receptacle of the female crab.

Summary and discussion

In this study of spermatogenesis in Menippe mercenaria the principal points brought out are as follows:

1. There is a single row of cells which persists on one side of the testicular tubule and gives rise to successive batches of spermatozoa.
2. The spermatogonia divide without the formation of a spireme. The chromatin simply aggregates into chromosomes which are then gathered into an equatorial plate.
3. The maturation divisions follow one another quickly. They are preceded by spireme formation and a long period of synapsis.
4. There also seems to be a relatively long resting stage after the nucleus of the spermatid is formed before the transformation into the spermatozoon begins.
5. In the transformation of the spermatid, three structures must be considered, namely, the nucleus, the capsule and the mitochondrial ring.
6. The nucleus becomes uniform in consistency, reduced in size and cup-shaped.

7. The capsule arises in the cytoplasm as a clear vacuole which may be stained with Lichtgrün. Its content is gradually changed to have a greater affinity for chromatic stains.

8. From a granule on the proximal side of the capsule the central body develops into the capsule. At the distal end of this body a vesicle arises, which is changed into the inner tubule.

9. The mitochondrial substance is segregated from the cytoplasm and deposited as a ring between the nucleus and the capsule.

Some of the theoretical questions connected with the development and structure of the spermatozoa of the decapods will be taken up at the end of this article. At this point I wish to say that the above description is in agreement with the principal observations made by Grobben ('78), Gilson ('86), Sabatier ('93), Brandes ('97) and Koltzoff ('06). These authors have all seen the same general structures and transformations. They all describe a nucleus which, during development, is modified in its staining reactions, reduced in size and often flattened or otherwise changed in its shape. They do not disagree as to which part of the cell is the nucleus. They likewise describe a vesicle which arises in the cytoplasm either against the nucleus or close to it, and they mention the substance of cytoplasmic origin which appears between the nucleus and the vesicle. Most of them see a structure like the central body and describe the inner tubule. There are many variations in the detail of the development of these last two structures, and different species seem to differ widely in this respect. There is much disagreement concerning the destiny of the nucleus and the origin and nature of the substance in the capsule. These points of disagreement do not however affect the statements I have made concerning the structure of the mature sperm. It is with this structure that we have to do in the further course of the present investigation.

3. COPULATION

We now come to the question of the transfer of the spermatophores from the body of the male to that of the female; from the deferent duct to the seminal receptacle. We therefore turn our attention from the sperm itself to some of the habits of these crabs. Menippe mercenaria lives in crevices under or between the rocks, or in burrows which it digs in the mud along the shore a little below low water line. Usually one crab is found in each burrow, but occasionally, and even frequently in the month of August, a male crab will be found guarding a hole in which there is a female. Sometimes the female thus found has a soft shell. If its shell be hard it molts within a few days after being brought into captivity. On August 17, a female with a soft shell and male crab which had been taken from the same hole about noon, were placed together in a compartment of a floating cage. At 5:45 P.M. they were observed to be copulating. On being disturbed they separated. Their behavior was then observed while copulation was resumed. The most significant point with regard to this behavior was the apparent care with which the male acted in order to inflict no injury upon the soft, delicate shell of the female.

During copulation the spermatophores are transferred from the deferent duct to the portion of the seminal receptacle which is lined with chitin, where they are deposited in a very compact mass. Here they remain until the next spawning of eggs. Only a portion of the spermatozoa are used for the fertilization of any one batch of eggs. One crab, kept by itself in a compartment of a floating cage for sixty-nine days during the summer of 1911, spawned six times and apparently all of the eggs in the six different batches of 500,000 to 1,000,000 eggs each, were fertilized and developed normally.

4. SPAWNING HABITS

The spawning habits and the development of this crab will be discussed in a later paper. Here we will present only such points as are necessary in order to make it clear how the stages in the entrance of the sperm and fertilization are obtained.

When a female is ready to lay a batch of eggs she assumes an upright position and holds the abdomen out from her body so that it and the exopods of the abdominal appendages form a basket into which the eggs are run. They there become attached to the hairs of the endopods of the appendages and pass through the embryonic stages of their development, which requires from nine to thirteen days. The eggs then hatch and the larvae escape. The female then cleans off the egg-shells and their stalks from the hairs of the pleopods and, after one day to three weeks, she spawns again. Eight days is a very common length for the period between the hatching of one batch of eggs and the spawning of the next. With these facts in mind I made a large floating cage with fifty compartments and collected a large number of females with eggs and placed one in each compartment. After the eggs of several of these had hatched so that there were some fifteen crabs without eggs I kept these under almost constant observation, day and night. When one assumed the position ready for spawning it was naturally supposed to contain eggs which were mature if they were not already fertilized. Before describing the process of fertilization we should consider briefly the structure of the genital organs of the female.

5. THE REPRODUCTIVE ORGANS OF THE FEMALE

Figure 121 is a diagrammatic representation of the ovary and one seminal receptacle and oviduct of this crab. The ovary is an *H*-shaped tube, the lumen of which opens directly into the seminal receptacle at a point a little posterior to the cross connection of the *H*. The eggs are produced in the wall of this tube and when mature are set free in the lumen.

The seminal receptacle is composed of two parts, a glandular portion (figs. 121 and 122, *g.*) into which the ovary opens and a portion lined with chitin (figs. 121 and 122, *c.*) from which the oviduct leads to the third segment of the sternum. The spermatophores are stored in the latter division. The cavities of the two portions communicate through a large opening (fig. 121, *o.*) in the chitinous lining. Just before the crab molts, the glandular portion secretes a mass of gelatinous material which

greatly distends it (fig. 122) and the spermatophores are by some means transferred to this part of the receptacle where they lie in the mass of jelly. This prevents them from being lost at the time of molting when the chitinous lining is shed. Whether they are returned to this part of the receptacle after the molting has not been determined. The glandular part of the receptacle is rapidly reduced after the shell is shed, but I do not know what becomes of the secretion. During spawning the glandular portion is very much contracted (fig. 121) so that it is little more than a tube connecting the ovary with the chitinous receptacle. There is one possibility which may be mentioned here; the glandular receptacle may secrete a semi-fluid substance and then, by contracting, force the spermatozoa into the lumen of the ovary just before spawning begins. As I shall show later, the spermatozoa are transferred to the ovary. This however is only a conjecture as to the method of the transfer. The only time at which the receptacle is known to be actively secreting a substance is just before molting and it may simply be a device for retaining the spermatophores at the molting period.

If a crab that has just begun to lay its eggs be opened, the lumen of the ovary and the oviduct will be found to be full of eggs. Some eggs were taken from the lumen of the ovary with a sterilized pipette and placed in filtered sea-water. Since these developed into embryos it is evident that fertilization takes place in the ovary. Sections were made of eggs taken from the lumen of the ovary and from the oviduct and from these the phenomena of fertilization were observed, but we will return to this later.

6. THE BEHAVIOR OF THE SPERMATOZOA

The spermatozoa of this crab are so very minute, the eggs so relatively large and opaque, and the conditions for sperm entrance so difficult to reproduce on the microscopic slide, I did not see the living spermatozoon enter the egg. It is easy, however, to interpret the structures seen in sections of eggs taken at spawning time, after one has observed the behavior of the spermatozoa under certain experimental conditions. We will proceed, therefore, to a description of this behavior.

Methods of study

Koltzoff ('06) by his careful analysis of the effects on the sperm of solutions differing in osmotic pressure, has cleared up many of the mysteries of the decapod sperm. According to his researches, the spermatozoa maintain their normal form in solutions of salts having the same osmotic pressure as sea-water. He also found that 5 per cent KNO_3, 2.8 per cent NaCl, 4.25 per cent $NaNO_3$, 18.5 per cent $MgSO_4$, 7 per cent glycerine and 25.65 per cent sugar solutions are isotonic with sea-water. Solutions of these salts at a lower concentration cause a deformation of the spermatozoa.

For my studies, solutions of KNO_3, NaCl and $NaNO_3$ were used. The spermatozoa taken from the seminal receptacle and placed in solutions of these salts isotonic with sea-water would remain many days without perceptible change. When they were placed in weaker solutions of these salts transformations occurred. In studying these changes I proceeded as follows: Spermatozoa from the seminal receptacle were placed in the serum of the crab's blood or in the isotonic solutions of KNO_3, NaCl, or $NaNO_3$. In these solutions they were transferred to the slide, covered and examined under the high power of the microscope. Then, by placing a weaker solution of one of the salts at the edge of the cover-glass and allowing it to diffuse underneath, a slow change in the form of the spermatozoon was obtained. This change was thus followed in detail. It is to these changes that we will now turn our attention.

By referring to figure 61, we may again call to our minds the normal condition of the mature spermatozoon which consists of a chitinous capsule, set in a protoplasmic cup. The capsule contains a tubule with an inner and outer cavity and, running through the inner cavity of the tubule, is the central body, the proximal end of which rests on the wall of the capsule.

Changes in the nuclear cup

When solutions with a lower osmotic concentration than sea-water come in contact with the nuclear or protoplasmic cup it becomes thicker and the pseudopodia are withdrawn so that the outline of the spermatozoon, viewed from the top or bottom of the cup, is circular instead of star-shaped. The disappearance of the pseudopodia proceeds by a swelling at the base while the outer portion tapers very gradually to an extremely fine point (compare fig. 62 with 63 and 65). As the base widens out still farther the rays are reduced to a very fine thread, which either breaks off or is contracted into the body. When the pseudopodia break loose from their attachment the whole spermatozoon is apt to move suddenly and then be borne away if there be any currents in the containing fluid. This sudden movement probably results from some of the pseudopodia breaking loose slightly before the others. This rather than the explosion of the capsule may be the explanation of the 'springing of the sperm' discussed by Koltzoff ('06). This rounding up of the protoplasmic portion of the sperm is apt to be completed before any change takes place in the capsule. Sometimes, however, the capsule may be completely changed before the disappearance of the pseudopodia. Probably, in rapid explosion, the two take place simultaneously.

Changes in the capsule

For the interpretation of the entrance of the spermatozoon into the egg the transformation of the capsule is much more important than the changes in the protoplasmic cup. We will therefore follow the capsular changes very carefully. The first change is the out-pushing of the outer cavity of the inner tubule (compare fig. 61 with 67). Here it is evident that the wall of the outer cavity of the inner tubule has been everted, while the wall of the inner cavity (fig. 61, *i.t.c.*) has been stretched. It is difficult to see just what change has taken place at this time in the central body. In some instances it appeared that it had been lengthened, and in some specimens I thought the end of

it could be seen at the summit of the out-pushed portion. It may be that the lengthening of this body is the force that turns this distal cavity inside out.

In the next step of the capsular inversion the thick covering of the out-pushed part shown in figure 67 becomes turned out laterally so as to form a collar (fig. 68, *r.*) and the inner tubule becomes farther everted. The collar formed at this stage persists unchanged throughout all the further modifications of the capsule. The central body may now become greatly increased in length so that it projects beyond the out-turned part of the inner tubule (fig. 69, *c.b.*, also figs. 70 to 72). From this stage on to the completion of the eversion there is little further increase in the length of this axis. The everted portion of the inner tubule, however, swells out more and more (figs. 77 to 79). The transition from the condition shown in figures 71 and 76 to that in figures 77 to 79 is brought about by a further eversion of the inner tubule. The part of the inner tubule involved in this second definite eversion is probably marked by the funnel-shaped portion in figure 76. The portion of the everted wall, derived from the part of the tubule turned by this second eversion, is indicated by the granule *g* in figure 77. At this stage there is another pause while the out-turned part continues to swell.

Finally, the tension becomes so great that another portion of the inner tubule is everted and, as it turns, the wall of the capsule is also turned through the collar formed in the early stage of the process of eversion. This last eversion is shown halfway completed in figure 80, and the completed process in figure 81. In the latter figure the central body stands on the apex of the eversion and the inverted capsule (*inv.c.*) is above the collar (*r.*). In dilute solutions of the salts used, the protoplasmic portion, which contains the nucleus and mitochondrial substance, swells up to a spherical body as shown in figure 82. Often one finds on the slides, bodies like the one represented in figure 83. It is evident that these are exploded spermatozoa from which the everted inner tubule has disappeared, leaving the central body (*c.b.*), the inverted capsule (*inv.c.*), the collar (*r.*), and the shrunken nuclear cup (*n.c.*).

Changes in the central body

We now return to a more complete consideration of the behavior of the central body and the part that it plays in the explosion of the spermatozoon. These spermatozoa are so very small it is difficult, in many cases, to distinguish the central body, especially in the live, unstained material. Some significant facts, however, have been observed. As is shown in figure 61, the central body is composed of two distinct parts, the distal part within the cavity of the inner tubule and a proximal part connecting the inner end of the tubule with the wall of the capsule. Whether the central body projects into the outer cavity of the inner tubule or ends against the shelf separating the inner and outer cavities of the tubule, was not definitely determined, but the latter seems to be the case.

In figure 66, which was drawn from a spermatozoon in the tubule of the testis, fixed in Worcester's fluid and stained in iron hematoxylin, the central body projects through the apex of the capsule. This condition may have been brought about by an elongation of the central body or by a shrinking of the capsule. In either case it indicates that the central body is more or less rigid. One should notice also that the fixing fluid has caused a shrinking of the nuclear cup, so that it is now more like a saucer than a cup.

In figures 69 to 72, which were drawn from living spermatozoa, the central body projects beyond the everted tubule like a rigid rod, giving the impression that its elongation may have had something to do with the stretching of the tubule and the lengthening of that axis of the spermatozoon. The idea that the central body is somewhat rigid is further supported by its appearance in figures 73 and 74, where it stands out above the everted tubule. The same condition is produced in figures 81 and 83. Probably the strongest evidence in favor of the rigidity of this structure is found in figure 75, where, in lengthening, it has pushed backwards through the wall of the capsule and pushed the nuclear cup away from the wall of the capsule.

There are some indications that the central body is not firm but a plastic, semifluid substance. This is supported by the fact that it sometimes glides out through the inner tubule at stages such as that shown in figure 76 and adheres to the surface of the everted tubule in one or more amorphous masses (figs. 77, *g.*, 79 and 82). This condition may have been brought about by a degeneration of the body as a result of keeping the spermatozoa in the serum of the blood or in salt solutions. Sometimes in unexploded spermatozoa the central body adheres to one side of the tubule instead of standing in the center, and it may be that it was only in such cases as this that it adhered to the everted wall of the tubule.

Dynamics of eversion

We may now consider the forces involved in the turning of the tubule and capsule inside out. We may divide this inquiry into two questions: (1) What are the *external* conditions necessary to initiate and carry on the process? (2) What are the *internal* conditions which respond to the external ones and determine the nature of the process?

As stated above, a decrease in the osmotic pressure of the medium in which the spermatozoa lie, will cause the eversion. Unexploded spermatozoa, taken from blood serum and placed in 5 per cent KNO_3, do not explode; placed in 3 to 4 per cent KNO_3 they take the forms shown in figures 67 to 70; in 2 to 3 per cent KNO_3, the forms in figures 70 to 72 and 76 are obtained; in 1.5 to 1 per cent KNO_3 the eversion proceeds to the stages shown in figures 77 to 82. Like results were obtained by treating the spermatozoa with dilutions of 2.8 per cent NaCl or 4.25 per cent $NaNO_3$. Not all the individuals are equally affected by these solutions. Many of the spermatozoa retain the unexploded conditions of the capsule for a long time in a 2 per cent KNO_3 solution, and often none of them attain to the stage represented in figure 82 when treated with 1 per cent KNO_3.

Spermatozoa kept for several days in 2.8 per cent NaCl exploded when transferred to 4.25 per cent $NaNO_3$. Here we had

an explosion when they were transferred from a solution of one salt to that of another with equal osmotic pressure. Fresh spermatozoa do not explode when placed in 4.25 per cent $NaNO_3$; therefore the spermatozoa must have been changed by the NaCl, or the presence of these two salts must have had an effect that neither had when acting alone. To determine the factors here acting will require further experimentation.

Some of the spermatozoa explode whenever they are transferred to a slide and covered with a cover-glass. The cause of such explosions was not determined. Koltzoff found that mechanical pressure would cause the explosion of the spermatozoa of some Decapods. I failed to produce any explosion by pressing on the cover-glass of a preparation containing them. Koltzoff ('06) made extensive researches to find some specific stimulus that would cause a certain definite explosion which he believed to be the normal one but failed to find one. It appears, however, that a careful investigation of the conditions which initiate the process, followed up by an analysis of the conditions which may increase the pressure within the capsular cavity (fig. 61, *c.c.*), would throw valuable light on this subject. My researches have been concerned with the exact changes which occur in the spermatozoon, rather than with the conditions that cause the changes.

The second question, the one concerning the internal conditions which determine the response of the spermatozoon to the external conditions, may now be considered. What is there in the spermatozoon which may react to a decrease of the osmotic pressure of the solution which surrounds it? An examination of figures 68 to 82 clearly shows that it is the capsular cavity which increases in size. It must therefore contain a substance which is isotonic with sea-water and with the blood of the crab and which absorbs water when placed in any solution which is of a lower concentration. This water is doubtless taken in through the wall of the inner tubule, which seems to be semipermeable, while the outer wall of the capsule is probably impervious.

Another striking feature of the explosion is the remarkable extensibility of the wall of the inner tubule which is everted

to form the wall of a structure many times larger than the capsule. The central body must also be considered as one of the structures taking a part in the explosion of the capsule. We have therefore three changing structures, a swelling mass, a stretching membrane, and an elongating body, each of which take a part in determining the form of the inversion. To these must be added two structures which do not change and are resistant in their nature. These are the wall of the capsule and the collar surrounding the hole in the capsule, through which the tubule is everted. Let us now follow the interaction of the forces involved in the behavior of these changing structures. For this purpose we shall divide the explosion into four stages.

Stage 1. The eversion of the outer cavity of the tubule (*fig. 67*). Two forces probably take part in this, the pressure in the capsular cavity and the elongation of the central body.

Stage 2. The elongation of the everted outer cavity (*figs. 68 to 71 and 76*). This results in the formation of the collar (fig. 68, *r.*). Here again two forces may be involved, the swelling of the material in the capsular cavity and the further elongation of the central body which stretches the portion of the inner tubule which bounds the inner tubular cavity. The fact that the everted portion is sometimes longer in the axis through which the central body passes, indicates that this body may be exerting an out-pushing force. If this be the case, we have here an elastic body which has become active by being released from compression; that is, the central body elongates like a coiled spring. This action is fully discussed by Koltzoff. The pressure in the capsular cavity is sometimes shown by the squeezing of the central body out through the outer end of the tubule when it has lost its resistant properties.

Stage 3. The second eversion of the inner tubule (*figs. 77 to 79*). From the condition shown in figures 71 and 76, the increasing pressure in the capsular cavity causes the wall of the everted tubule to swell to the form shown in figure 72. Finally, the pressure becomes so great that the ring which formed the division between the inner and outer tubular cavities (fig. 61, *i.t.c.* and *o.c.*) gives away and a part of the tubule bounding the inner

tubular cavity becomes everted. Portions of the central body often adhere to the wall of the tubule and are carried outward and so mark the extent of this second eversion (fig. 77, *g.*).

Stage 4. The third eversion of the tubule, accompanied by the inversion of the capsule (figs. 80 to 82). The internal pressure continues to increase, as is shown by the bulging out of the walls of the everted portion (figs. 78 and 79). This brings a strain upon the axis in which the tubule and central body lie. This tends to stretch these structures as is shown by the incurving of the apical wall of the everted portion in figures 78 and 79. This causes the base of the everted part to press on the sides of the capsule. This pressure on the sides of the capsule, together with an up-pulling along the line of the central body, results in turning the capsule through the collar when the last section of the tubule is everted. In figure 82 the portions of the everted wall contributed by the second, third and fourth stages of the eversion are probably indicated by the granules g^1 and g^2. Thus we see that the whole transformation may be explained by the increase of pressure in the capsular cavity together with tensions along the line of the inner tubule and the central body.

Efforts were made to reverse this process by placing spermatozoa in very concentrated solutions of the salts used. The only effect of this treatment was a shrinking of the everted portion, which would again swell up and the process of eversion continue when dilute solutions were again used. After the explosion had reached the stage presented in figure 82 the only part affected by concentrated solutions was the protoplasmic portion at the bottom. It is also true that this is the only part that takes methylene blue, methyl green or thionin stains when these are applied to the living spermatozoa. It was rather surprising that the contents of the everted capsule were not stained by these stains. Sometimes a few granules can be seen in this cavity.

Figures 73, 74, 84 and 85 were made from spermatozoa which had been kept in a 5 per cent KNO_3 solution for fifty-one days. We observe here that partial explosion had taken place. Those shown in figures 84 and 85 had reached the stage corresponding to figure 70. When these were treated with a solution of lower

concentration the eversion continued, but the wall of the part already everted seemed to be hardened, so that it made an elongated collar through which the further inversion took place.

The effect of reagents on the everted spermatozoa

After trying several fixing reagents it was found that for imbedding and cutting the crab's eggs Morgan's fluid gave decidedly the best results. This fluid is a saturated solution of picric acid in 30 per cent alcohol, to 100 cc. of which 2 cc. of H_2SO_4 are added. Spermatozoa in various stages of the process of inversion and those in the normal mature condition were mounted under the microscope and killed with this fluid in order to determine its effect upon them. A considerable amount of shrinking was observed, but no decided change in the relationship of the parts seemed to take place. Figures 86 and 87 were drawn from spermatozoa exploded in distilled water, fixed in Morgan's fluid, stained in thionin and eosin, dehydrated in alcohols, cleared in xylol and mounted in balsam. The general relations of the parts is the same as in figures 81 and 82. The space between the everted tubule and the inverted capsule seems to have shrunk relatively more than the inverted capsule. Having now observed the behavior of spermatozoa under experimental conditions we may proceed to our observations concerning the extrance of the spermatozoon and the process of fertilization.

7. THE ENTRANCE OF THE SPERMATOZOON

Eggs were taken from the lumen of the ovary just after the crab began to spawn, and were fixed in Morgan's fluid, imbedded in paraffin and sectioned. A microscopic examination of these eggs showed the spermatozoon in the act of entering the egg. The best stain for the study of these sections is thionin, for it stains the chromosomes in the mitotic figure of the nucleus of the egg and the nuclear cup of the spermatozoon a deep blue. It stains the everted portion of the spermatozoon faintly and the food material, cytoplasm and egg-shell are unstained or only

faintly stained. This treatment makes it possible to find these minute structures in the relatively immense egg. After one has become familiar with these structures and their position on the egg it is possible to find them quite readily with other stains such as Delafield's hematoxylin, iron-hematoxylin, or safranin and Lichtgrün.

The relation of the spermatozoa to eggs taken from the lumen of the ovary is shown in figures 88 to 92. Spermatozoa in the same condition are also found in eggs taken from the oviduct as is shown in figures 93 and 94. Now, when these figures are compared with figures 86 and 87, which have been treated with the same fixing reagents, it is evident that it is the everted portion of the spermatozoon which has gone through the shell of the egg. The nuclear cup (*n.c.*, figs. 91 and 92) is on the outside of the shell. The everted tubule forms a vesicle within which one sees the inverted capsule (*inv.c.*, fig. 91). Hereafter I shall call this everted tubule and capsule, the 'sperm-vesicle.' At the inner-end of this sperm-vesicle the ejected central body may be seen (figs. 88, 91 to 94, *c.b.*). That the part which remains outside is the nuclear cup with its radiating pseudopodia can be more clearly seen by a surface view of the structure as it lies upon the egg, such as is presented in figure 95. Furthermore, the staining reactions are in accord with those observed in the mature spermatozoon and the artificially exploded ones and are as follows:

Thionin and eosin { the part outside of the shell, blue
the part inside of the shell, red

Safranin and Lichtgrün { part outside of shell, red
part inside, green mixed with red

Iron-hematoxylin { part outside of shell, black
part inside, brown except the central body which is black

Koltzoff ('06) claimed that in certain Decapods the spermatozoa settled on the egg with the nuclear cup towards the egg and the capsule pointed away from the egg. He was also of the opinion that the rebound from the explosion of the capsule was sufficient to drive the nucleus into the egg. On eggs taken

from the ovary of Menippe mercenaria, I found a few spermatozoa attached, as shown in figure 96, with the nuclear cup next to the shell of the egg. That the eversion of the capsule does not force the nucleus through the shell in this case is shown in figure 97, where a spermatozoon has exploded with the nuclear cup against the egg. So far as my observations go there is no evidence whatever that the eversion of the capsule causes any sudden movement of the spermatozoon body as a whole.

The number of spermatozoa which have pierced the shells of the eggs is much greater for eggs taken from the oviduct than for those taken from the lumen of the ovary. The number was counted in a few eggs which had just been spawned and the number per egg was as follows: 28, 44, 52, 52, 54, 71, 73 and, in one exceptional case, 679.

So far there seems to be no doubt as to the behavior of the spermatozoon in entering the egg, but we may ask: Is this the final stage in the entrance of the spermatozoon? Is not the nuclear cup drawn through the shell at a later stage? If not, what becomes of it?

That the nuclear cup does not enter the egg, but falls off is shown in figures 98 to 101. Here we see that the nuclear cup has moved away from the egg-shell and that a strand of some substance, by which it was probably attached to the bottom of the capsule, is drawn out with it. Sometimes the nuclear cup breaks loose from the strand and leaves it projecting through the shell into the capsule (fig. 100). In eggs taken from the oviduct or from the pleopods just after spawning, large numbers of sperm-vesicles are found sticking to the inside of the shells after the nuclear cup has fallen off (fig. 101). It is very clear then that, in most cases at least, the nuclear cup does not enter the egg. But does it thus fall off from the particular spermatozoon which fertilizes the egg, or only from those which have failed to perform the work of fertilization? This question can best be answered by a further study of the events of fertilization.

8. FERTILIZATION

In eggs taken from the oviduct or just after leaving it, many sperm-vesicles may be found lying on the edge of the cytoplasm as shown in figures 102 and 103, while *one* is found down in the cytoplasm (figs. 104 to 108). Here it is evident that the movement of the cytoplasm has carried the vesicle below the surface. Before the sperm-vesicle enters, there is a layer of cytoplasm just inside of the egg-shell. The rest of the egg is filled with spherules of food material, in the interspaces of which the cytoplasm extends from the peripheral layer, by fine strands, all through the egg. The fact that the spherules of food move apart and a small mass of cytoplasm accompanies the sperm-vesicle into the egg, is best explained by supposing that the first vesicle which comes in contact with the cytoplasm, initiates a flowing movement of the latter along the inner surface of the shell, from all sides towards the newly entered vesicle. The cytoplasm, moving thus along the inner surface of the shell towards one point, would be deflected in towards the center of the egg, and would tend to carry the vesicle in with it. We may suppose further that, when the cytoplasm has once responded to such a stimulus, its physiological state is so changed that it will not respond to another. As a result only one vesicle becomes imbedded in the cytoplasm of the egg where it is to be transformed into the male pronucleus.

The vitelline membrane (fig. 104, *v.*) is formed just after the entrance of the sperm-vesicle into the cytoplasm and the vesicles which failed to enter, lie between it and the shell of the egg. The first polar body (fig. 114), which is cast off while the eggs are passing through the oviduct, is also found between the vitelline membrane and the shell.

The first step in the transformation of the sperm-vesicle into the male pronucleus is a thickening of its lateral walls. This may be observed in figures 105 to 111. Accompanying this there is an increase of affinity for the stains used (thionin and Delafield's hematoxylin). Next, there seems to be an extrusion of the old capsular wall which, if we recall the method of the ever-

sion of the spermatozoon, we know forms the inner lining of the sperm-vesicle. The discarding of this capsular wall was not clearly made out, but figures 109 and 110 indicate that such a change takes place. In eggs taken soon after spawning the sperm-vesicle has increased considerably in size and the wall has taken on a vesicular appearance. This is shown in figures 110 and 111, which show the sperm-vesicle in two different aspects. The cavity of the vesicle gradually disappears, leaving only a notch on one side (figs. 108, 109, 110 and 112). Figure 112 was about an hour old. In eggs two hours old the vesicle has taken on the appearance of an ordinary nucleus containing granules of chromatin (fig. 116). At this time it has gone about one-fourth of the distance from the circumference to the center of the egg (fig. 117).

We will now turn our attention to the egg-nucleus. When the eggs are set free in the lumen of the ovary, just before spawning begins, the spindle for the first maturation division is already formed and its long axis is parallel with the surface of the egg (fig. 113). As the egg passes through the oviduct the spindle turns to a position perpendicular to the surface of the egg and the first polar body is cut off. This is shown in figure 114, which is from an egg that has just passed out of the oviduct. Efforts were made to count the chromosomes just after this division. Twenty-five to twenty-eight were counted. Thus the double number would be fifty to fifty-six. They are however so small and placed so closely together that it is difficult to distinguish them accurately.

Between one and one-and-a-half hours after spawning the second maturation division takes place. The second polar body is apparently not cut off, but remains in the egg where it degenerates and is absorbed by the cytoplasm.

The female pronucleus is now formed and proceeds to the center of the egg, where it meets the male pronucleus. Figures 115 and 116 are drawn from nuclei fixed at two hours and fifteen minutes after spawning. At this time it is not possible to tell which is the male and which the female pronucleus unless the

slightly concave side of the one shown in figure 116 indicates that it is related to the nucleus shown in figure 112. In this case it would be the male pronucleus. The nuclei have grown rapidly and continue to do so until they reach the center of the egg. Their contents are finely granular. These granules increase in size as the nuclei become larger. Figures 118 and 119 show the position of the nuclei four hours after spawning and figure 120 (from an egg six hours old) shows them lying side by side in the center of the egg. They have become elongated and many times larger than they were when first formed. From the above description of fertilization it is evident that the nuclear cup takes no part in the formation of the male pronucleus, for the latter is derived from the sperm-vesicle which is the inverted capsule.

This completes the description of the structure and behavior of the male cells in the stone crab. We have here something unique in the method by which the sperm enters the egg and something exceptional in the phenomena of fertilization. These observations raise several theoretical questions, some of which we will now briefly consider.

9. DISCUSSION

We have here a case in which an infolded vacuole which arose in the cytoplasm is everted through the shell of the egg and fertilizes it. How may such an event be brought into harmony with the existing theories concerning the chromosomes? In all other cases of fertilization the nucleus with its chromosomes or at least its chromatin is considered the essential thing; the bearer of the paternal qualities to the egg. The part that they play in the theories concerning heredity is too important and useful to be lightly discarded. But, granting all that is claimed for the chromosomes, we are nevertheless face to face with the fact that in most cases they disappear during the telophase and are reformed in the next prophase of cell division. Between the miotic divisions they can be followed from one spindle to the next and in some other cases, some investigators have claimed to have

been able to observe the continuity of individual chromosomes from one division of the cell to the next one. But these are exceptions. The problem of the origin of the chromosome is a real problem. For many reasons, in our final analysis, we must go back of the chromosome. So without attacking the proposition that chromosomes are the means for distributing the hereditary elements at the time of division, we may take up the question of the origin of the chromosome before division.

The phenomena described in this paper force us to consider this question if we are to bring the fact concerning fertilization in this crab in line with existing theories. Does any of the chromatin from the nucleus of the spermatid enter the egg? We have shown that it is the substance in the wall or in the cavity of the capsule that enters and fertilizes the egg. Now is there any evidence that the chromatic substance in the nucleus is transferred to the capsule during spermatogenesis?

Grobben ('78) claimed that the capsule is derived from the nucleus of the spermatid. He described a change in the consistency and a reduction in size of the nucleus which occurred simultaneously with the development of the capsule. He seemed to be of the opinion that the nuclear material was transferred by diffusion from the nucleus to the capsule.

Herrmann ('90) suggests that when one follows the parallel transformations of the capsule and the nucleus, one gets the impression that there is a sort of migration of the chromatic substance from the nucleus to the capsule.

Brandes ('97) found two substances in the nucleus of the spermatid. One was stained blue with methylene blue, the other red with acid fuchsin. The latter settles to one side of the nucleus and then passes out into the cytoplasm. The later workers, Koltzoff ('06) and Spitschakoff ('09), describe no such process.

In my own investigations I have noted a decrease in affinity for chromatic stains and in the size of the nucleus. The capsule, on the other hand, showed an increasing affinity for iron-hematoxylin and safranin. These facts suggest a transfer of nuclear material.

Finally, if we may accept the views of Stauffacher ('10) and Derschau ('11), that basichromatin is derived from oxychromatin, the former being deposited from the latter, we may postulate a theory for the explanation of the phenomena of fertilization in this crab. I do not claim that the facts establish the theory; they only suggest it. Some of the basichromatin in the nucleus of the spermatid is dissolved by the oxychromatin and transferred to the capsule. After the capsule is everted into the egg and has entered the cytoplasm of the latter, the basichromatin is redeposited and thus the granular structure of the male pronucleus appears. It may be possible to explain the number of chromosomes which appear, by supposing that there are a certain number of different kinds of molecules which are deposited out of the oxychromatin and that these have such an affinity for each other that they are aggreagted into a definite number of groups, or they may be of such a structural nature than they can fall only into certain groups. Of course, I claim for this only that it is a possible explanation of phenomena which are apparently not in accord with the conception of an individual continuity of the chromosomes.

10. SUMMARY

1. The seminal elements in Menippe mercenaria arise from a single row of primary spermatagonial cells which persist along one side of the testicular tubule.

2. The tubule is divided into three or four regions by longitudinal partitions composed of epithelial cells. The seminal elements in the division next to the row of primary spermatogonial cells are younger than those in any other division. The region on the opposite side contains mature spermatozoa. The seminal elements in one end of a given division are further along in their development than those in the other end.

3. The spermatogonial nuclei lie in a common cytoplasmic mass and multiply irregularly without the formation of a spireme.

A spireme and synapsis occur in connection with the first miotic division. The second mitotic division follows soon after the first.

4. In the mature spermatozoon the protoplasmic portion, containing the nucleus, is cup-shaped. From the rim of the cup pseudopodia project like the rays of a star. There is a capsule half-imbedded in the cup. An inturned tubule is connected with an opening in the distal portion of the capsular wall, and a rod-like central body arises from the proximal side of the capsule and projects into the inner tubule.

5. In the transformation of the spermatid, the nucleus becomes uniform in consistency, reduced in size and cup-shaped. A mitochondrial ring is formed between the nucleus and the capsule. The capsule arises as a vacuole in the cytoplasm. In the course of its development it shows an increasing affinity for nuclear stains.

6. The central body develops from a granule which appears on the proximal side of the capsule. The inner tubule is formed from two vesicles which arise at the distal end of the central body.

7. Hypotonic solutions of various salts and possibly other stimuli cause a lengthening of the central body, an eversion of the inner tubule and an inversion of the wall of the capsule.

8. When the spermatozoa come in contact with the egg under normal conditions, the capsule is usually applied to the shell of the egg and the nuclear cup is directed away from the egg. In this position eversion takes place and the ejected central body, the inner tubule, and the capsule with its contents are thus turned through the shell into the egg.

9. The nuclear cup is left on the outside of the egg; it soon falls off.

10. The wall of the capsule, together with its everted contents, which we now call the sperm-vesicle, sinks into the cytoplasm of the egg, where it is enlarged and transformed into the male pronucleus.

11. The contents of the capsule may be derived from the nucleus of the spermatid and is probably oxychromatin which deposits basichromatin after it enters the egg and so gives rise to the chromosomes in the male pronucleus.

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PLATE 1

EXPLANATION OF THE FIGURES

1 Transverse section of a testicular tubule of a small crab, fixed in Petrunkewitsch's fluid; spermatogonia at the top; spermatozoa at the bottom, spermatids between. × 450.

2 Transverse section of testicular tubule, *p.s.*, primary spermatogonial cell. The mass of spermatogonia larger than in figure 1. × 450.

3 Transverse section of testicular tubule, showing larger mass of spermatogonia; *p.s.*, primary spermatogonial cell. × 450.

4 Transverse section of testicular tubule, showing a large mass of spermatocytes in synapsis; *p.s.*, primary spermatogonial cell. × 450.

5[2] Resting phase of a spermatogonial nucleus.

6 Prophase of a spermatogonial nucleus showing the paired chromosomes which were seen in the upper one-half of the nucleus.

7 Optical section of a spermatogonial nucleus showing the peripheral arrangement of the paired chromosomes in the prophase.

8 The miotic figure in a spermatogonial division.

9[3] Early prophase of the first miotic division of the spermatocyte.

10 Synapsis in the first miotic division of a spermatocyte.

11 to 12 The stage following synapsis, showing the spireme loosened up and separating into chromosomes.

13 First miotic division; stage just preceding the formation of the equatorial plate.

14 Equatorial plate and spindle in first miotic division.

15 A tripolar division of the nucleus of a spermatocyte of the first order. Figure 15a is a drawing of the same nucleus made from a lower plane and showing a portion, *e*, of the chromatin which was not included in the equatorial plate.

16 Chromosomes found in the equatorial plate of the first miotic division.

16 a and b Portions of the first miotic figure in the metaphase, showing the chromosomes.

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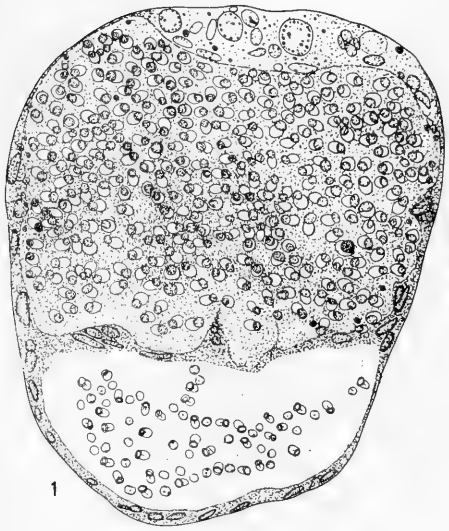
[2] Figures 5 to 112 (except 70 to 72) were all drawn with the camera lucida and a Zeiss 1.5 mm. apochromatic objective and a compensating ocular (either a No. 6 or No. 8). Then the drawings were enlarged so that in the plates there is a magnification of 3000 diameters. In making figures 70 to 72 the camera lucida was not used.

[3] Figures 9 to 58 were copied by Mr. E. A. Morrison.



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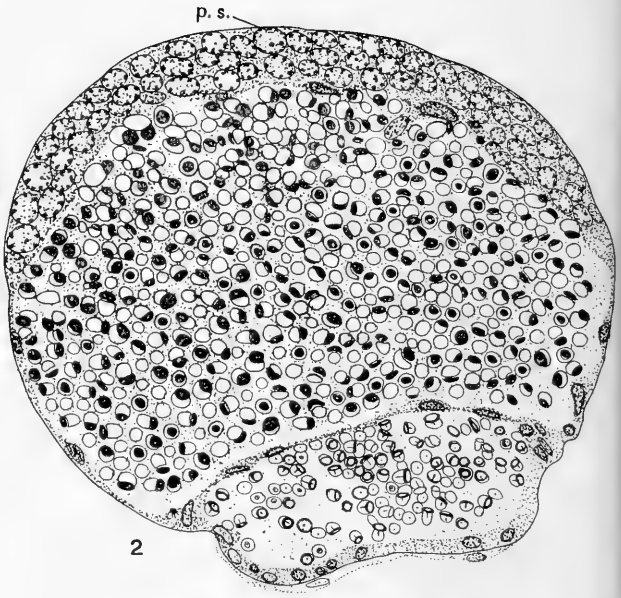

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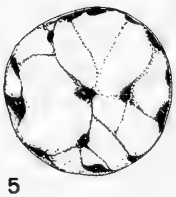

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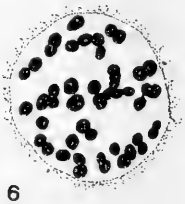

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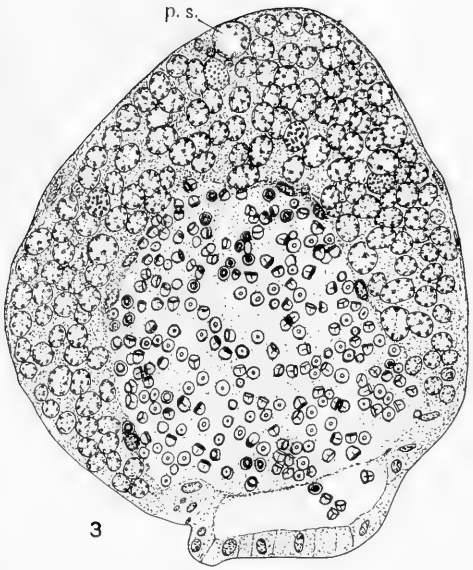

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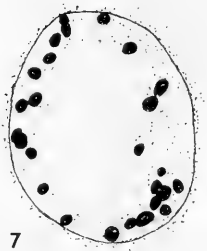

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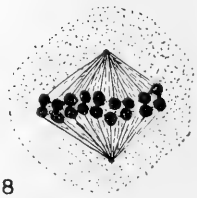

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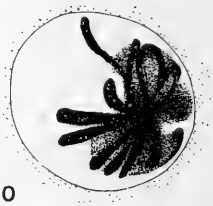

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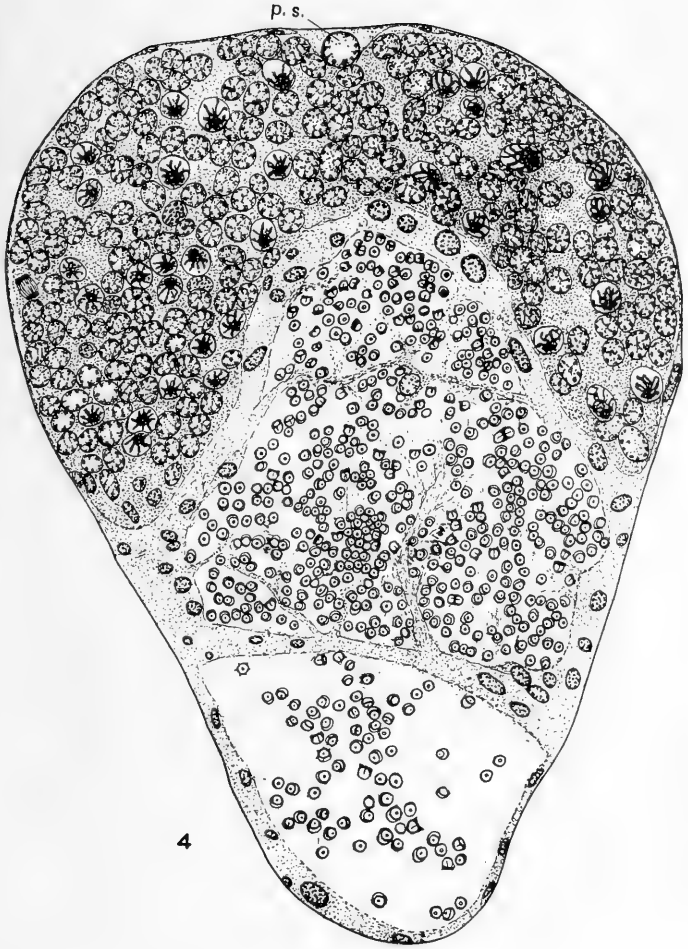

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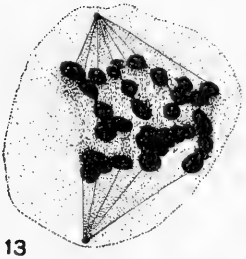

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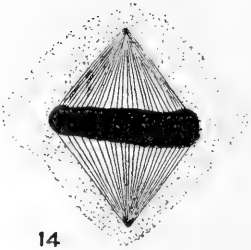

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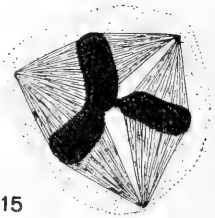

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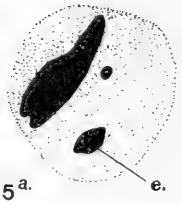

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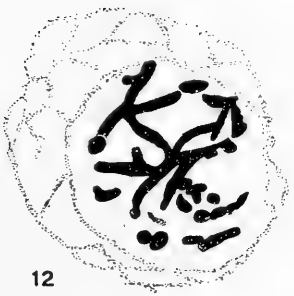

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16a.

16b.

PLATE 2

EXPLANATION OF FIGURES

17 to 18 Two stages in the anaphase of the first miosis.

19 to 22 Show different stages and variations in the early telophase of the first miotic division. Interzonal fibers are shown stretching between the masses of chromatin, the mid-body apparently forming a band around the fibers.

23 to 25 The second miotic division: stages in the formation of the equatorial plate.

26 The metaphase of the second miotic division.

27 Telophase of second mitotic division, showing interzonal fibers and the mid-body.

28 Later telophase. The centrosome is still visible here and is still attached by fibers to the nucleus. A clear area also surrounds the nucleus.

29 to 32 Different stages in the formation of a reticulate nucleus in the spermatid. A black granule, the centrosome may be seen and a more or less complete zone free from granules around the nucleus.

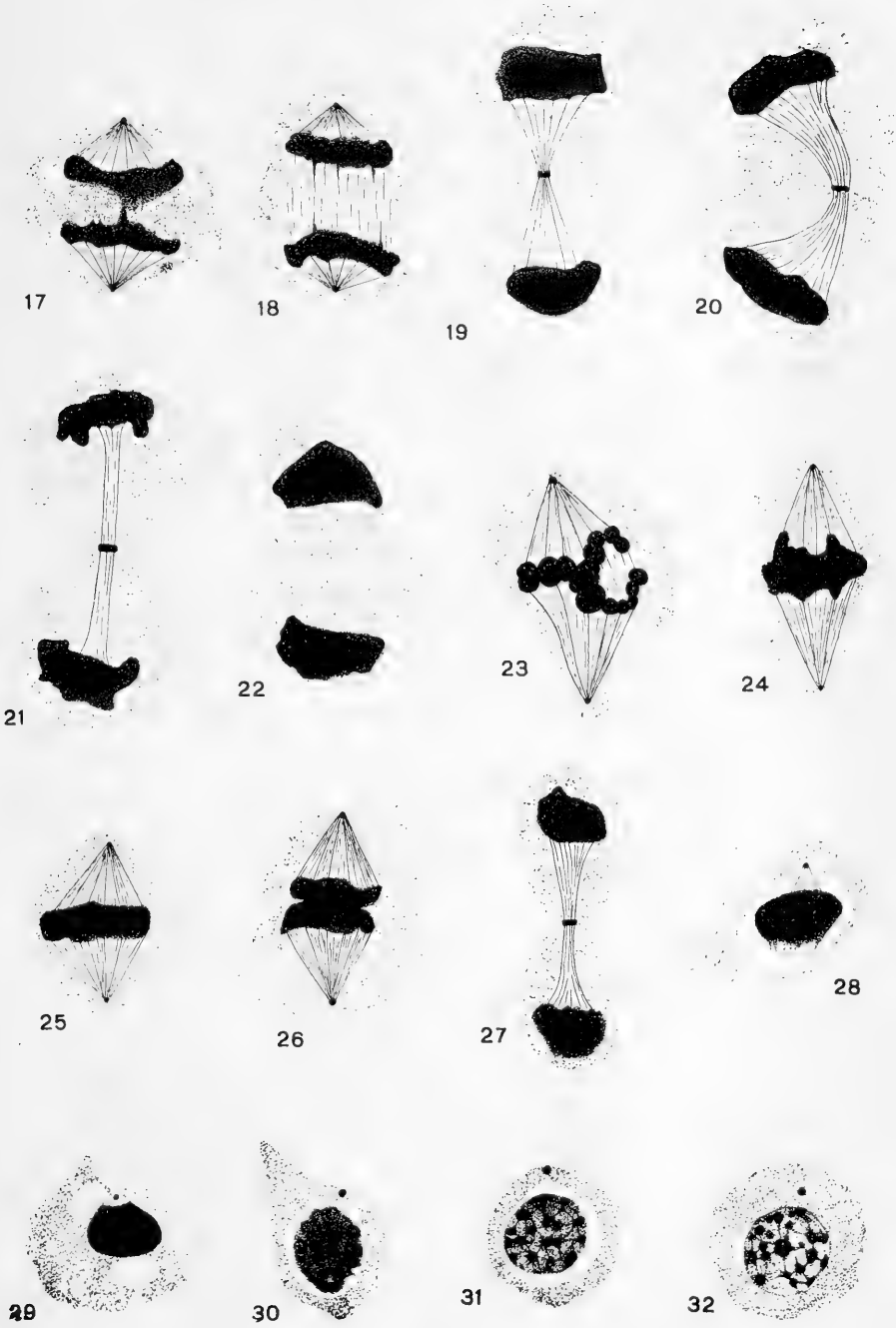
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PLATE 3

EXPLANATION OF FIGURES

33 to 36 Spermatids with vacuoles in the cytoplasm; *c.a.*, mass of finely granular cytoplasm which may be the mitochondria.

37 Spermatid with vacuole or capsule and nucleus; the periphery of nucleus more densely stained than center.

38 Spermatid showing a small clear vacuole in the nucleus.

39 to 42 Later stages in the transformation of the spermatids; *mt.*, mitochondrial substance.

43 Spermatid showing two sides of a dark ring, *d.*, in the capsule; *mt.* mitochondria, also the central body on the nucleus at the bottom of the capsule.

44 Spermatid showing two granules on the border line between the nucleus and capsule.

45 to 47 Spermatid from different view points, showing the mitochondria, *mt.*, and a granule, in bottom of capsule.

48 Spermatid with mitochondrial ring completed.

49 to 60 Spermatids showing stages in the development of the central body and the inner tubule within the capsule. A clear space appears for a time between the capsule and the nucleus; *i.t.*, a vacuole which forms the inner tubule; *c.b.*, central body; *n.*, nucleus.


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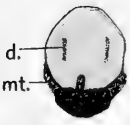

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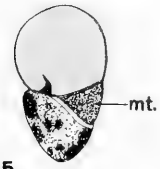

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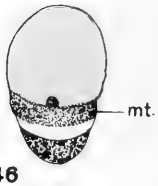

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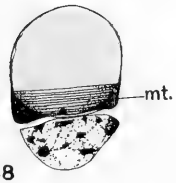

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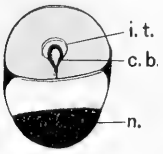

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PLATE 4

EXPLANATION OF FIGURES

61 Mature spermatozoon, fixed in vapor of osmic acid and stained in gold chloride preparation; *n.c.*, nucleus cup; *p.s.*, pseudopodia; *c.*, wall of the capsule; *c.c.*, cavity of the capsule; *i.t.*, inner tubule; *i.t.c.*, inner cavity of the inner tubule, *c.b.*, central body; *o.c.*, outer cavity of the inner tubule.

62 Spermatozoon viewed from the top, mounted in 4 per cent KNO_3.

63 Spermatozoon mounted in the serum of the crab's blood.

64 Side view of spermatozoon in the serum of the blood.

65 Spermatozoa in serum with pseudopodia all turned to one side by currents in the serum.

66 Spermatozoon in testicular tubule, fixed in Worcester's fluid. Stained in iron-hematoxylin, central body projecting from the top.

67 A spermatozoon treated with 3 per cent KNO_3, fixed with Morgan's fluid, and stained with Delafield's hematoxylin.

68 A spermatozoon treated with 3 per cent KNO_3, fixed with Morgan's fluid and stained with eosin.

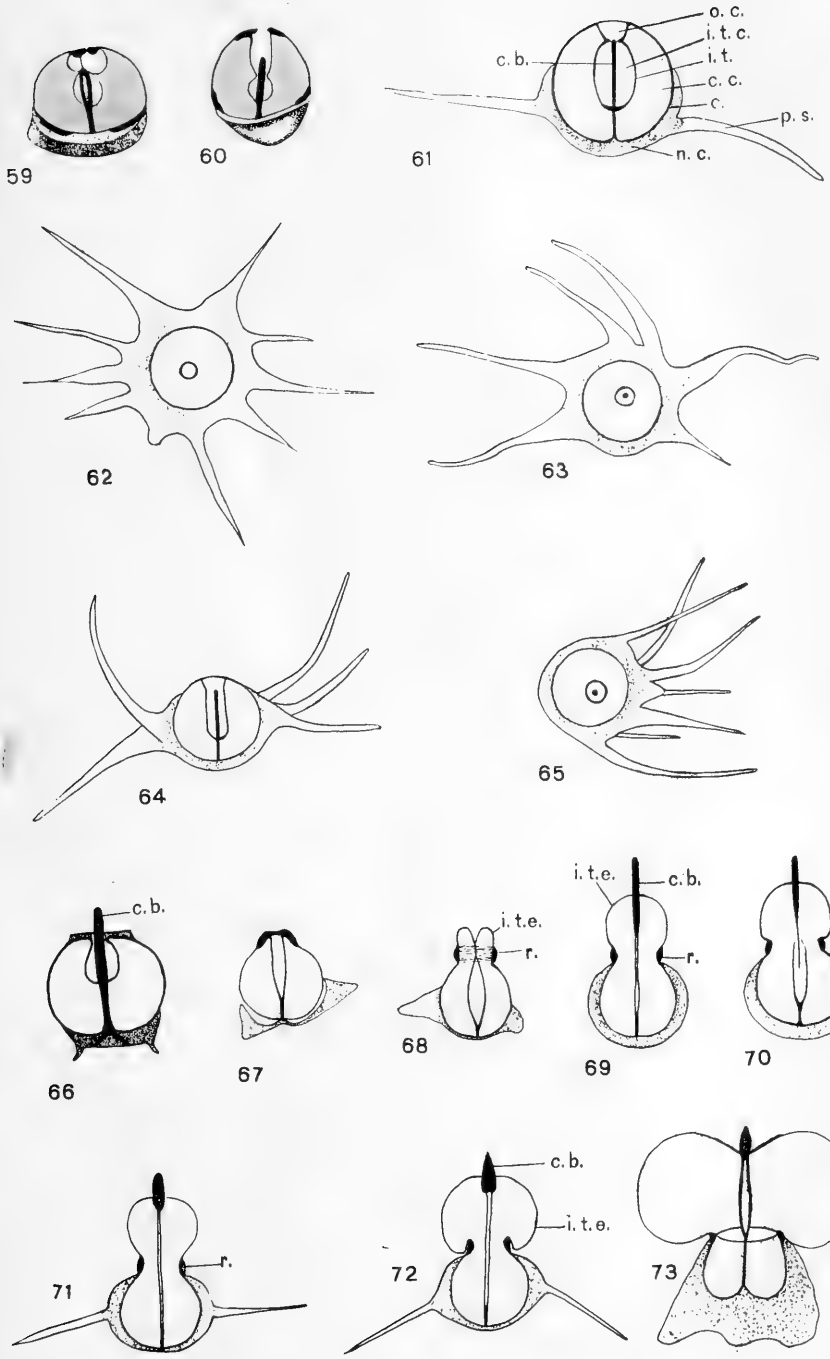
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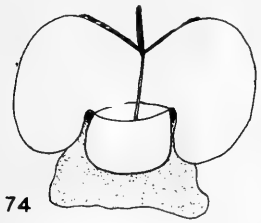
PLATE 5

EXPLANATION OF FIGURES

69 to 85 Were all drawn from living spermatozoa which had been treated with hypotonic solutions of salts, and show various stages in the eversion of the capsule; *r.*, the collar; *c.b.*, central body; *i.t.e.*, everted inner tubule; *inv.c.* inverted capsule; *g*, g^1 and g^2, pieces of the central body on the everted wall of the inner tubule.

86 to 87 Spermatozoa which were exploded in distilled water, fixed in Morgan's fluid, then stained with thionin and eosin.

88 to 92 Everted spermatozoa extending through the shell of the egg, from the lumen of the ovary; fixed in Morgan's fluid. In figure 89 the shell of the egg is badly warped; *c.b.* ejected central body; *inv.c.*, inverted capsule; *n.c.*, nuclear cup; *r.*, collar of the capsule; *e.*, shell of the egg; *i.t.e.*, inner tubule everted.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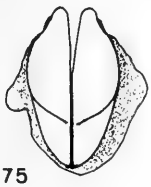
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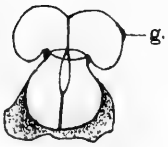

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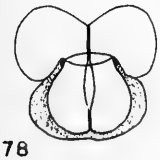
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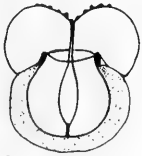
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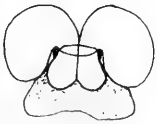
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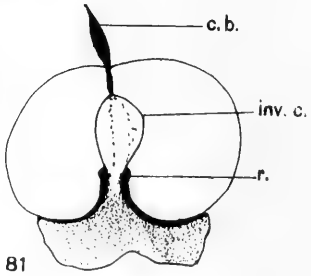

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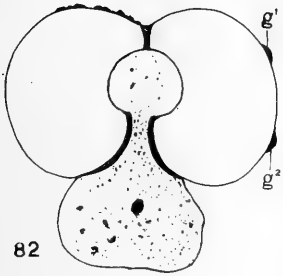

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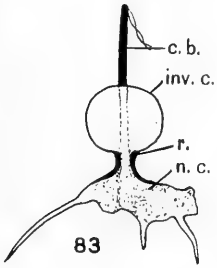

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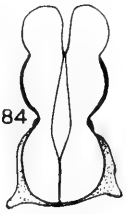
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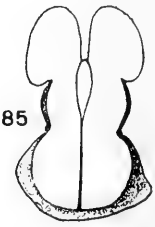
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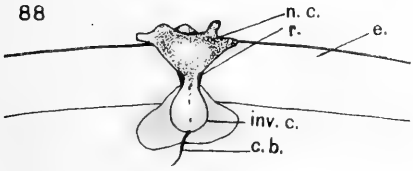

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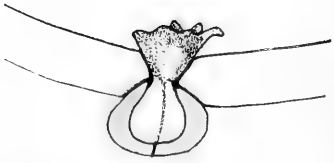
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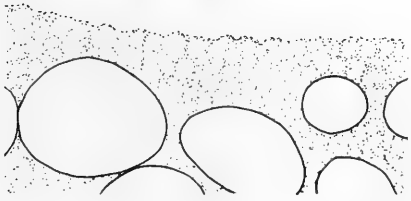


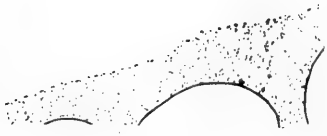


PLATE 6

EXPLANATION OF FIGURES

93 to 94 Everted spermatozoa in the shells of eggs from the oviduct; *c.b.*, central body ejected; *r.*, collar.

95 The portion of the spermatozoon which remains on the outside of the egg, seen from the bottom of the nuclear cup; *p.s.*, pseudopodia.

96 Spermatozoon on the shell of the egg with nuclear cup next to the shell.

97 A spermatozoon which has exploded, with nucleus next to the egg.

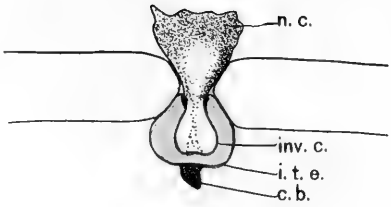
98 to 111 Portions of eggs with spermatozoa, taken from the oviduct or soon after leaving it.

98 to 99 Show the nuclear cup falling away from the egg and pulling a strand of some substance out with it; *n.c.*, nuclear cup.

100 Sperm-vesicle with the strand from which the nuclear cup has broken away, projecting through the shell into the vesicle.

101 Sperm-vesicle just inside the shell.

102 to 103 Sperm-vesicles lying on the cytoplasm.



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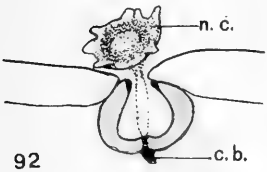

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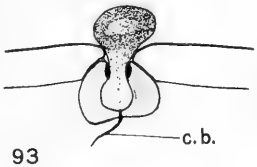

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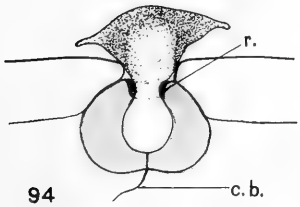

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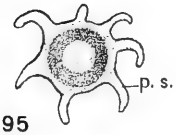

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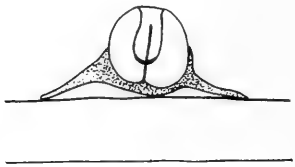

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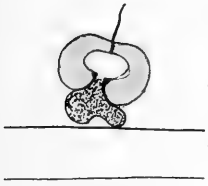

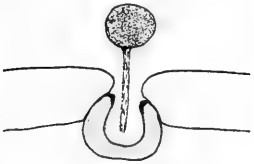

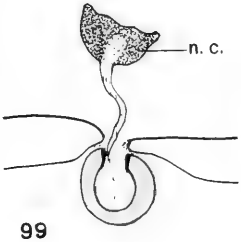

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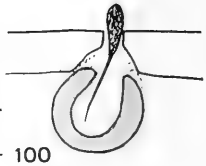

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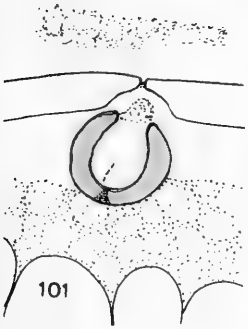

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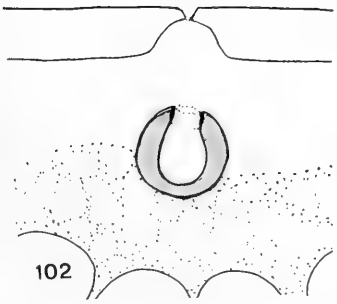

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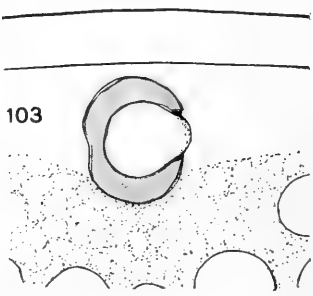

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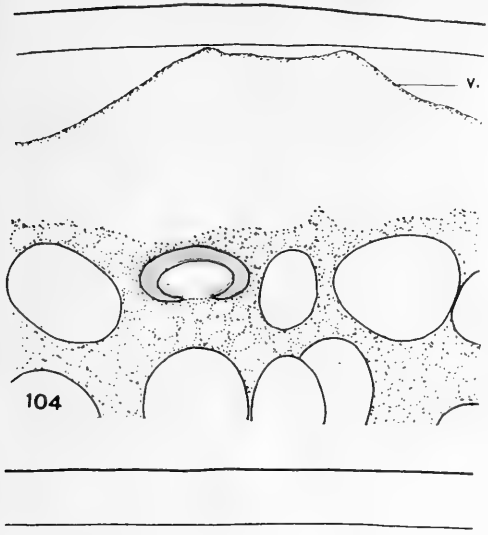
PLATE 7

EXPLANATION OF FIGURES

104 Sperm-vesicle which has just entered the cytoplasm; *v.*, vitelline membrane.

105 to 108 Sperm-vesicles down in the cytoplasm.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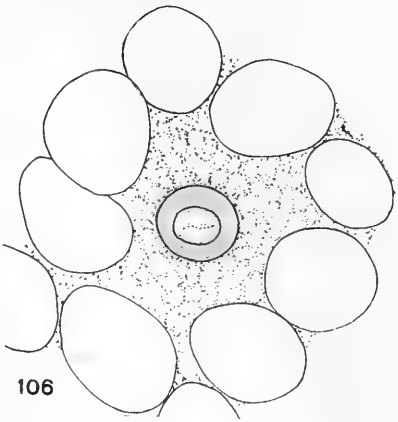
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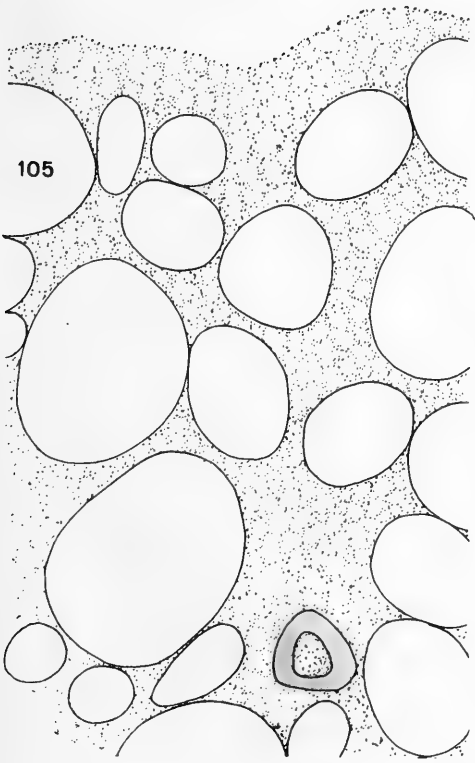
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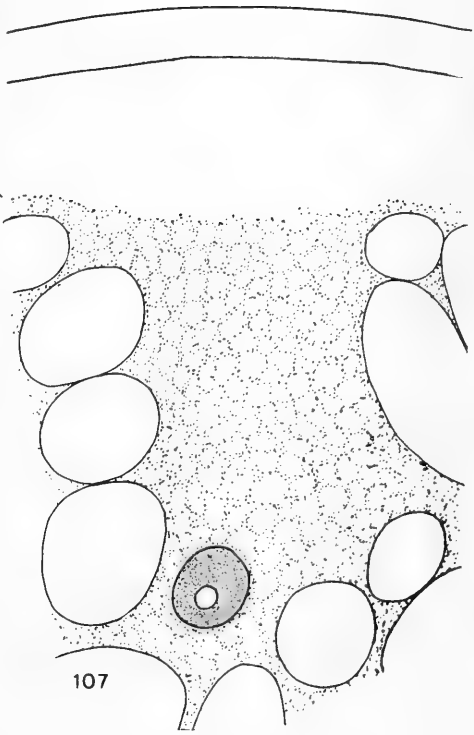
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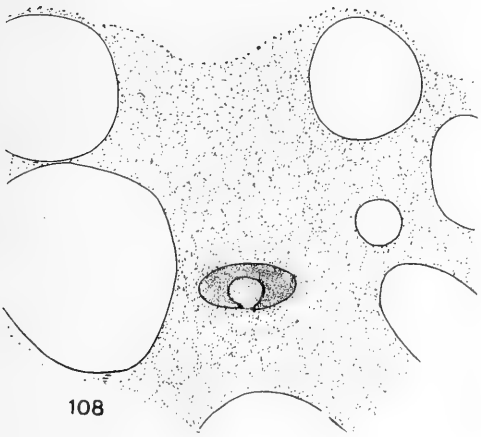
PLATE 8

EXPLANATION OF FIGURES

109 Sperm-vesicle with capsular wall projecting out of it.
110 Sperm-vesicle seen from one side; shows vesicular structure.
111 Sperm-vesicle seen from top—vesicular structure.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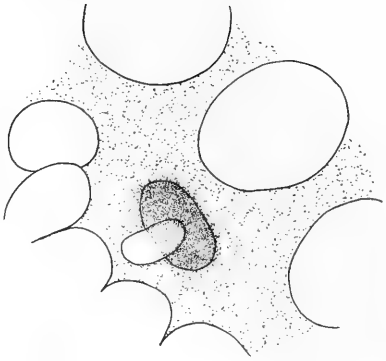
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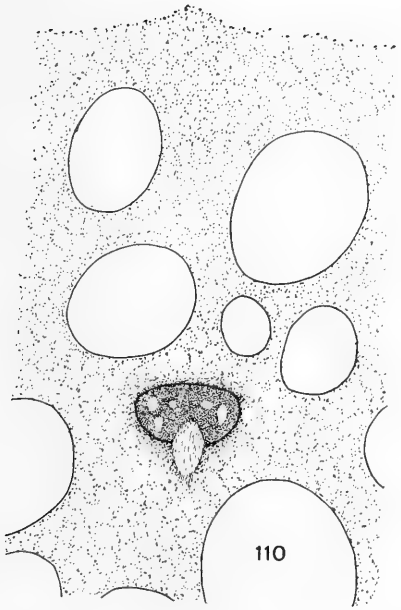
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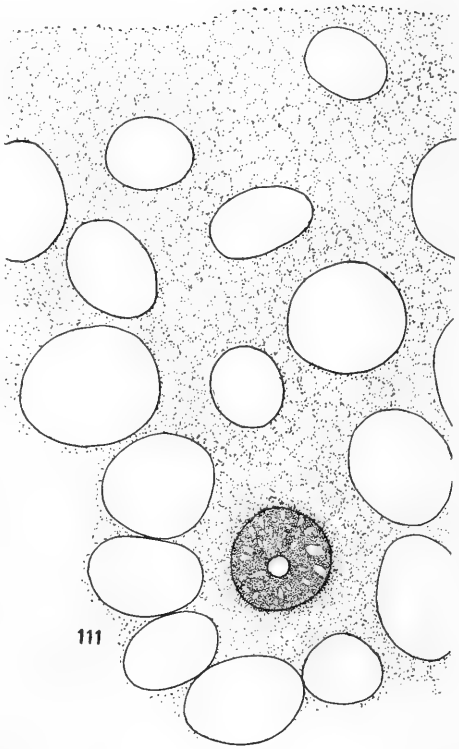
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PLATE 9

EXPLANATION OF FIGURES

112 Sperm-vesicle in egg, one hour and fifteen minutes old. This may now be called the 'male' pronucleus.

113 Egg from the lumen of the ovary showing the spindle of the first miotic division. × 3000.

114 First polar body of an egg just spawned. × 3000.

115 to 116 Two pronuclei found in one egg, two hours and fifteen minutes old. × 3000.

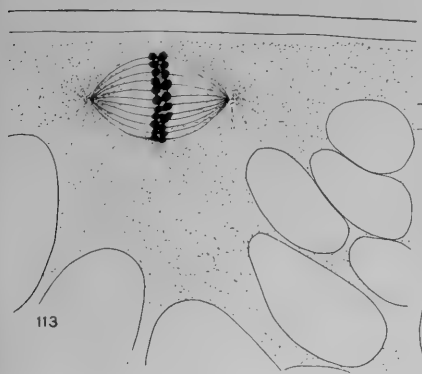
117 Egg two hours and fifteen minutes old; *h.*, transverse section of a hair of the pleopod with the shell of the egg wrapped part of the way around it; *n.*, pronucleus, probably the male; *v.*, vitelline membrane. × 320.

118 to 119 Egg four hours old, showing the pronuclei. Figure 118, × 366; figure 119, × 320.

120 Egg six hours old, showing the pronuclei side by side in the center of the egg. × 320.

121 Diagram of the ovary, the seminal receptacle and oviduct. The latter turned to one side so as to bring it in the same plane with the rest of the ovary; *c.*, portion of the seminal receptacle lined with chitin; *g.*, glandular portion of the seminal receptacle; *o.*, opening between the glandular and chitinous portion of the receptacle; *od.*, oviduct. Natural size.

122 Seminal receptacle at the time of molting; *c.*, chitinous portion; *g.*, glandular portion. Natural size.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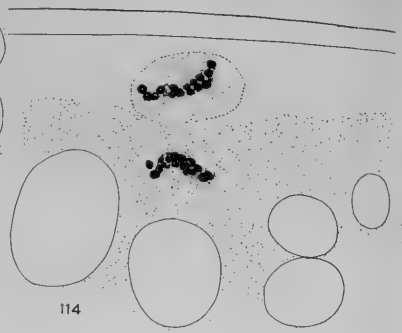
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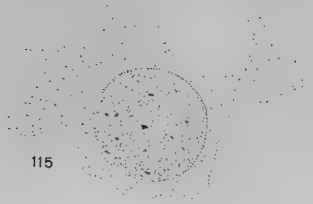
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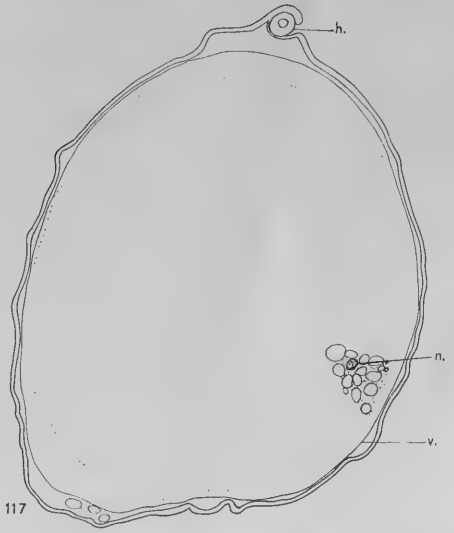

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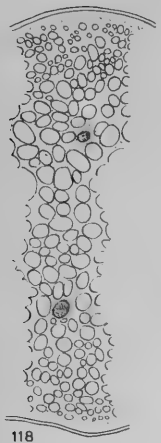
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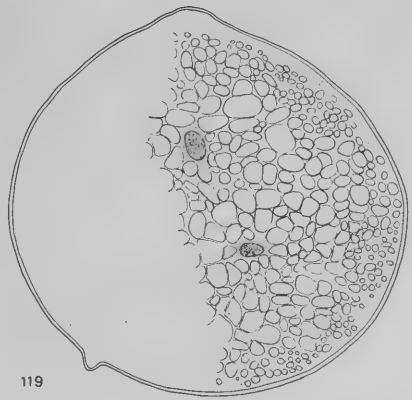
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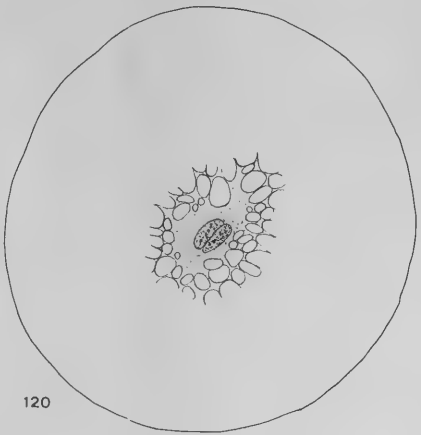
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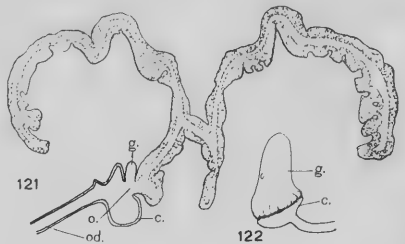

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METHOD OF CELL-DIVISION IN THE SEX CELLS OF TAENIA TENIAEFORMIS.[1]

MARY T. HARMAN

Kansas State Agricultural College, Manhattan, Kansas

EIGHT PLATES

CONTENTS

INTRODUCTION

Flemming ('92) in his review of the literature on amitosis from 1841 to the beginning of 1893, concludes that investigation has shown that amitosis is connected with a high specialization of the cell and may be a forerunner of degeneration. This is in harmony with the views of Ziegler and Vom Rath, that those

[1] Contribution from the Zoological Laboratory, Indiana University, no. 129. Offered as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

cells which divide amitotically are in process of degeneration. In a discussion of the question of amitosis Vom Rath ('91) states that when a cell has once divided amitotically it never again divides mitotically. Since 1892 a number of observers have recorded the occurrence of amitosis both in the somatic cells and the sex cells, but its unquestionable occurrence has been shown in few cases.

In the present paper the division of the sex cells only will be considered. Whether the sex cells or their progenitors ever divide amitotically is a question of interest on account of its bearing on a number of theories, among which are those of heredity, the continuity of chromosomes, and the relation of the sex chromosomes to the determination of sex. A number of observers have described amitotic division in both the testes and the ovaries of many animals. Meves ('94) describes the spermatogonial cells of Salamandra as dividing amitotically in the autumn and mitotically in the spring. However, his description of amitosis is very different from the process as usually described. He describes and figures the nucleus as being divided by the apparent constricting power of a ring-shaped centrosphere.

Preusse ('95) describes the appearance of amitosis in the ovaries of Hemiptera. Gross ('01) also finds amitosis in the ovaries of Hemiptera, but he contends that those cells which divide amitotically are degenerating or are secretory cells and therefore do not give rise to ova.. Amitosis is recorded as occurring in the spermatogonial cells of Amphiuma by McGregor ('99). He says: "Amitosis occurs among primary spermatogonia and is, apparently, a normal process. Secondary spermatogonia divide only by mitosis and contain the somatic number of chromosomes."

In 1904 Child gave a brief account of the occurrence of a mitosis in Moniezia in the early stages of segmentation. In a series of papers ('07) he described amitosis as taking place in the development of the reproductive organs, in the spermatogonial divisions, in the oogonial divisions, and in later segmentations. He also finds mitoses in all these places except in the later segmenta-

tions, but states that the mitoses are not at all frequent and that amitosis is the usual method of division. In his conclusions, in speaking of amitosis and mitosis, he says: "There can I think be little doubt that the two forms of cell division correspond to different physiological conditions of the nucleus. Judging from the visible phenomena, it also seems probable that mitosis is associated with cyclical and amitosis with acyclical processes." And again he says: "The regions where mitoses are most abundant may be regions of slowest division instead of the only regions where division is occurring."

Hargitt ('06) with reference to cell-division in Clava leptostyla, says: "During the early cleavage, even up to the sixteen-cell stage, no evidence of mitosis has been found." Later in the same paper he says: "The facts seem clearly to justify the general conclusion that for a time in the early history of the development of the egg, nuclear activity differs greatly from the ordinary forms of mitosis, and appears to involve direct of amitotic division."

Beckwith ('09) in working on the same form, finds no evidence whatever of amitotic division, and states that both maturation and early cleavage take place by means of mitosis and not amitosis. She explains the nuclear nests of Hargitt by the condition of the nuclear reconstruction after cleavage and the lack of the appearance of the maturation divisions and fertilization by the fact that the eggs were not found at the right time of the day. Eggs collected from 4 to 6 A.M. show typical stages of maturation and fertilization.

Richards ('09) finds no amitosis in the oogenesis of Taenia and concludes that his observations "on the process of oogenesis point to mitosis as the usual method of cell-division." The same author (Richards '11) in his conclusions on his work on Moniezia, says: "In the early stages of sex cell development mitosis unquestionably occurs (probably periodically), while amitosis is not evident in my preparations; and finally there cannot be the slightest doubt that the cleavage of the ovum takes place by mitosis."

Child ('11) in a paper in which he bases his observations on slides of Moniezia prepared by Richards, finds what he interprets as numerous cases of amitosis. In speaking of his work and that of Richards, he says: "The difference between us seems to me to rest now on Richards's failure to recognize, or interpret as I have done, what his material actually shows." Further in the same paper he says: "I am perfectly well aware that none of these figures and likewise none of my earlier figures constitute a real demonstration of the occurrence of amitosis, for such a demonstration is impossible in fixed material."

The occurrence of amitosis both in the oogonial and the spermatogonial cells of Leptinotarsa signaticollis is recorded by Wieman ('10). He says that "amitosis is merely transient and inconspicuous in the ovogonia. In the spermatogonia it is more prominent, persists longer and is involved in the formation of the cysts."

Foot and Strobell ('11) describe amitosis as taking place in the ovaries of Protenor. Payne ('12) has shown in Gelastocoris that the cells which Foot and Strobell describe as dividing amitotically are food cells and therefore do not give rise to ova.

To the above observations might be added those of Glaser, Johnson, Young and others. In most cases, however, where amitosis has been described as occurring in the sex cells, subsequent observers have shown that a different interpretation is possible and that it yet remains to be proved that the sex cells may divide amitotically and afterward give rise to new individuals.

In September, 1910, at the suggestion of Dr. Payne,[2] I began my work on cell-division in the sex cells of the tape worm. On account of the theoretical interest of the question, I have confined my work to the sex cells. If amitosis can be proved to take place in the cells which afterward go to form new individuals, the occurrence of it in the somatic cells will not be questioned.

[2] I wish to thank Dr. Fernandus Payne for many helpful suggestions during the course of my work and to express my indebtedness to B. H. Ransom for identifying my material and to C. E. Wilson for assistance in securing material.

MATERIAL AND METHODS

The form which I have selected for my studies is Taenia teniaeformis (Bloch, 1780) (Stiles and Stevenson, '05), a cestode which is a common parasite in the small intestine of the domestic cat. Both fixed and live material have been used.

1. Fixed material

In order to ascertain whether or not the time of the year has any influence upon the character of cell-division or the apparent frequency of cell-division, cats were killed every month in the year and often three or four times during the month. As soon as the host was dead the intestine was opened and the tape worms were put into killing fluid. Also to determine whether or not the time of the day has any influence upon cell-division, material was fixed at all hours during the day from five o'clock in the morning until eleven o'clock at night. Some of the cats were fed all they would eat for three or four days previous to killing, others were starved for the same length of time, and still others were killed as soon as they were obtained. This was done in order to determine whether or not the food supply has any effect on the character of the cell-division. Most often the host was chloroformed, but to make sure that the chloroform has no effect upon the character of cell-division some cats were stunned by a blow upon the head and then bled to death. The character of cell-division or the apparent frequency of cell-division did not seem to be influenced by the time of the year, the time of the day, the amount of food material, or the use of chloroform.

The following killing and fixing agents were used; sublimate-acetic (a saturated aqueous solution of corrosive sublimate, one-hundred parts and ten parts glacial acetic acid); picro-acetic (a saturated aqueous solution of picric acid one part, distilled water two parts, and 1 per cent glacial acetic acid); alcohol-acetic (equal parts absolute alcohol and glacial acetic acid); Flemming's strong solution; Bouin's fluid; Gilson's mercuric-nitric mixture; Carnoy's fluid; and a mixture of Gilson's fluid and 4 per cent chromic acid

in the proportion of ninety parts Gilson to ten parts chromic acid. When a host contained more than one tape worm usually two or three killing fluids were used.

The following stains were used: Heidenhain's iron-alum-hematoxylin with a counter stain of eosin and without counter stain; safranin and gentian violet; safranin, gentian violet, and lichtgrün; Auerbach's fluid; alum-carmin and osmic acid; Delafield's hematoxylin; and Conklin's hematoxylin. Although all the above stains show nuclear structure, the combination which shows centrosomes and spindle fibers best is Heidenhain's iron-alum-hematoxylin without counter stain, following a fixation in Flemming's strong solution. These structures were more clearly visible when, before being stained, the sections were bleached twenty-four to forty-eight hours in turpentine at a slightly elevated temperature.

After fixing, the tape worms were cut into pieces, each containing from two to twenty-five proglottids, depending upon the size of the tape worm and the size of the proglottids. Two entire tape worms, a small one indicating that perhaps it was young and a large one probably older, were sectioned. Of the others, pieces at intervals of four to twenty-five proglottids were sectioned. The sections were cut 3 μ thick. Some sections were cut cross and others sagittal. The pieces not sectioned were preserved in 85 per cent alcohol and saved for further reference. The character of cell-division was found to be the same both in the large and the small worms.

2. Living material

At two different times I made observations on living material, believing with Richards that "for amitosis there is but one absolutely certain criterion, the observation of living material and subsequent study of fixed material under observation" and with Child that a real demonstration of the occurrence of amitosis is impossible in fixed material.

I found by experiment that Taenia teniaeformis, when placed in Ringer's solution and kept at a temperature of 39°C., will live outside the body of the host forty-eight hours or more.

The Ringer's solution was put into an open vessel in an open paraffin oven. By means of a Bunsen burner the oven was kept at such a temperature that the Ringer's solution was 39°C. As soon as the host was killed, the tape worms were taken from the intestine and transferred to the Ringer's solution. Immediately a proglottid from that portion of the worm which, in accordance with my former observations, contained segmenting ova, was transferred to a slide on a constant temperature stage which had previously been heated to 39°C. Here the proglottid was opened and some of the ova were teased out in the Ringer's solution and covered with a cover-glass. To prevent rapid evaporation the cover-glass was sealed with a ring of vaseline. Besides preventing evaporation the vaseline supported the cover-glass. The first preparation was observed two hours with a 4 mm. objective and a Zeiss 12 compensating ocular. Other slides were prepared in a similar manner and observed for different lengths of time, varying from ten minutes to three hours. Typical resting nuclei were seen, but in no case could there be determined any indication of cell-division or even a slight constriction in the form of the nucleus. Frequently the cells collected in groups, usually of twos but sometimes more than two cells were in a group. At first sight these groups of cells might have been mistaken for constricting cells but closer observation revealed that they were not cells in the process of constriction but merely cell groups, or more exactly speaking, groups of ova, for it was possible to distinguish two and even three nuclei in a single ovum.

To make sure that cell-division was taking place in the worms, this experiment was repeated about a week later and pieces of the worm upon which the experiment was being made were killed in Flemming's strong solution. The results of the observations on the live material were the same as in the first experiment but when the fixed material was sectioned it showed mitotic division.

Soon after the above experiments were made there appeared an article by Morse ('11) in which he reports similar results. In his experiments, which were upon Calliobothrium and Crossobothrium, he used the plasma of the host as a medium. Although

he does not state definitely how long a time a single observation was extended, evidently it extended over a much longer time than mine, for he says:

> I am under the impression that cells which are cultured in this way do not undergo cell-division at all. I made charts of my slides which I had under observation for a week at a time and checked the behavior of all the cells in each slide with camera lucida drawings, comparing those made one day with those made on the day previous. If any increase in number of cells had occurred, I should have noticed it, of course.

Since the cells in Morse's experiments behaved in every way similar to the cells in my experiments, the comparatively short time that my experiments were under observation can make no difference. The only conclusion that can be drawn is that, under the conditions of the experiment, the nuclei do not divide.

OBSERVATIONS

Observations have been made upon the spermatogonial divisions and spermatogenesis, oogonial divisions, synapsis and the growth period, maturation divisions, and the cleavages. More attention has been given to the cleavages because it is here that the character of cell-division is most uncertain.

1. Spermatogonial divisions and spermatogenesis

My observations on the spermatogonial cells show nothing that I can in any way interpret as amitosis. It is true that I find few cells which are in the characteristic stages of mitosis. Most of the spermatogonial cells are in the so-called resting stage. I find no constricting nuclei and no cells which contain nuclei in contact. In early post synapsis the chromatin assumes the form of a more or less connected spireme, usually in contact with a large nucleolus which lies to one side of the nucleus (fig. A, plate 1). A group of cells in the anaphase of the first spermatocyte division is shown in figure B. The spindle fibers are very delicate, and, at the poles, there are small but very distinct, deeply staining centrosomes. No aster and no definite cell-wall is distinguishable. The second spermatocyte division follows

the first without any intervening resting stage. In figure D are shown two cells in the metaphase of the second spermatocyte division while in figure C are shown two cells in the metaphase and two in the anaphase. In the late anaphase the chromatin is collected in a mass at the poles. Occasionally there is a small portion of chromatin which lags behind the mass. This is illustrated in 1 and 2 of figure E and 1 of figure F. The spindles are even more delicate than in the earlier anaphases and the metaphase. I have seen no division of the centrosome.

2. Oogonial divisions

In the oogonial divisions mitoses are very frequent and there are no constricting nuclei nor nuclei in close contact. Plate 2 shows oogonial cells in different stages of mitotic division. The spindle is very similar to the spindle in the spermatocyte divisions but the centrosome is different. Here the centrosome is globular in form (showing a circle in section), comparatively large and stains a little more deeply than the spindle fibers. No aster is present. Some parts of the cytoplasm stains a little more deeply than others, thus giving it a mottled appearance. In the so-called resting stage the nucleus is very large and contains a large nucleolus which usually lies to one side of the nucleus and stains like chromatin. The chromatin is more or less scattered through the nucleus as a finely granular reticulum. The nuclear membrane stains like the chromatin and chromatin granules are distributed around the periphery of the nucleus. Figure E, plate 2, shows two oogonial cells in the resting stage and figure A, plate 3, shows one in the resting stage and the other in the telophase. The nuclear membrane has not yet formed, nor has the cell completely constricted.

3. Synapsis and the growth period

At the beginning of the growth period a marked change takes place in the appearance of the cells. There is a slight increase in the amount of the cytoplasm and a great increase in the mass of the chromatin. The chromatin takes the stain very readily

and forms into an indistinct spireme which becomes a greatly tangled mesh, lying to one side of the nucleus (fig. E, plate 3), The nucleus is large and lies to one side of the cell.

Figure C, plate 3, shows a group of cells in the early post-synaptic stage. There is not much change in the cytoplasm but the chromatin forms a definite, coarsely granular spireme which is in contact with a large nucleolus. In synapsis no nucleolus could be distinguished. This change in the chromatin goes on until it forms a finely granular reticulum which is in contact with the nucleolus. The chromatin remains in this condition during the remainder of the growth period.

4. Maturation and fertilization

Since both Child and Richards agree that maturation takes place by mitotic division, I have done no more upon this than figure a few maturation divisions. Figure F, plate 3, shows the metaphase of the first maturation division. The mitotic figure in maturation differs from the mitotic figure in segmentation, in having a smaller centrosome, and in the form of the chromosomes. The chromosomes in maturation are irregular, while in segmentation they are more definitely limited. The maturation spindle is always very long and one pole lies near the periphery of the cell. The segmentation spindle may be long or short and may lie in almost any position.

Child states that in no case has he observed asters at any stage in maturation in Moniezia but thinks that they probably do occur. Richards finds faint astral radiations in the same form. In Taenia I find asters, but in some cases they are very faint, as also are the achromatic spindle fibers. However, after being fixed with Flemming's strong solution, followed by a slow bleaching in turpentine and being stained with Heidenhain's iron-alum-hematoxylin, the asters and spindles are more plainly visible. Figure H, plate 3, illustrates the more plainly visible astral rays.

Inasmuch as fertilization does not bear directly upon the character of cell-division I have not included that in my observations.

5. Cleavages

Since fertilization has not been considered the relation of the two pronuclei previous to the first cleavage spindle has not been observed. In Taenia, Richards describes cleavage as taking place by mitotic division. In Moniezia, he finds frequent mitoses and no facts which he is able to interpret as amitosis. Child says, in his earlier paper, that in Moniezia the first cleavage is usually or always mitotic but cases of mitotic divisions are rarely seen after the first cleavage. He finds what he interprets as frequent cases of amitosis. In his later paper he says that mitosis occurs much later than the first cleavage but that the prevailing method of segmentation is by amitotic division.

a. Character of the cleavage. In Taenia, I find frequent cases of mitosis, not only in first segmentation but also in later segmentations. In fact, there are very few sections in my material where segmentation is taking place which do not have evidences of mitotic figures. The figures of mitosis that I have shown in plates 4, 5 and 7 might be duplicated many times. In Taenia teniaeformis it is not a question of whether mitosis occurs in some stages of segmentation and not in others, nor is it a question as to the frequency of its occurrence. The question is, whether or not mitosis is the only method of segmentation. The figures of plate 6 are essentially similar to Child's figures of amitotic division. I find these figures about as numerous as those which are unquestionably mitosis. These conditions occur, not only in later segmentations, but in early segmentation and many that I have figured are taken from first and second segmentations. The question is, do these admit of any interpretation other than that the nucleus has divided amitotically? I will discuss this question later.

In cleavage, nuclear division takes place very much in advance of cytoplasmic division. In the early divisions it is the exception and not the rule to find even a constriction in the cytoplasm. This gives rise to a syncytial condition. This syncytium persists until very late cleavage. Richards says that, in properly fixed material, he has never seen an egg syncytium. It cannot be the lack of proper fixation or stain which gives this syncytial

condition, for I find it with all the different fixations and stains. There is no indication whatever of poor fixation in those slides from which I have taken my drawings.[3]

Judging from the comparative size of the nuclei, the first division is an equal one. This is what would be expected if the division is mitotic. Soon after the first division there is a difference in the rate of nuclear division. I cannot say at just which division this difference in rate occurs. All the figures in plate 7 show nuclei of different sizes. I have never found two nuclei differing appreciably in size in which I could find evidence with any degree of certainty that they came from the same mother nucleus. For this reason I account for the difference in size of the nuclei by a difference in the rate of nuclear division.

b. Length and position of the spindle. As I have said before, the segmentation spindle may be long or short and may lie in almost any position with reference to the periphery of the cell. It may lie near the center of the cell with the cytoplasm almost equally distributed around it, as in figures A and B, plate 4, or near the periphery, as in figures E and F of the same plate. In these cases the spindle is comparatively short. Figures C and D, plate 4, show the position of the spindle intermediate between those shown in figures A and F of this plate, but of the same relative length. In figure H of the same plate the spindle is very long, extending from one side of the cell to the other. Figures I and J, plate 4, show cells in very late anaphase of first segmentation. The spindle fibers are not visible, but the centrosome and the masses of chromatin are very plainly visible. There is no doubt that in these cases the spindle was long. Figure G of the same plate shows a cell which has a spindle comparatively longer than those shown in figures A to F inclusive, and shorter than those shown in figures H to J inclusive, of this plate. All these are examples of the first segmentation spindles.

The same variation of the relative length and position of the spindle is found in later segmentations. However, here the length may depend somewhat upon the size of the nucleus, which is,

[3] Dr. Richards has seen some of my slides and says there is no question but that the fixation is good. However, he did not examine them with reference to the question of a syncytium.

perhaps, determined by the rate of division as described before. Therefore, the comparisons of length are by no means so certain as the position. Figure A, plate 7, shows a spindle, one pole of which is far from the periphery of the cell and the other lies in contact with a nucleus in the prophase. In figure C of the same plate is shown a spindle which has cytoplasm distributed almost equally around it, while figure B shows a spindle, neither pole of which lies near the periphery, yet one pole is very much farther from the periphery than the other. In figures D, E, G and I of the same plate are shown metaphase plates lying in different positions with reference to the other nuclei. In figure D are two metaphase plates which lie rather near each other, one of which is in close proximity to a small nucleus in which the chromatin is in a more or less perfect spireme. In figure I is a metaphase plate. surrounded by nuclei, the chromatin of which is in a reticulum or broken spireme. These nuclei are of different sizes. In figure N, plate 5, the metaphase plate is somewhat removed from the other nucleus in the cell. From these facts it may be concluded that the mitotic figure may lie in almost any position with reference to the periphery of the cell and the other nuclei in the cell.

c. Reconstruction of the nucleus. In the reconstruction of the nucleus the first mitotic structure to disappear is the spindle. Soon after its disappearance, the chromosomes become somewhat scattered and the centrosome becomes less clearly visible. The chromatin becomes more or less ragged and a light area appears around it. With the further breaking up of the chromatin the definite boundary between the cytoplasm and the nucleus appears. Some of the chromatin is distributed around the periphery of the nucleus. The reconstruction, or the rearrangement of the chromatin material may begin some time before the chromosomes have reached the poles, or it may not begin until they are very near the poles. Figures A to H inclusive, plate 5, show nuclei in different stages of the process of reconstruction, while figures I to J of plate 4, show the chromatin near the poles, but the rearrangement of the chromatin has scarcely begun. In figure F, plate 5, the chromosomes have begun to assume the ragged ap-

pearance, showing that they are in the process of reconstruction. The light area is appearing around the mass of chromatin, but the centrosomes are still faintly visible. Judging from the position of the centrosomes, this spindle was evidently a long one, and evidently reconstruction began when the chromatin was some distance from the poles. If these daughter nuclei should completely reconstruct and should reach the usual size of nuclei of the resting stage of cell-division, there is no doubt that they would lie in close contact if not even press against each other. It can easily be seen that such nuclei could give rise to nuclei having the relative positions of the nuclei shown in figures H to K inclusive of plate 6, or to daughter nuclei which would be in as close contact as those shown in figures F and G of the same plate. On the other hand, it would be pretty hard to imagine how the daughter nuclei, arising from the reconstruction of the chromatin masses as shown in figures I and J, plate 4, could lie in close contact. They would undoubtedly give rise to daughter nuclei with a relative position similar to that of those shown in figure L of plate 6.

Figure G, plate 5, shows the process of reconstruction of the nucleus more nearly completed than is shown in figure F of the same plate. The centrosomes have disappeared and the chromatin is in a more finely divided state. Although the reconstruction is by no means completed and the nuclei have not reached the usual size, the two nuclear areas lie against each other. If reconstruction should be completed, it is entirely possible that it would give rise to a condition such as is shown in figure E of plate 6. The reconstruction of the chromatin in a division like the one shown in figure D of plate 5, could very easily give rise to daughter nuclei having the relative position of the nuclei shown in figure J of plate 6. Here the nuclei lie in contact with the periphery of the cell on the opposite sides and yet they touch each other.

The reconstructions just described are reconstructions after the first segmentation spindle. Reconstructions of later segmentations are shown in figures G and H of plate 7. The nuclei shown in the process of reconstruction in figure H, are evidently

the result of the division of a large nucleus. If reconstruction should be completed, one daughter nucleus would lie very near to, if not in actual contact with, two other nuclei of the same ovum. The other daughter nucleus would lie very near the periphery of the ovum. In figure G of the same plate is a reconstruction which is the result of the division of a smaller nucleus than that in figure H. If reconstruction were to be completed in this case, one daughter nucleus would lie in contact with one other nucleus of the same ovum, if not with two. These figures show, at least, that nuclei may lie in contact without having arisen from the same mother nucleus. If this be true, then the fact that two nuclei lie in contact is no evidence that they have arisen by amitotic division.

d. The condition of the chromatin in the nucleus. Wilson says: "Amitosis, or direct division, differs in two essential respects from mitosis. First, the nucleus remains in the resting state (reticulum), and there is no formation of a spireme or of chromosomes. Second, division occurs without the formation of an amphiaster." In my preparations many of the nuclei which lie in close contact have the chromatin in a more or less perfect spireme. This is true, even in those nuclei which lie in such close contact that a definite boundary between them is not visible. The nuclei in the figures of plates 6 and 7 show this condition. Figure I, plate 5, shows four smaller nuclei in which the chromatin is in a more or less finely granular reticulum, and a large nucleus in which the chromatin is in a connected spireme.

Child has suggested that it is possible that the smaller cells are the result of amitotic division and the larger ones of mitotic division. His reason for this conclusion is that he has observed mitotic figures more often in large cells than in small ones. He also says that the cells are small because they have divided more often than the large ones. Bu t,he further assumes that the process of division by amitosis is a more rapid process than by mitosis. I think that this assumption is hardly justified. It is true that there are more changes taking place in mitotic division; that is, that it is a more complicated process than amitotic division, but the length of time required for a cell to pass completely through

a process of division may be determined only by observation. If the time had been determined for a cell to divide mitotically and for a cell of the same material under the same conditions to divide amitotically, Professor Child's statement would have been more nearly justified.

If the fact that the chromatin is in the form of a spireme be an indication that cell-division is taking place by mitosis, in figure I, plate 5, we might interpret the large nucleus as dividing mitotically and the smaller nuclei amitotically. However, figures H and I, plate 7, show both large and small nuclei in a connected spireme. It seems that it is perfectly possible that the difference in the character of the chromatin in the nuclei shown in figure I, plate 5, might be interpreted as different stages of the prophase. Mitosis unquestionably occurs in the smaller nuclei as well as in the larger ones. Figure C of plate 7, shows a spindle in the metaphase of mitotic division. Since the segmenting ovum is a syncytium, the size of the cell cannot be determined, but the nucleus giving rise to the spindle was undoubtedly smaller than the largest nucleus in the ovum. The nuclei in the process of reconstruction shown in figure G of the same plate, are undoubtedly smaller than the large nucleus of the same ovum and could not greatly exceed in size any nucleus shown in the figure. The mitotic figures shown in figures A and B of this plate could not produce daughter nuclei which would exceed in size the other nucleus of the respective ova.

Granted that nuclei which lie in close contact afford evidence that nuclear division may have taken place amitotically and that the imperfect spireme is no indication that mitosis is taking place, how could the position of the nuclei forming a triangle in figure L, plate 7, be explained? The nuclei are of nearly the same size and lie in very close contact, although the boundaries between them are clearly visible. If they have come about by the process of amitosis, which nuclei were the first to constrict off? It seems that it would be necessary to assume an unequal division and that one division has followed the other very closely before the nuclei have moved apart. Without these assumptions, I see no possible explanation for this arrangement of the

nuclei if they have arisen by the process of amitosis. The same statement will apply to the nuclei shown in figure K of the same plate. If we assume on the other hand that nuclear division has taken place by mitosis the condition shown in figure A offers an explanation for the condition figured in K and L. In the process of reconstruction the nuclei have come to lie in close contact. The sister nucleus of at least one of these nuclei is in another section of the same ovum.

COMPARISON WITH MONIEZIA

When I had almost concluded my work on Taenia, I received some of Dr. Richard's slides of Moniezia.[4] The examination of these slides shows that the character of cell-division in Moniezia is for the most part similar to that of Taenia. The figures of plate 8 have been taken from Moniezia. They show late segmentation and the cell boundaries are not visible. Early cleavage takes place by mitosis and the blastomeres are distinct. Later, as Child ('11) states, the cell boundaries become less distinct and entirely disappear, giving rise to a syncytial condition. When the cell boundaries are distinct (in the early cleavages) in Moniezia, the nuclei do not lie in contact nor is there any condition which indicates that cell-division has taken place by amitosis. Later, when the cell boundaries have disappeared, numerous cases of nuclei which lie in contact are found, and sometimes they are so close together that the surfaces between them are flattened. The unquestionable cases of mitosis are fewer in these regions, but that mitosis does take place here is shown in figures B, D, and E. In Taenia, where, in the early cleavages, the ovum is a syncytium, nuclei may lie in close contact any time after the first cleavage, as has already been shown.

Since, among the slides of Moniezia, that I have examined, there is only one of the late segmentations, the comparison of the length and position of the spindle has not been made, but the reconstruction of the nucleus is similar to that of Taenia. The nuclei in the process of reconstruction shown in figure E, plate

[4] I take this opportunity of expressing my indebtedness to Dr. Richards for his kindness in loaning me these slides.

8, are essentially like those shown in figure G, plate 5. In figure E the section passes through the middle of only one nucleus, but it shows a small portion of the other. However, the light areas appearing around the chromatin lie almost in contact, and reconstruction is far from being completed. The nuclear membrane has not yet appeared and the chromosomes have not lost their identity although they have the ragged appearance described above in the reconstruction of the nucleus in Taenia. If reconstruction should be completed, the nuclei would undoubtedly lie in as close contact as the two nuclei shown at *1* of the same figure, if not closer.

There is also the same variation in the condition of the chromatin in Moniezia as there is in Taenia. Some of the nuclei show the chromatin in a finely granular reticulum, typical of a resting stage, others a more coarsely granular reticulum, bordering on the formation of the spireme, and still others show the spireme in almost all degrees of perfection. No one of these conditions is confined to any particular sized nucleus. The reticulum is found in the large as well as the small nuclei. The same is true of the spireme. In figure D are shown three nuclei in a row and in close contact. The chromatin of the middle nucleus is in a spireme, while the chromatin of the other two nuclei is in a finely granular reticulum. In figure C two nuclei lie so close together that the surfaces of contact are flattened and yet the chromatin in each nucleus forms a perfect spireme which is in contact with a large nucleolus. The two large nuclei of figure A are in as close contact as those described in figure C, but the spireme is much less perfect, while the chromatin in the two smaller nuclei of the same figure forms an almost perfect spireme. The same variation is shown in all the figures of this plate. If the fact that the chromatin is in the form of a spireme, be an indication of mitosis, the two small nuclei of figure A are in the prophase of mitotic division.

The character of the cleavage, the reconstruction of the nucleus, and the character of the chromatin in the nucleus in Moniezia offer no more indication of amitotic division than they

do in Taenia. In fact, the indication of amitosis in Taenia is greater than in Moniezia, for, as has been said before, in Taenia nuclei are found lying in contact, even in the first segmentation, while in Moniezia this condition is not found until late segmentations.

DISCUSSION

My observations have not shown that amitosis does not take place in Taenia or Moniezia, but they have shown no condition which cannot be as readily explained as the result of mitotic, as of amitotic division. Since all those who have worked on cell-division in the cestodes record the occurrence of mitotic division, at least occasionally, those conditions which would be difficult to explain, if amitosis were the only method of cell-division, need not be discussed again. The absence alone of unquestionable cases of amitosis is, of course, no absolute proof that it does not take place. Whether a cell divide mitotically or amitotically is, in itself, of no significance. From the standpoint of the mere increase in the number of cells, it matters not what the character of the cell-division is. The question becomes of interest beyond the mere fact of its occurrence, when its bearing upon other biological questions is considered. Among these questions are the theories of heredity, the continuity of chromosomes, and the relation of the sex chromosomes to the determination of sex.

The fact that the germ cell is a single cell which gives rise to a new individual, however simple or complex that individual may be, must be the foundation for a discussion of any theory of heredity. This single cell may be the entire individual as in some protozoa; it may be a cell similar to the somatic cells, as in reproduction by budding; it may be the unfertilized ovum, as in parthenogenetic reproduction; or it may be the fertilized ovum, as in bisexual reproduction. This cell, whatever it is, contains in it the sum-total of the heritage of the species. The characteristics of the species are transmitted to the next generation by the division of this one cell. No matter what the character of the cell-divi-

sion is, it must be such that at least those cells which give rise to the new germ cells will contain the bearers of the characteristics of the species, or the characteristics themselves in potentia.

Strasburger, Weismann, Kölliker, and Oscar Hertwig, independently and almost at the same time, identified the nucleus as the bearer of the hereditary qualities. This view, while held by many, is by no means universally accepted. If it be true that the nucleus is the bearer of the hereditary qualities, and if division be amitotic, it must be assumed that the nucleus as a whole is the bearer of the characteristics, and, so far as the hereditary qualities are concerned, there can be no differentiation of the nuclear material.

The theory of the continuity of chromosomes is far from being absolutely proved or universally accepted. However, Rabl, Zur Strassen, Boveri, Van Beneden, Morgan, Wilson, Payne and others have made observations that give some very strong evidence in favor of it. If it should be proved that amitosis does not take place in cells which are the progenitors of new individuals, this would give no direct proof of the theory. On the other hand, if it should be proved that amitosis does occur in cells that are the progenitors of new individuals, it would offer very strong evidence against it. If the nucleus divide amitotically, the chromatin which goes to one daughter nucleus gets into that particular daughter nucleus rather than into the other, by chance. Such a condition would be one of the strongest evidences against the continuity of chromosomes.

If there be a mass division of the chromatin, and consequently no continuity of chromosomes, the accessory chromosome and the idiochromosomes and their relation to the determination of sex have no significance unless as Wilson ('06) and Morgan ('09) have suggested, sex is determined by the quantity of the chromatin. Their regular occurrence and their uniform behavior in a species would be difficult to harmonize with amitotic nuclear division.

SUMMARY

In conclusion, the evidence presented in these observations show that:

1. Neither the character of cell-division nor the apparent frequency of cell-division is influenced by the time of the year, the time of the day, the amount of food material, or the use of chloroform.

2. Under the conditions of my experiment the ova do not segment outside the body of the host.

3. Division in the spermatogonial cells is unquestionably mitotic. I find no condition that might be interpreted as amitotic division.

4. The spermatocyte divisions are mitotic.

5. The second spermatocyte division follows the first without an intervening resting stage.

6. In oogonial divisions mitosis is very frequent and there is no evidence of amitosis.

7. The maturation of the ovum takes place by mitotic division.

8. Mitotic division occurs both in late and early cleavages.

9. In the cleavages nuclear division takes place very much in advance of cytoplasmic division which results in a syncytial condition of the ovum.

10. The mitotic figure in the cleavages may lie in any position with reference to the periphery of the ovum and to the other nuclei of the ovum.

11. The cleavage spindle may be long or short and the reconstruction of the nucleus may begin when the chromatin is some distance from the poles of the spindle.

12. By the time reconstruction is completed the daughter nuclei become very large and consequently may lie in close contact.

13. The close contact of nuclei is no indication of the character of the cell-division.

14. In Taenia teniaeformis I have found no condition that cannot be as readily explained as the result of mitotic division as of amitotic division.

15. The character of the cleavage, the reconstruction of the nucleus, and the character of the chromatin in the nucleus in Moniezia offer no more indication of amitotic division than they do in Taenia.

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EXPLANATION OF PLATES

All figures were drawn with a Zeiss compensating ocular no. 6 and a 1.5 mm. objective at table level with the aid of a camera lucida and then enlarged two-and-a-half diameters. The figures on plates 1, 2, 3, and 8 were reduced one-third and those on plates 4, 5, 6, and 7 were reduced one-half. The figures on plates 1 to 7 inclusive were made from slides of Taenia teniafermis and those on plate 8 were made from slides of Moniezia.

PLATE 1

EXPLANATION OF FIGURES

A Early post synapsis spermatogonial cells.

B Anaphase of first spermatocyte division.

C Metaphase and anaphase of second spermatocyte division.

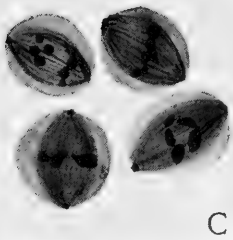
D Metaphase of second spermatocyte division.

E Late anaphase of second spermatocyte division; *1* and *2*, a portion of chromatin which lags behind the mass of chromatin.

F Late anaphase of second spermatocyte division; *1*, a portion of chromatin which lags behind the mass of chromatin.


A


B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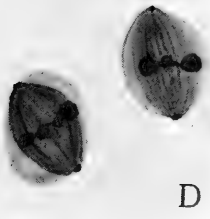
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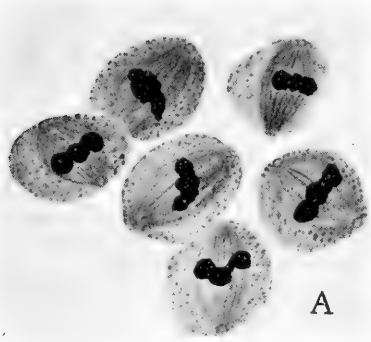
PLATE 2

EXPLANATION OF FIGURES

A, B and C Metaphase of oogonial cells.
D Metaphase and late anaphase of oogonial cells.
E Resting stage of oogonia.
F and G Metaphase and early anaphase of oogonial division.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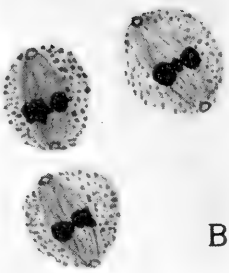
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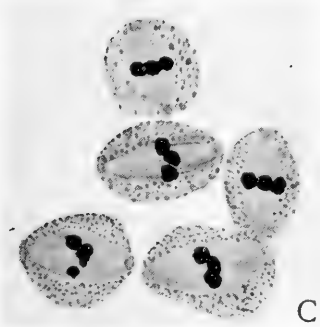
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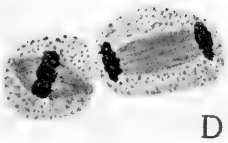
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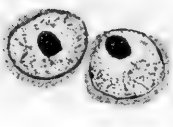
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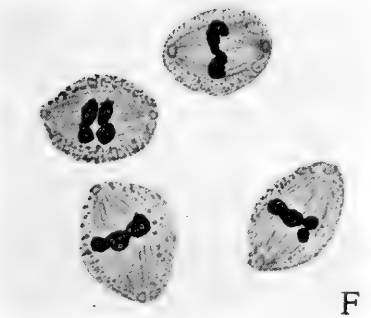
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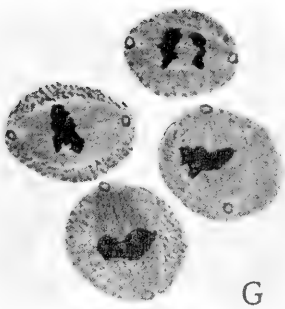
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PLATE 3

EXPLANATION OF FIGURES

A Telophase and resting stage of oogonial cells.
B Post-synapsis of oogonial cells.
C Early post-synapsis of oogonial cells.
D Metaphase of second maturation division.
E Synapsis of oogonial cells.
F, G and H Metaphase of first maturation division.

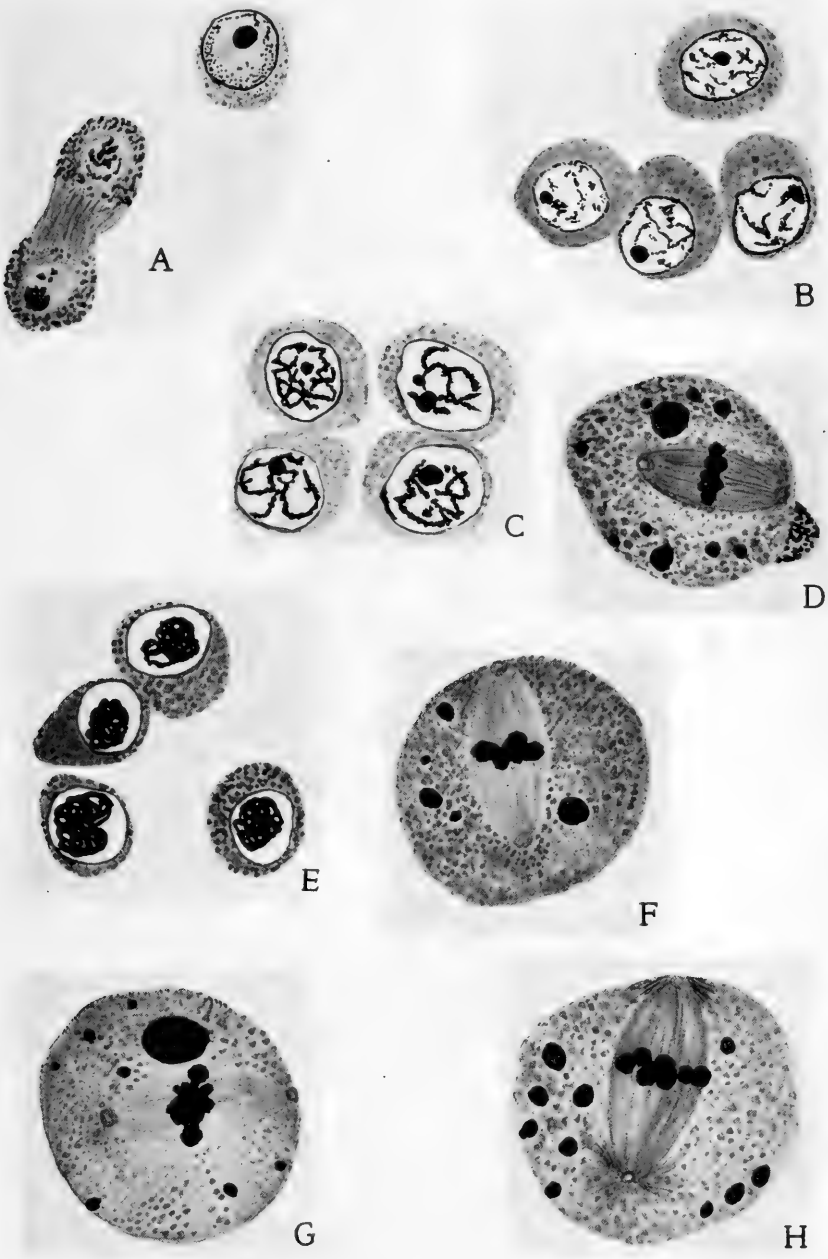
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PLATE 4

EXPLANATION OF FIGURES

A Metaphase of first segmentation.
B and C Early anaphase of first segmentation.
D Metaphase of first segmentation.
E Anaphase of first segmentation.
F Metaphase of first segmentation.
G and H Anaphase of first segmentation.
I and J Late anaphase of first segmentation.
K and L Later segmentations, showing resting nucleus and spindle.

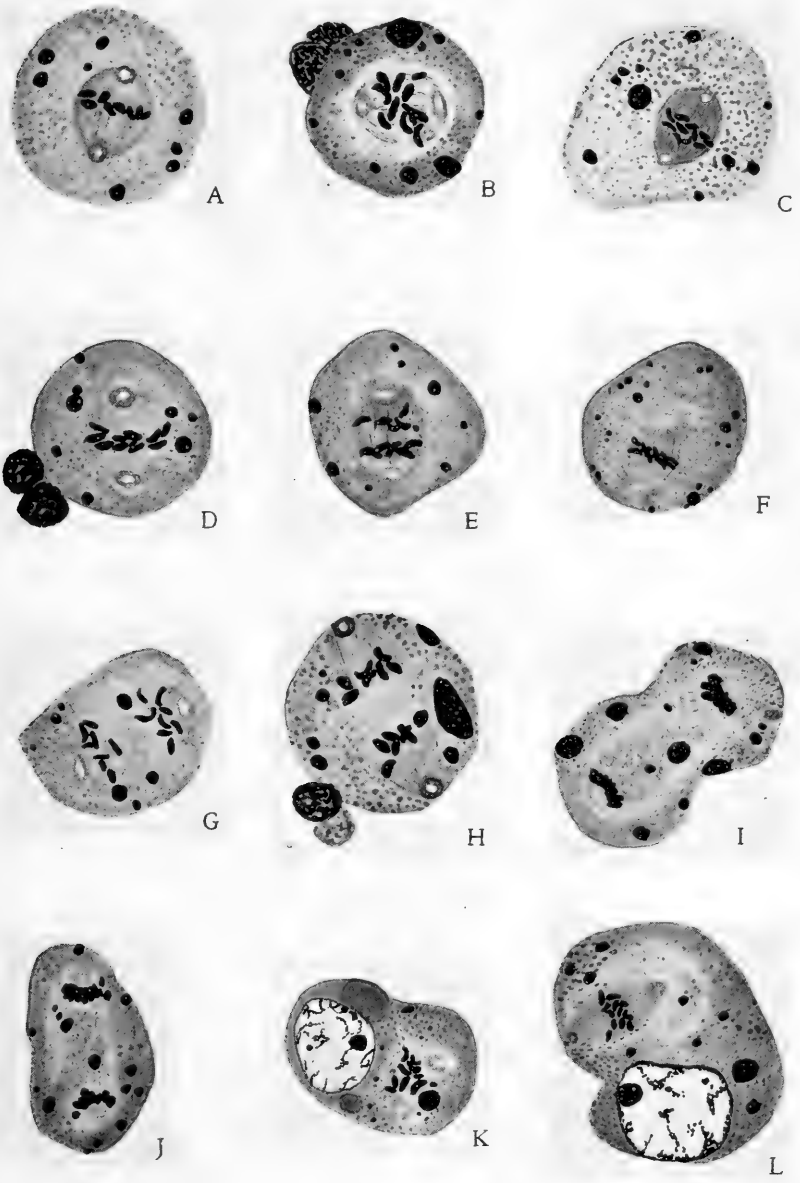
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PLATE 5

EXPLANATION OF FIGURES

A, B, C and D Anaphase of early segmentation.

E, F, G and H Telophase of early segmentation.

I Later segmentation, showing a metaphase plate, a large nucleus with the chromatin in a spireme, and smaller nuclei with the chromatin in a reticulum.

J and K Early segmentation showing one nucleus with chromosomes and the other with the chromatin in a spireme.

L Early segmentation with a nucleus apparently constricted and the chromatin in a spireme.

M Metaphase plate of early segmentation.

N Early segmentation showing a metaphase plate and the other nucleus with the chromatin in a spireme.

O Early segmentation with a nucleus apparently constricted and the chromatin in a spireme.

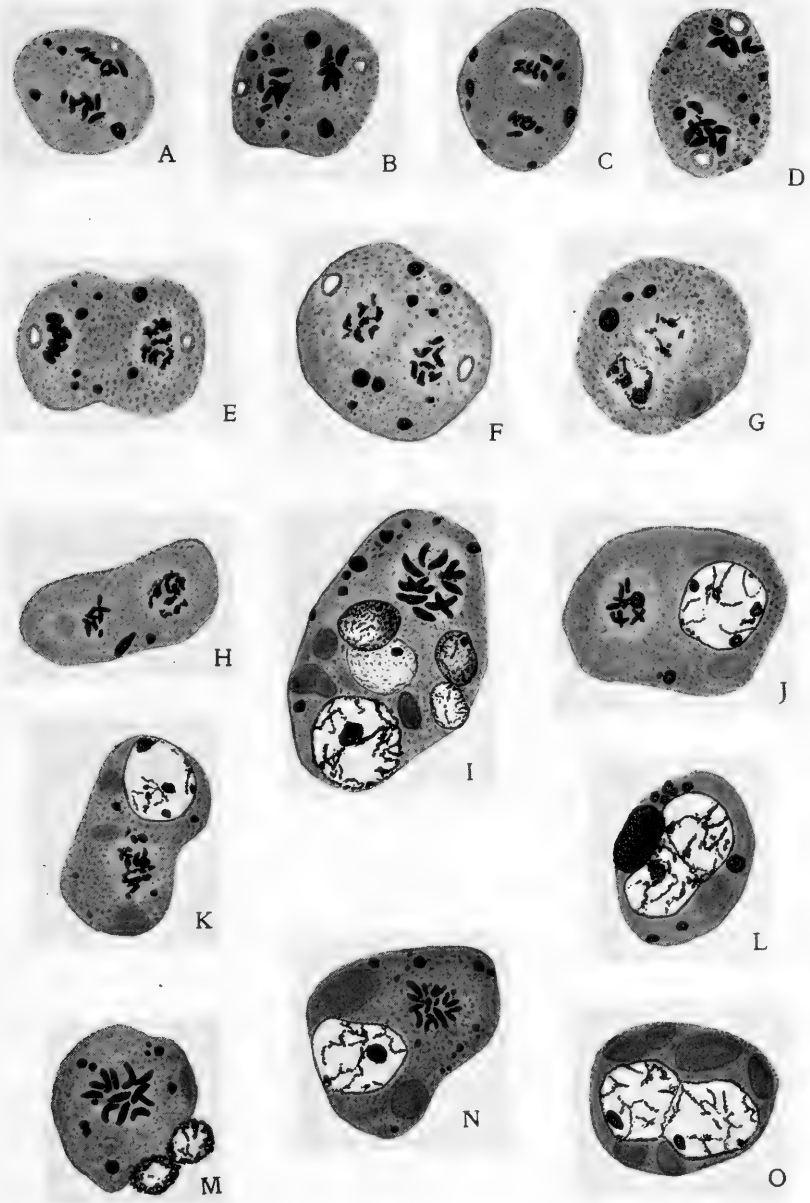
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PLATE 6

EXPLANATION OF FIGURES

All the figures on this plate show early segmentation and the chromatin in a more or less perfect spireme.

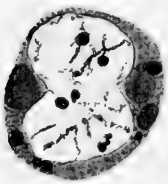
A and B Nuclei between which no definite boundary is visible.

C, D and E Nuclei in which a more or less definite plate marks the boundary.

F and G Nuclei close together; the sides which are almost in contact are flattened.

H, I, J and K Nuclei in contact.

L Nuclei some distance apart.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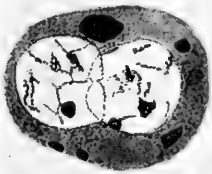
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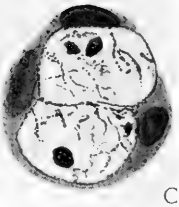
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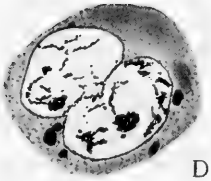
D


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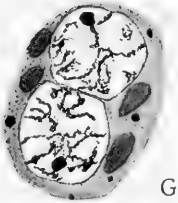
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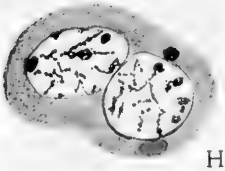
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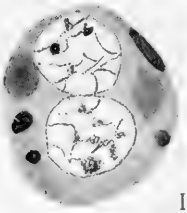
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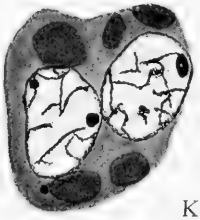
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PLATE 7

EXPLANATION OF FIGURES

A Late segmentation with one nucleus in metaphase one pole of the spindle in contact with the other nucleus which is in prophase.

B Segmentation, showing one nucleus in prophase and one in metaphase; the nuclei are some distance apart.

C Late segmentation with one nucleus in metaphase and the others in different stages of prophase.

D Two metaphase plates and other nuclei in prophase.

E Segmentation, showing one large nucleus and two smaller nuclei in prophase, and one metaphase plate.

F Late segmentation, showing chromatin in the process of reconstruction and nuclei in prophase.

G Late segmentation, showing metaphase plate, early telophase and prophase.

H Late segmentation, showing nuclei in early telophase and both large and small nuclei in prophase.

I Segmentation, showing metaphase plate and nuclei in prophase.

J Late segmentation, showing different sized nuclei in contact in which the chromatin is in prophase.

K Segmentation, showing the nucleus apparently being constricted into three parts.

L Late segmentation, showing the nuclei in contact; the chromatin is in a fine spireme.

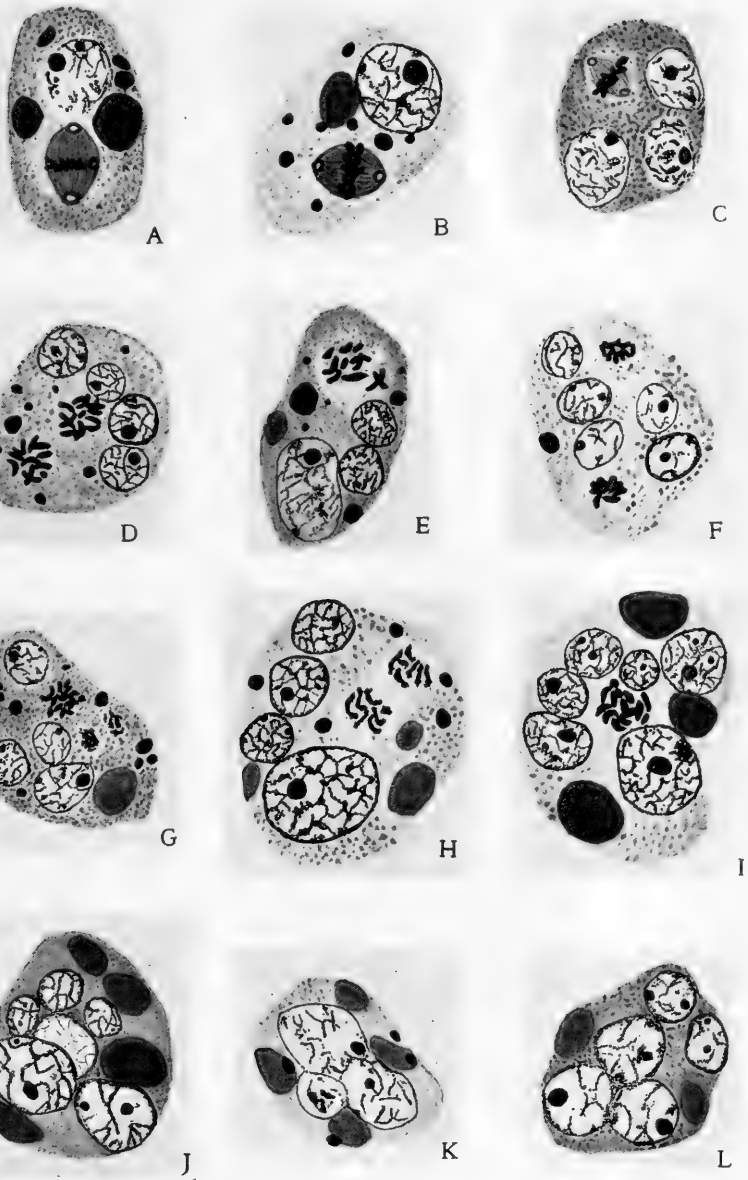
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PLATE 8

EXPLANATION OF FIGURES

The figures on this plate are from slides of Moniezia showing segmentation.

A Two large nuclei in contact, in which the chromatin is in a fine spireme, three smaller nuclei in which the chromatin is in the same form, and two still smaller nuclei in contact in which the chromatin is in a coarse spireme.

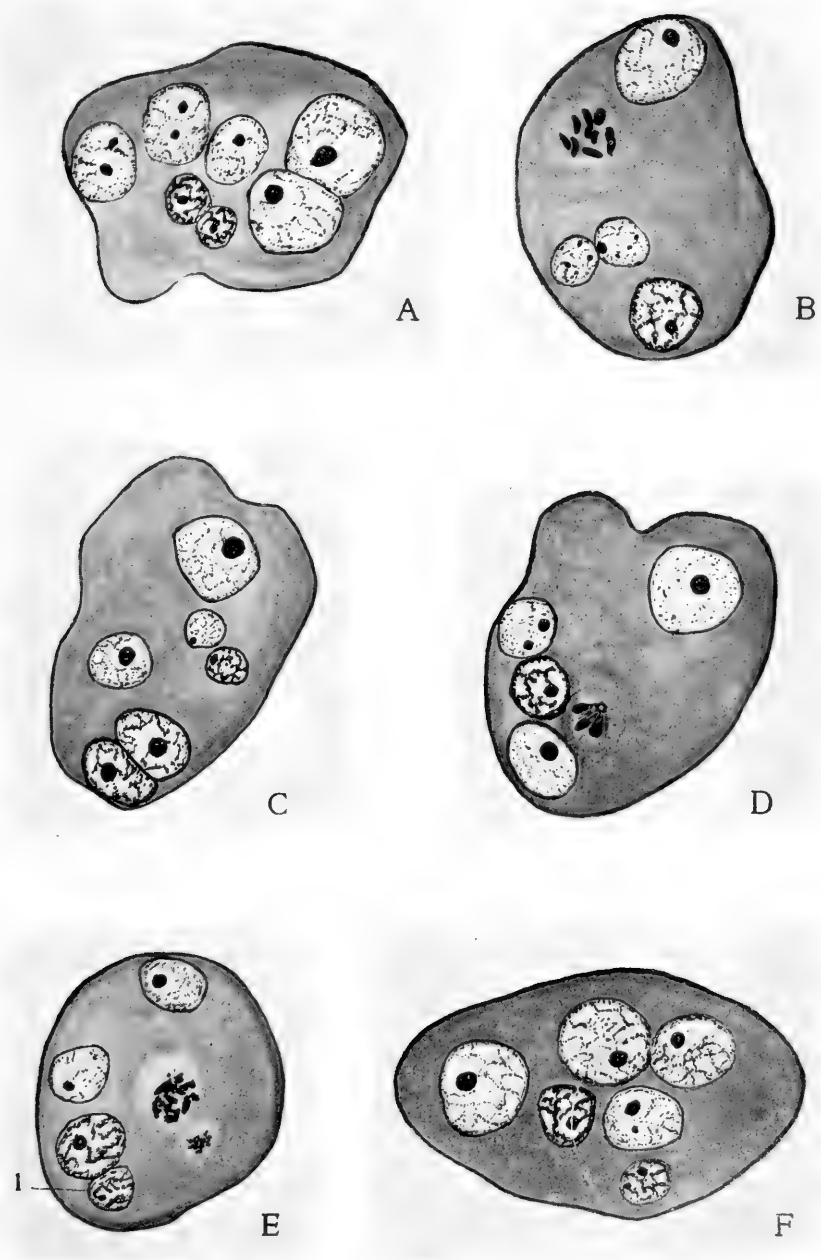
B A metaphase plate, two nuclei in contact, in which the chromatin is in a fine spireme, a large nucleus in contact with the periphery of the cell and the chromatin in a fine spireme, and a large nucleus in which the chromatin is in a coarse spireme.

C Three nuclei with the chromatin in a coarse spireme and three in which the chromatin is in a fine spireme.

D Part of a segmentation spindle, three nuclei in a reticulum and one with a coarse spireme.

E Two nuclei with chromatin in telophase, two in reticulum, and two in a coarse spireme, *1*, a small nucleus in contact with a large nucleus.

F Four nuclei of different sizes and of different relative positions with the chromatin in a fine spireme and two smaller nuclei with the chromatin in a coarse spireme.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THE CRANIAL NERVES OF SIREN LACERTINA

H. W. NORRIS

The Zoological Laboratory of Grinnell College

FORTY-FOUR FIGURES

CONTENTS

INTRODUCTION

Fischer (1864) seems to be the first to give any accurate description of the nervous system of Siren, for the account given by Vaillant ('63) is hardly worthy of mention. Fischer describes the seventh, ninth and tenth nerves, and gives some figures of the skeletal and muscular features of the head which show incidentally some of the minor nerve branches. In his description of the seventh nerve he overlooks the ramus mentalis externus, and confuses the ramus communicans vagi cum faciali with a "Kopftheil des Sympathicus." Parker ('82) in his description of the skull of Siren, figures and mentions the exits from the skull of most of the main trunks of the cranial nerves. Wilder ('91) describes the nerves and muscles of Siren as shown by a general dissection of the head, although his analysis of the IX-X complex is far from satisfactory. Drüner's account ('04) deals with the cranial nerves, only as they are related to the branchial musculature. His descriptions are given with his characteristic clearness and with very few inaccuracies. Upon the subject of the nerve components in Siren previous writers have thrown little light.

In the matter of nomenclature the writer has followed Fischer, Drüner and Osawa ('02) chiefly, attempting to avoid, as far as possible, on the one hand the formation of new names, and on the other the slavish subserviency of the systematist to priority. The BNA terminology must be applied with caution to amphibian structures until exact homologies are more satisfactorily determined.

METHODS AND TECHNIQUE

The present paper is based upon the study of young adults of Siren lacertina 140 to 220 mm. in length, fixed and stained in vom Rath's solution of the following composition:

Picric acid, saturated solution	250 cc.
Platinic chloride (dissolved in 5 cc. of water)	2.5 grams
Osmic acid	1 gram
Glacial acetic acid	5 cc.

The duration of the fixation and accompanying decalcification was about ten days. This was followed by washing in running water twenty-four hours, in 50 per cent and 70 per cent alcohol until the excess of picric acid was removed. The customary treatment with pyroligneous or pyrogallic acid was omitted, as the increased blackening of the general tissues thus produced has been found to be detrimental to the tracing of the peripheral nerve fibers. The material was imbedded in celloidin after months of infiltration, beginning with 0.5 per cent solution and ending with a 20 per cent solution. The sections, cut 20μ in thickness, were counterstained on the slide in Van Giesen's picro-fuchsin. Sections prepared by the celloidin method, as thus employed by the writer with specimens of considerable size, have many advantages over those prepared by the paraffin method. Their clearness, freedom from distortion and contraction, and absence of displacement of parts, more than offset their thickness (20μ) and the tedium of the prolonged section cutting. When the treatment with pyroligneous acid is omitted in the vom Rath procedure the hot melted paraffin and its subsequent removal by xylol, and so forth, in the paraffin method bleach out to a considerable extent the osmic acid stain. By the celloidin method the stain is apparently unaffected, even when the infiltration is continued six months. Sections were cut in the three conventional planes. Despite the thickness of the sections the fixation and differentiation have been so precise that there has been little difficulty in tracing and distinguishing the various nerve components. Even the fine twigs to the individual neuromasts

have been traced with absolute certainty, so that with a few exceptions, due to imperfect sections, the innervation of each neuromast on the head has been traced.

THE OLFACTORY NERVE

The olfactory nerve is double in origin and distribution. A posterior series of rootlets, arising from the olfactory glomeruli in three groups, dorsal, lateral and ventral, produces a nerve trunk that is distributed chiefly to the anterior nasal epithelium and Jacobson's organ (fig.1,*Ip.*). The nerves supplying the latter structure (*Ijo.*) apparently enter the ventral group of rootlets, but do not comprise the entire group. An anterior series of rootlets (*Ia.*), arising mostly from a single group situated laterally on the olfactory lobe and at the inner border of the trunk formed by the posterior series of rootlets, innervates chiefly the posterior nasal epithelium. In addition, the anterior series possesses a small ventral rootlet that arises on the ventro-lateral border of the lobe. Into this small ventral rootlet the nervus terminalis enters. The nerve, formed by the anterior series of rootlets, leaves the brain on the inner border of the other trunk and almost immediately curves over the dorsal border of the latter in passing to its destination. It cannot be said, however, that the two trunks are absolutely distinct in origin and distribution. A horizontal section through the origin of the nerve from the olfactory lobe shows that there is some commingling of fibers of the two groups of rootlets (fig. 2.). The Caecilians seem to be the only other amphibians in which a double olfactory nerve has been reported, although Lee ('93, pp. 10, 11) has pointed out the double nature of the olfactory lobe. According to Wilder ('91, p. 689) the olfactory nerve of Siren enters the nasal "capsule through a large foramen in the ethmoid bone" (orbitosphenoid). In specimens 140 mm. in length the writer finds the olfactory nerve passing through a notch at the anterior end of the orbitosphenoid, but in older individuals of 180 mm. length the orbitosphenoid completely surrounds the exit of the nerve. Wilder's statement that the fibers of the olfactory nerve "take a direction almost laterally outward from the brain, thus lying nearly at

right angles with the other nerves which lie near it" (l.c.), requires some qualification. It is true of the root which passes to the posterior portion of the nasal epithelium, but is not true of the anterior trunk. Figure 2 shows the direction the roots take on emerging from the brain, and also the double origin of the nerve from the glomeruli.

ABBREVIATIONS

alv., r. alveolaris VII
alv.1, dorsal branch of r. alv. VII, in close relation with md. 4a
alv.2, ventral branch of r. alv. VII
alv.-pal., common trunk of rr. alveolaris and palatinus VII
ao., antorbital cartilage
aop., antorbital process of orbito-sphenoid bone
auo., ossification of the ear capsule
aur., r. auricularis X
bhy., basi-hyal cartilage
br., brain
br.1,X.1, branch of the second branchial nerve supplying the first gill
br.2,X.1, branch of the second branchial nerve supplying the second gill
br.2,X.2, branch of the third branchial nerve supplying the second gill
br.3,X.2, branch of the third branchial nerve supplying the third gill
buc., r. buccalis VII
buc.1, r. buccalis, ventral division
buc.2, r. buccalis, division anastomosing with r. oph. prof. V(op.4)
buc.3, r. buccalis, lateral division
buc.2+mx.2, union of buc.2 with fibers of r. max.V, to anastomose with r. oph. prof. V(op.4)
buc.3+mx.3, union of buc. 3 with fibers of r. max. V, innervating side of head
cbr.1, *cbr.2*, *cbr.3*, *cbr.4*, first, second, third and fourth ceratobranchial cartilages
cc., cranial cavity
ccbl., cerebellar commissure
ch., cerebral hemisphere
che., m. ceratohyoideus externus and branches of the r. jgl. VII innervating it
chi., m. ceratohyoideus internus and branches of the IX and X nerves innervating it
chy., ceratohyal cartilage
cp., petrosal cartilage = cartilaginous base of ear capsule
cplx., choroid plexus
dbr.1,dbr.2,dbr.3, depressor muscles of the first, second and third gills and the nerves innervating them
dent., os dentare
dl.1, branch of tr. int.-acc.X, innervating dorsal portion of m.dorso-laryngeus
dl.2, branch of tr. int.-acc.X, innervating middle portion of m. dorso-laryngeus
dl.3, branches of r. int.rec.X, innervating ventral portion of m. dorso-laryngeus
dm., m. depressor mandibulae = digastricus = cephalo-hyo-mandibularis
dma., anterior inner division of m. depressor mandibulae, and nerves innervating it
dmp., posterior division of m.depressor mandibulae, and nerves innervating it
en., os ethmo-nasale of Parker, nasale of Cuvier
eo., os exoccipitale
fc., fasciculus communis

gac., ganglion acusticum
gacs., ganglion acusticum, posterior saccular division
gacv., ganglion acusticum, anterior vestibular division
gen., ganglion geniculi
gg., ganglion Gasseri
ggc., general cutaneous ganglion of the seventh nerve
ggl., ganglion glossopharyngeum
gh., m. geniohyoideus
glld., ganglion lineae lateralis dorsale facialis
gllv., ganglion lineae lateralis ventrale facialis
glo., glomeruli olfactorii
gon., os goniale=angulare, auct.
gspt., ganglion r. supratemporalis X
gv., ganglion vagi
h., horny covering of jaws
hbc.1,hbc.2, first and second hypobranchial cartilages
hgl., n. hypobranchialis (including n. hypoglossus)
hhy., hypohyal cartilage
hm., tr. hyomandibularis VII
hthl., hypophysis and hypothalamus
ib.1, m. interbranchialis 1, and branches of r. jugularis VII innervating it
ib.4, m. interbranchialis 4, and branch of r. int. recurr. X innervating it
ih., m. interhyoideus, and branches of r. jugularis VII innervating it
im., m. intermandibularis
inc., internasal cartilage
infro., small nerves of mixed composition arising from the base of tr. infraorbitalis
int.-acc., r. intestino-accessorius X
int.rec., r. intestinalis recurrens X
io., m. obliquus inferior
jc., Jacobson's anastomosis
jgl., r. jugularis VII
jo., Jacobson's organ
l., lens
la., lobus auricularis
lab.1,lab.2,lab.3,lab.4, levator muscles of the branchial arches and the nerves innervating them respectively
lar.rec., m. laryngeus recurrens X
lat.d., r. lateralis dorsalis X
lat.m., r lateralis medius X
lat.v., r. lateralis ventralis X
lbr.1,lbr.2, lbr.3, levator muscles of the gills and the nerves innervating them
lg., branch of r. posttrematicus IX innervating the tongue
lhs., hyo-columellar ligament
lhy., branch of r. jugularis VII innervating m. levator hyoidei
ling., tongue
ll., lobus lineae lateralis
lm., lemniscus system of brain fibers
lvao., levator muscle of the antorbital cartilage
mao., branch of r. mandibularis V innervating the levator and retractor muscles of the antorbital cartilage
mas., masseter
mast., tendon of the masseter muscle
max., maxilla
mck., Meckel's cartilage
md., r. mandibularis V
md.1, rrm. musculares of r. md. V, innervating the temporal, masseter and pterygoid muscles
md.2, rm. malaris of r. mandibularis V
md.3, rm. labialis of r. mandibularis V
md.4, rm. mandibularis externus V
md.4a, rm. alveolaris of rm. mandibularis externus V
md.4b, a small branch of rm. md. ext. anastomosing with the preceding and extending anteriorly upon the upper lip
md.5, rm. intermandibularis V
mes., mesencephalon
mo., medulla oblongata
mth., Mauthner's fibers
mthc., Mauthner's cells
mtl.ext., r. mentalis externus VII
mtl.int., r. mentalis internus VII
mx., r. maxillaris V
mx.1, r. maxillaris V, ventral division
mx.2, r. maxillaris V, fibers with buc.2 joining the profundus-palatine anastomosis

mx.3, r. maxillaris V, fibers associated with the lateral division of r. buccalis VII (buc. 3)
nas., nose and nasal cavity
nc., nasal cartilage
ne., nasal epithelium
ngm., median nasal gland=gland of Jacobson
oc., eyeball
occ., occipital condyle
oma., m. omo-arcualis
op., r. ophthalmicus profundus V
opc., os operculare=spleniale
op.1, rm. ophthalmicus profundus minor V
op.2, rm. nasalis internus V
op.2a, small median branch of the preceding innervating the extreme tip of the snout
op.3, rm. nasalis externus V
op.4, rm. palatinus profundus V
op.4l, rm. pal. prof. V, lateral division
op.4m., rm. pal. prof. V, medial division
op-mx., union of ophthalmicus profundus and maxillaris fibers sharing in the profundus-palatine anastomosis
op-pal., anastomosis of r. ophthalmicus profundus V with r. palatinus VII
op-pal.l., lateral portion of the op-pal. anastomosis
op-pal.m., median portion of the op-pal. anastomosis
os., r. ophthalmicus superficialis VII
osph., orbitosphenoid bone and cartilage
pa., os parietale
pal., r. palatinus VII
pal.1, median division of r. palatinus VII
pal.1a, median branch of the preceding
pal.2, lateral division of r. palatinus VII
pc., palatinus caudalis
ph.i-a, a posterior pharyngeal branch of the r. int-acc. X
phe., pharyngeal epithelium
pmx., premaxilla
po., postorbital process, or cartilage
psph., parasphenoid
pt., pterygoid muscle and nerve branches innervating it
qu., quadrate cartilage
rext., m. rectus externus
rinf., m. rectus inferior
rint., m. rectus internus
rs., m. rectus superior
rtao., retractor muscle of the antorbital cartilage
sao., mm. subarcuales obliqui
sar., m. subarcualis rectus
sh., vestigial muscle to which the palatinus caudalis is related
so., m. obliquus superior
spro., nerves of mixed composition from base of supraorbital trunk
s-r., r. recurrens sensitivus X
s-r.1, first pharyngeal branch r. recurrens sensitivus
s-r.2, second pharyngeal branch r. recurrens sensitivus
spt., r. supratemporalis *X*
sp.1, first spinal nerve
sp.2, second spinal nerve
sq., os squamosum
st., stapes=columella
tbs., tractus tecto-bulbaris et spinalis
th., thymus gland
thl., thalamencephalon=interbrain
tm., m. temporalis and nerve branches innervating it
tmv., m. temporalis, inner ventral portion, with origin on orbitosphenoid
tmo., roof of medulla oblongata
to., tectum opticum
trap., m. trapezius and nerve innervating it
tr.io., truncus infraorbitalis
trm., muscles of the trunk
tro., tractus opticus
tr.so., truncus supraorbitalis
tso., tectum synoticum
vp., vomero-palatine ossicles with teeth
I., nervus olfactorius
Ia. division of n. olf. from anterior rootlets

Ip., division of n.olf. from posterior rootlets
Ijo., branch of n.olf. innervating Jacobson's organ
Ira., anterior rootlets of n. olf.
Irp., posterior rootlets of n. olf.
II., nervus opticus
IIped., hollow optic stalk
III., nervus oculomotorius
IV., nervus trochlearis
IVdcs., decussation of the trochlear nerve
V., nervus trigeminus
V ad VII., trigeminal fibers entering lateral line trunks of the facial nerve
Vm., radix motor trigemini
Vrm., radix mesencephalica trigemini
Vrml., internal lateral portion of radix mes. V going to posterior part of tectum opticum
Vrmp., posteriorly directed rootlet given off from the radix mes. V
Vrr., radices trigemini
Vsp., radix spinalis trigemini
VI., nervus abducens
VII., nervus facialis
VII ad X., lateral line anastomosis between the facial and vagus nerves
VIIc., radix communis facialis
VIIgc., radix spinalis facialis = general cutaneous root
VIIll., radices lineae lateralis facialis
VIIm., radices motores facialis
VIIm.1, r. motor primus facialis
VIIm.2, r. motor secundus facialis
VIIm.3, r. motor tertius facialis
VIIrlld., radix dorsalis lineae lateralis facialis
VIIrllv., radix ventralis lineae lateralis facialis
VIII., nervus acusticus
VIIIa., radix anterior acustici
VIIIp., radix posterior acustici
VIIIs., posterior saccular division of the auditory nerve
VIIIv. vestibular division of the auditory nerve
IX., nervus glossopharyngeus = n. branchialis primus
IXph., r. pharyngeus IX
IXprt., r. pretrematicus IX
IXpst., r. posttrematicus IX
IXr., radix glossopharyngeus
X., nervus vagus
X.1, nervus branchialis secundus
X.1,ph., r. pharyngeus of second branchial nerve
X.1,prt. r. pretrematicus of second branchial nerve
X.1,pst, r. posttrematicus of second branchial nerve
X.2, nervus branchialis tertius
X.2,ph., r. pharyngeus of third branchial nerve
X.2,prt., r. pretrematicus of third branchial nerve
X.2,pst., r. posttrematicus of third branchial nerve
X.3,prt., r. pretrematicus of fourth branchial nerve
X.4,prt., r. pretrematicus of fifth branchial nerve
X. ad VII, r. communicans vagi cum faciali
X. ad IX, communis fibers passing from vagus roots into ninth nerve along with radix spt.
Xr.2, radix secundus vagi
Xr.3, radix tertius vagi
Xrll., radix lineae lateralis vagi
Xrspt., radix supratemporalis vagi

Figures 1 and 42 to 44 are projections upon the sagittal plane of plottings from drawings made with the camera lucida. Figures 2 to 41 are drawn from sections with a camera lucida or a projection lantern. Only the minuter details are schematic. Blood vessels are omitted. After the descriptions of most of the cross-sections is given the number of the section in figure 44 which corresponds approximately (or exactly) to the section described.

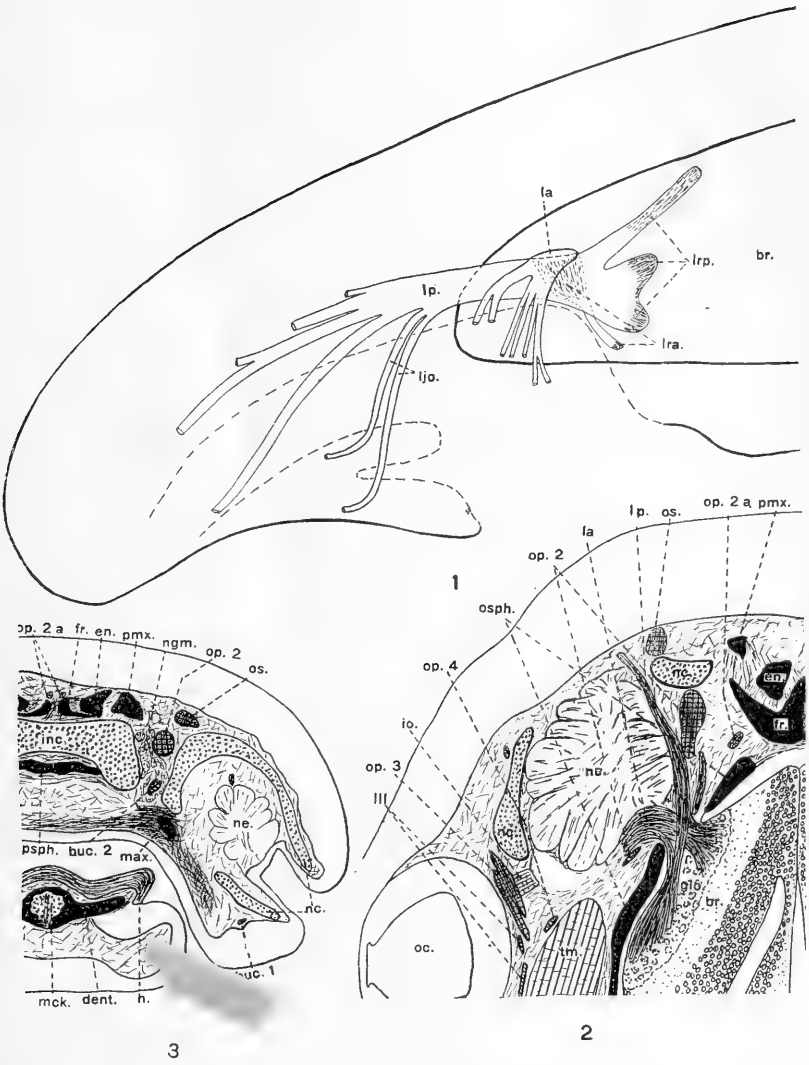

Fig. 1 A projection of the olfactory nerve upon the sagittal plane, showing its double origin and distribution. ×25.

Fig. 2 A horizontal section through the olfactory glomeruli at the level where the olfactory nerve passes out through the orbitosphenoid bone. The double origin and distribution of the nerve is shown. ×20.

Fig. 3 A cross-section through the anterior nasal region, showing chiefly skeletal features. The rudimentary maxilla is shown. Section 60. ×20.

NERVUS TERMINALIS

Herrick ('09) has described the nervus terminalis of the frog, larval and adult, tracing it from its position in the olfactory nerve to the region of the anterior commissure. McKibben ('11) describes the same nerve in Necturus, Amblystoma tigrinum Diemyctylus torosus, Amphiuma, Acris, Hyla pickeringii, Rana catesbiana, and Bufo lentiginosus americanus, and finds its relations quite like those described by Herrick in the frog. In Necturus, Diemyctylus, Amphiuma and Amblystoma he finds the nervus terminalis connected with the hypothalamus and the interpeduncular region.

In Siren a tract of fibers may be traced from the base of the anterior series of rootlets of the olfactory nerve postero-ventrally between the olfactory glomeruli and the posterior root of the olfactory nerve to the ventro-lateral border of the olfactory lobe. Thence the tract passes caudo-medially, but soon enters the substance of the brain wall, The nerve was traced posteriorly, ventral to and past the anterior commissure into the hypothalamic region, and there was lost, but its direction seemed to be toward the ansulate commissure. The material studied was not favorable to the tracing of non-medullated fibers and in consequence the ultimate distribution of the fibers of the nervus terminalis in the brain could not be determined. But in general, as far as traced, it showed a similarity to the corresponding tract in other Urodela, as described by McKibben. Peripherally the nerve was not traced farther than the base of the olfactory nerve. That it enters the anterior series of rootlets of the olfactory nerve which innervates the posterior nasal epithelium seems almost certain.

THE OPTIC AND EYE MUSCLE NERVES

Along with the rudimentary condition of the eye the optic nerve and the eye-muscle nerves have undergone a considerable degree of atrophy. All are present, however, and easily traced from external origin to distribution. Their distribution and arrangement seem to be the characteristic ones. No relations to ciliary ganglia were found.

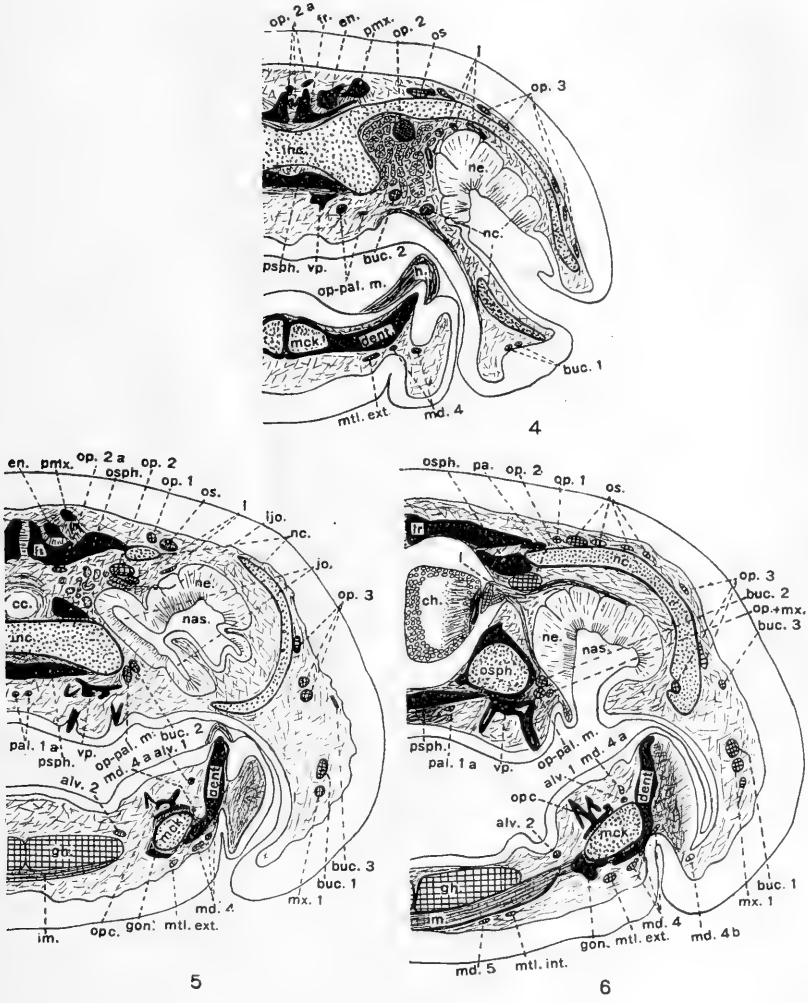

Fig. 4 A cross-section of the head through the external nostril. Section 70. ×20.

Fig. 5 A cross-section of the nasal region through Jacobson's organ. Section 115. ×20.

Fig. 6 A cross-section of the nasal region through the internal nares, and exit of the olfactory nerve. Section 140. ×20.

The optic nerve fibers enter the brain at the end of a slender hollow stalk (fig. 10). Cells of the ventral wall of this stalk extend out into the nerve trunk and form a central core that extends through the nerve even to the entrance of the nerve into

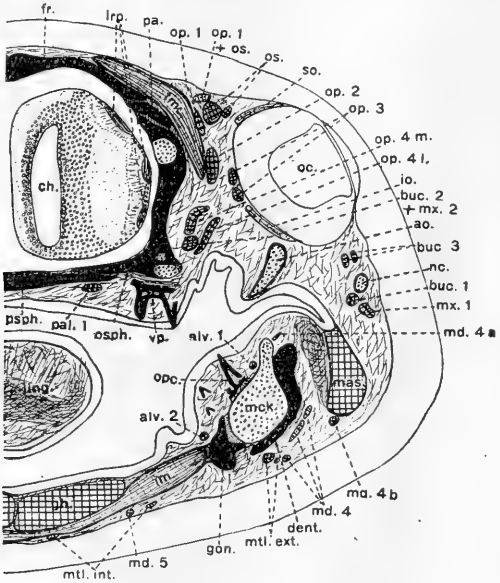

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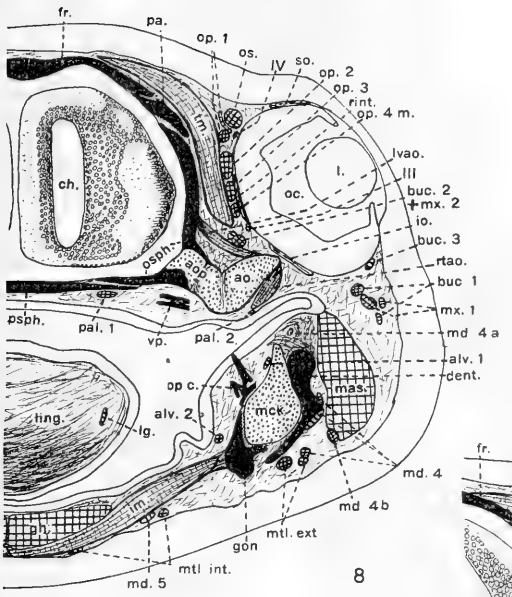
Fig. 7 A cross-section through the anterior part of the eyeball and posterior edge of the postnares. The anterior end of the antorbital cartilage (*ao*) is shown. Section 160. ×20.

the eyeball. The fibers of the optic nerve run into the brain along the posterior wall of the hollow stalk. Externally to the brain the optic nerve extends anteriorly along the medial border of the trabecula (orbito-sphenoid cartilage). Emerging from the

Fig. 8 A cross-section through the middle of the eyeball. Shows the antorbital cartilage, its attachment to the skull, and the two antorbital muscles (*rtao.* and *lvao.*). The alveolar branch of the ramus mandibularis V (*md. 4a*) is seen passing over the jaw to join the alveolaris VII (*alv. 1*). Section 170. ×20.

Fig. 9 A cross-section through the eyeball, a little posterior to the preceding. Section 175. ×20.

Fig. 10 A cross-section through the external origin of the optic nerve. All the eye-muscle nerves are shown. The trochlearis is passing out through a foramen in the parietal bone. Section 290. ×20.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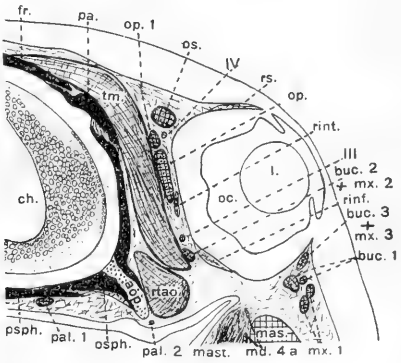
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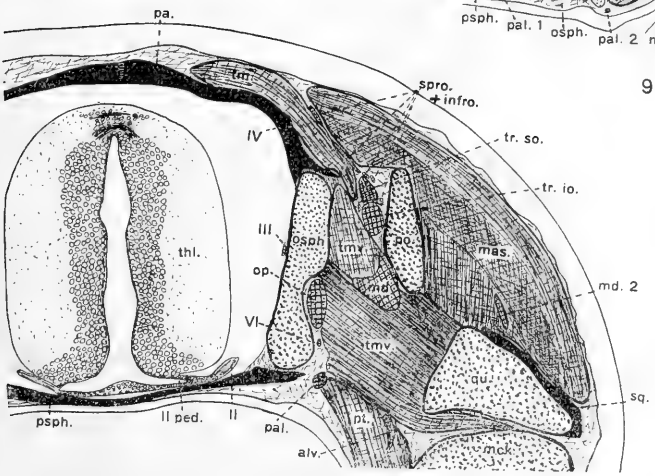
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skull through its foramen in the orbito-sphenoid bone it passes anteriorly along the inner border of the ramus ophthalmicus profundus V, and, after reaching the transverse level of the posterior border of the eyeball, passes around the ventral border of the above-mentioned ramus to its destination.

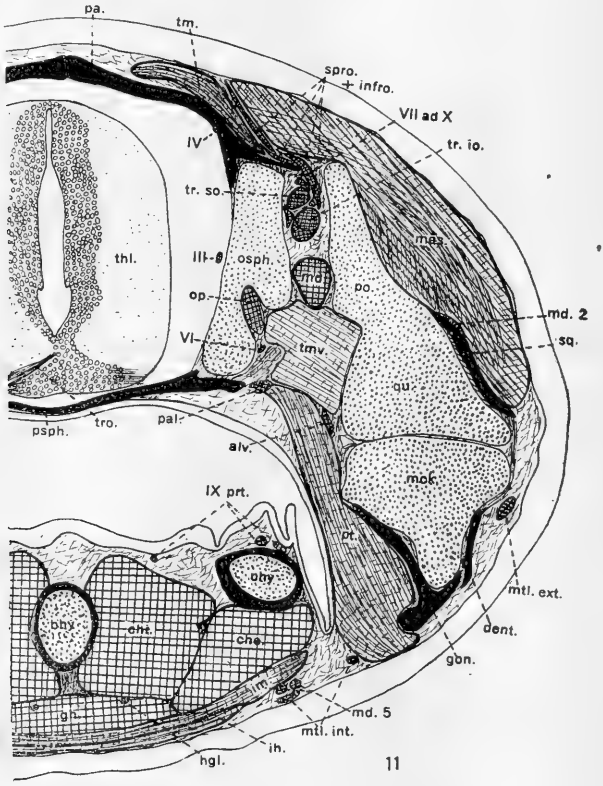
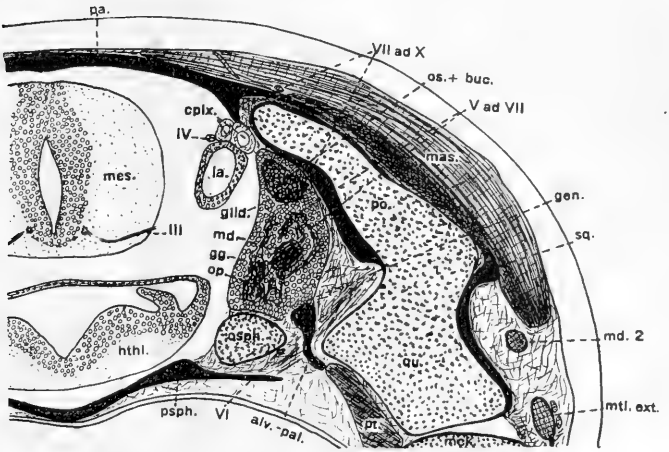


Fig. 11 A cross-section slightly posterior to the preceding. Section 300. ×20

The oculomotor nerve arises in the usual fashion from the ventral longitudinal column in the midbrain (fig. 12). On leaving the brain it runs antero-laterally out to the inner border of the orbito-sphenoid cartilage (figs. 10, 11) along which it passes to its foramen in the latter. While passing through this foramen it divides into two branches, one of which passes anteriorly dorsal to the ramus ophthalmicus profundus V to innervate the rectus



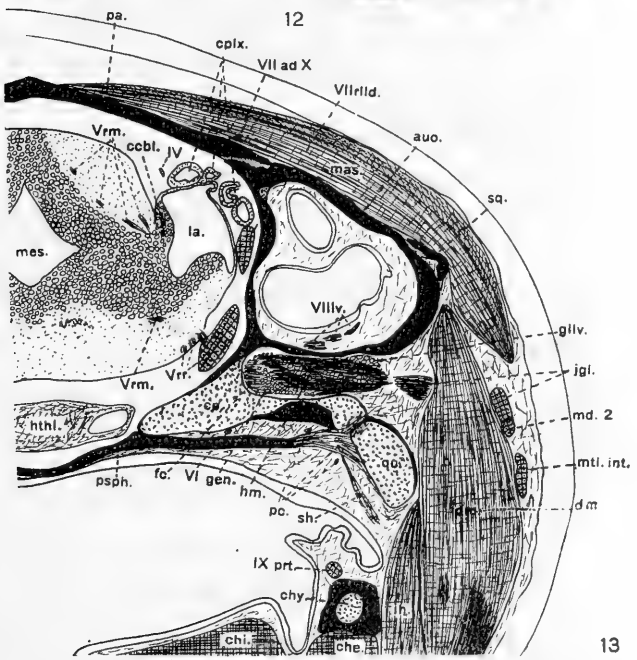
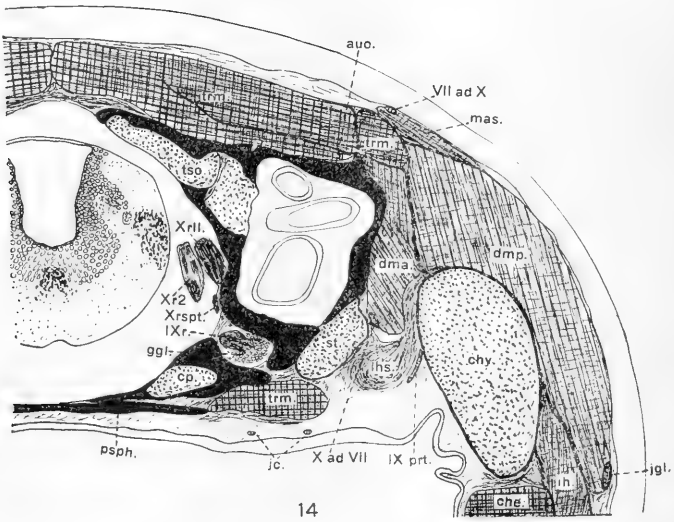


Fig. 12 A cross-section through the Gasserian and dorsal lateral line ganglia, and origin of the common trunk of the rr. palatinus and alveolaris VII. The origin of the oculomotor nerve is shown. Section 320. ×20.

Fig. 13 A cross-section through the facial canal and anterior part of the ear capsule. The vestigial muscle (*sh.*) running between the suspensory apparatus and the ceratohyal cartilage is shown. Section 345. ×20.



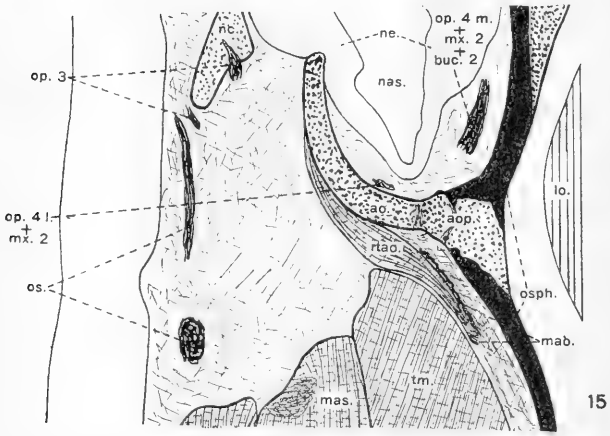
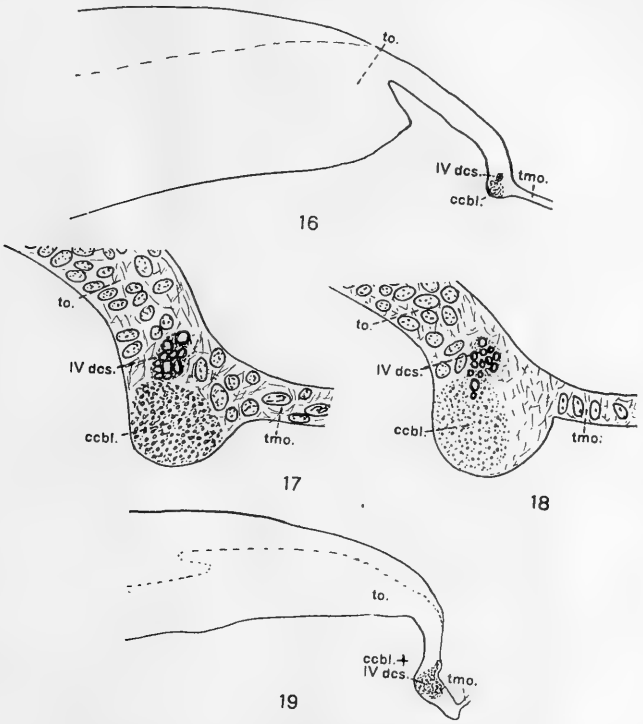


Fig. 14 A cross-section through the posterior part of the ear capsule and the roots of the IX–X nerves. Section 443. ×20.

Fig. 15 A horizontal section through the attachment of the antorbital cartilage to the skull, showing the relations of the cartilage to the choana. ×30.

superior muscle, the other branch (figs. 8,9, *III.*) runs ventrally around the ventral border of the optic nerve as the latter emerges from the skull and passes along with it until the latter enters the eyeball. It supplies the internal and inferior rectus and the internal oblique muscles.

The trochlear nerve arises externally from the extreme posterior border of the midbrain. The two trochlear nerves decussate along the dorsal border of the cerebellar commissure (decussatio veli), the 'commissura intertrigemina' of Bindewald ('11) (fig. 16, *dcs.*). Sagittal sections of the roof of the midbrain, a little



Figs. 16 to 19 Sagittal sections through the posterior part of the optic tectum, the cerebellar commissure and the decussation of the trochlear nerve.

Fig. 16 Section near the middle line. ×50.

Fig. 17 Cerebellar commissure and trochlear nerve of the same section more highly magnified. ×250.

Fig. 18 Same structures situated more laterally. ×250.

Fig. 19 Similar to figure 16, but of an adult Necturus. ×50.

at one side of the middle line, show the two nerves distinct from each other, but at the middle line their individual identity is not so evident (fig. 17). Near the point where a trochlearis passes from the brain wall of its respective side, the tract of the other

nerve may be seen emerging from the cerebellar commissure (fig. 18) and may be traced back toward its internal origin anteroventrally along and in the inner border of the commissure to the point of junction of the cerebellum and midbrain (figs. 20–27). It is at this point that the trochlearis enters the commissure. From here it may be traced ventro-medially (figs 26, 27) through the radix mesencephalica V into the region of the longitudinal ventral columns. The small size of the tract and its diffuse condition render the detection of its nucleus of origin very uncertain. Anteriorly from its emergence from the brain the nerve runs along the dorsal border of the chorioid plexus (figs. 20–27, 12, 13). It passes from the skull in a foramen in the pars orbitalis of the parietal bone (figs. 10, 11). In this passage through the parietal bone we see an agreement with the conditions found in the Urodela in general (Gaupp, '11 a), but in contrast to the condition in Amphiuma, where the nerve emerges between the orbito-sphenoid and the parietal bones. After leaving the skull the nerve passes anteriorly, closely pressed between the parietal bone and the temporal muscle, until the transverse level of the eyeball is reached, where it passes anteriorly, ventrally and laterally, through the temporal muscle, to its termination in the superior oblique muscle. For a short distance before reaching the oblique muscle the trochlearis comes into close relations with a small posteriorly-directed general cutaneous branch of the ramus ophthalmicus profundus minor V (fig. 8). In some cases the two nerves fuse, in other instances they are merely in contact. This intimate association between the trochlearis and a branch of the ramus ophthalmicus profundus is not uncommon in the Urodela. Miss Bowers ('00) reports it in Spelerpes, and Norris and Buckley ('11) find a somewhat similar arrangement in Necturus. In Amblystoma Coghill ('02) found a close association between the trochlear nerve and a twig of the ramus ophthalmicus profundus. It does not, however, appear certain that the relations in Siren in this respect correspond to those in these other Urodela.

The abducens nerve arises by three or four fibers from the ventral border of the medulla oblongata, slightly posterior to the level of the origin of the trochlearis. Its fibers can be traced in-

ternally into the immediate vicinity of the ventral longitudinal columns. After emerging from the brain the abducens passes antero-ventrally across the arachnoidal and sub-dural spaces into a small foramen in the cartilaginous base of the cranium, thence antero-laterally between the basal cartilage and the parasphenoid bone (fig. 13). A little anterior to the level of the anterior border of the ear capsule it passes laterally and dorsally around to the lateral border of the orbito-sphenoid cartilage in the connective tissue between that cartilage and the quadrate (fig. 12), taking a position ventral to the ramus ophthalmicus profundus V as the latter leaves its ganglion (fig. 11). For some distance the abducens runs anteriorly, ventral to the profundus, but at the level of the oculomotor foramen it has taken a position at the ventro-medial border of the profundus, between the latter and the orbito-sphenoid cartilage. This position it maintains until the rectus externus muscle is reached. Thus it will be seen that the abducens nerve in Siren is completely independent of all other nerves, from its origin to its termination, and thus retains what may be considered a primitive arrangement.

THE TRIGEMINAL NERVE

1. The roots of the trigeminal nerve

Three fiber tracts, or groups of rootlets enter into the composition of the fifth cranial nerve: (1) The bulk of the fibers of the fifth nerve, on entering the medulla, turn posteriorly into the tractus spinalis trigemini (*Vsp.*) which may be traced as far as the level of the third spinal nerve. In Necturus Kingsbury ('95 a, p. 189) finds a tract of fibers related to a nucleus of cells in the gray matter adjoining the spinal V tract and connected with the latter just after the latter enters the brain. Osborn ('88, p. 68) recognized a similar tract in Cryptobranchus. In Amphiuma the writer ('08, p. 530) found a corresponding tract, but very doubtfully considered it distinct from the spinal V tract. In Siren such a tract does not appear distinct from the spinal V, unless it be found in the small posteriorly directed tract (*Vrmp.*) given off from the radix mesencephalica as the latter enters the brain. (2) Motor fibers enter the trigeminal nerve

by three to six rootlets from a nucleus of large cells in the ventrolateral gray matter. (3) Radix mesencephalica trigemini. As the nature and origin of this root of the fifth nerve have received considerable attention of late (Johnston '09; Bindewald '11; van Valkenburg '11) a somewhat detailed account of its course in Siren will be given. Kingsbury ('95, p. 190) and Osborn ('88, p. 69) both describe a mesencephalic constituent of the trigeminus in Necturus and Osborn in Cryptobranchus. In Amphiuma the writer ('08, p. 531) has recognized a tract of fibers from the mesencephalic roof which appears to contribute to the fifth nerve. Sections through the entrance of the trigeminal nerve into the medulla in Amphiuma show a tract of fibers running anteromedially through the acusticum nearly to the ventral border of the gray matter, then dorsally and laterally through the posterior border of the lateral wall of the midbrain into the tectum. Of the course of the radix mesencephalica V in Necturus, Johnston ('05 a, p. 370) says:

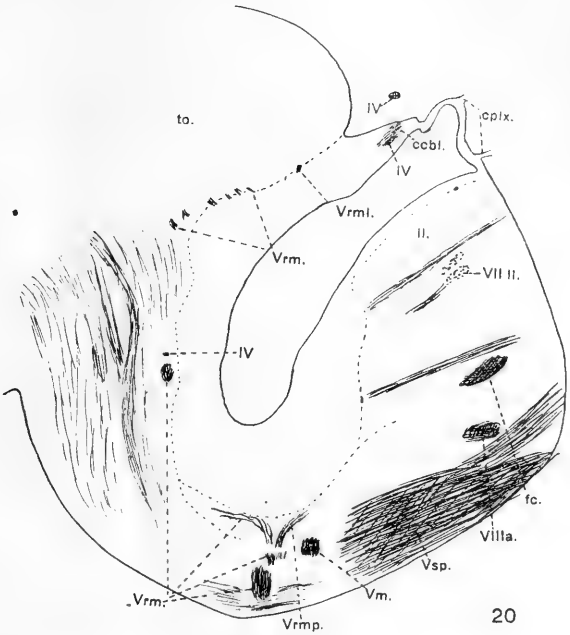
> When the junction of the cerebellum and tectum is reached the tract has collected into a compact bundle, which is imbedded in the thickness of the brain wall. The bundle now turns dorsally and divides into mesial and lateral parts. The lateral part is finer fibered. It arches up around the lateral lobe of the cerebellum close to the junction with the tectum and forms a commissure in the dorsal wall of the cerebellum which in Necturus lies forward over the tectum opticum.

On superficial examination this description seems to answer for the conditions in Siren, but after careful study the relations as above described are seen to be considerably modified. The radix mesencephalica V in Siren enters the brain (usually) in two tracts of coarse fibers (figs. 20, 24, *Vrm.*). The more posterior of these divides, one division passing anteriorly and joining the

Figs. 20 and 21 Sagittal sections through the auricular lobe of the brain, showing the internal distribution of the radix mesencephalica V, and the relations of the trochlear nerve to the radix and to the cerebellar commissure. ×50. Compare with figures 22 to 27.

Fig. 20 Shows the division of the radix into anterior and posterior parts as it enters the brain.

Fig. 21 Three sections lateral to the preceding. The relation of the radix to the tractus tecto-bulbaris et spinalis is shown. The roots of the facial nerve are seen.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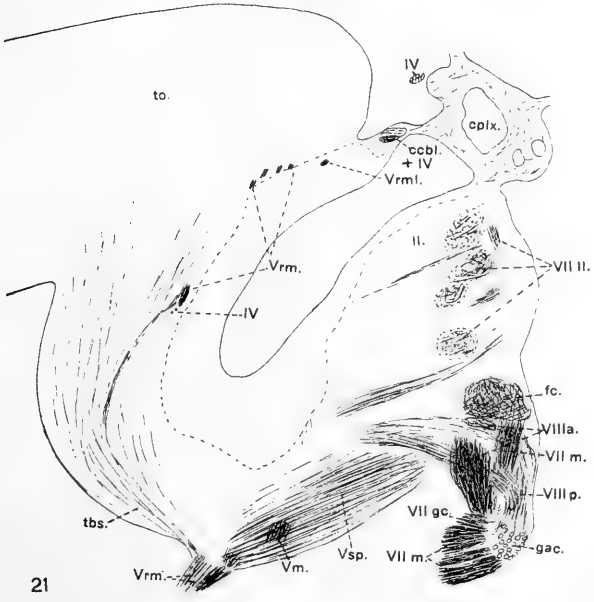
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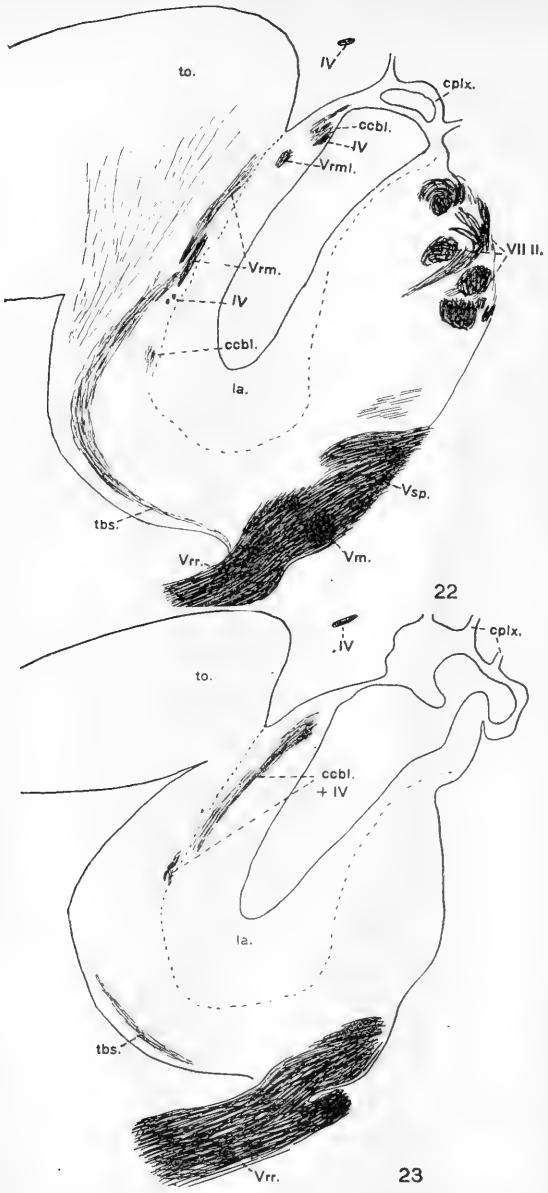
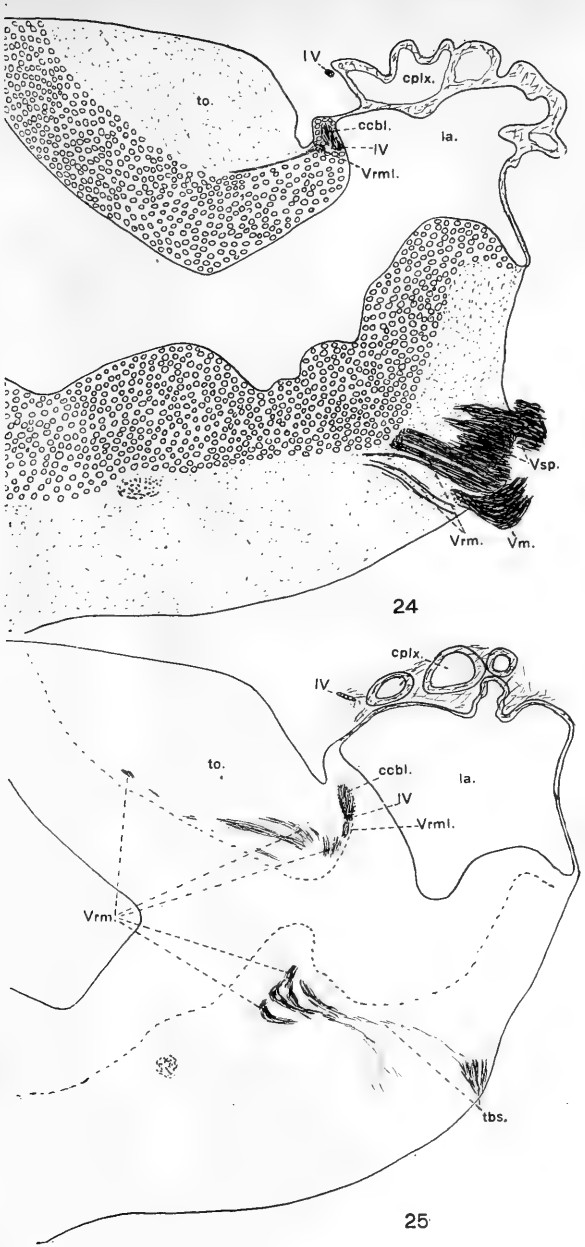


Figs. 22 and 23 Sagittal sections through the auricular lobe of the brain, showing the internal distribution of the radix mesencephalica V, and the relations of the trochlearis nerve to the radix and to the cerebellar commissure. ×50. Compare with figures 20, 21, 24 to 27.

Fig. 22 Five sections lateral to that shown in figure 20; dorsal and ventral parts of the cerebellar commissure, and lateral and medial divisions of the radix are shown.

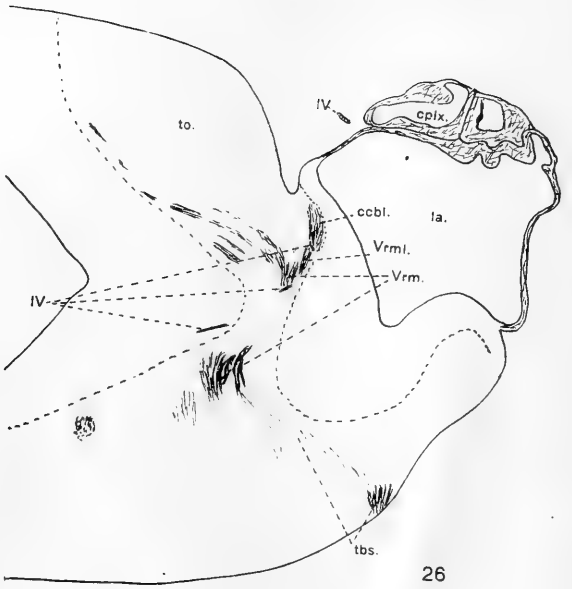
Fig. 23 Eight sections lateral to that shown in figure 20. The dorsal and ventral parts of the cerebellar commissure are united in this section.

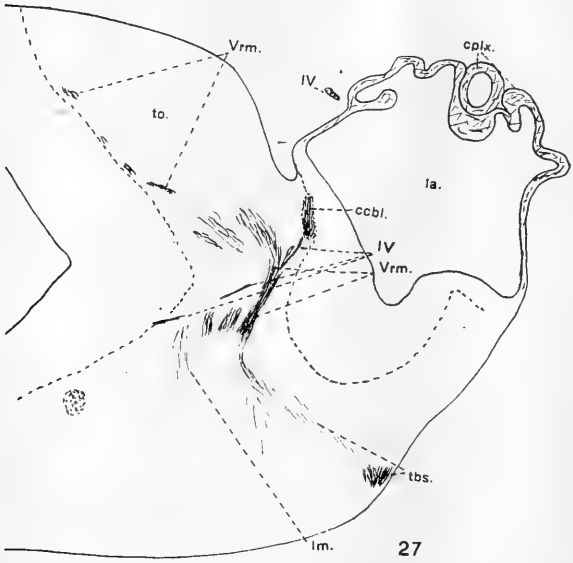


Figs. 24 and 25 Cross-sections through the auricular lobe of the brain, showing the internal distribution of the radix mesencephalica V, its relation to the cerebellar commissure and to the trochlear nerve. ×50. Compare with figures 20 to 23, 26, 27.

Fig. 24 Shows ventrally the entrance of the radix into the brain; dorsally the cerebellar commissure, the trochlearis and the lateral part of the radix (*Vrml.*).

Fig. 25 The radix is joined by fibers of the tractus tecto-bulbaris et spinalis (*tbs.*) ventrally; dorsally the radix is seen divided into medial (*Vrm.*) and lateral (*Vrml.*) portions.





Figs. 26 and 27 Cross-sections through the auricular lobe of the brain, showing the internal distribution of the radix mesencephalica V, its relation to the cerebellar commissure and the trochlear nerve. ×50.

Fig. 26 The trochlearis is seen leaving the cerebellar commissure and passing ventro-medially toward its place of internal origin.

Fig. 27 The dorsal and ventral parts of the radix are here seen to be continuous; fibers of the lemniscus system associate with the radix fibers; the trochlearis as in the preceding. Compare with figures 20 to 25.

anterior tract to form the radix mesencephalica V proper, the other passing posteriorly at the ventral border of the gray matter, (fig. 20, *Vrmp.*), and can be traced as far posteriorly as the level of the root of the seventh nerve (figs. 35–32). The writer's preparations of Necturus indicate a similar arrangement and division of the rootlets forming the radix mesencephalica V. The fibers of the radix mesencephalica V proper pass anteriorly, dorsally and medially (figs. 20–27), then posteriorly. As stated by Johnston for Necturus, "when the junction of the cerebellum and tectum is reached," the radix mesencephalica V has been joined by other groups of fibers, coarse and fine (figs. 21, 22, 25–27, *tbs.*, *lm.*), so that it becomes very difficult to distinguish accurately between it and the other fibers. The fibers of the mesencephalica V are, however, coarser and more heavily medullated. These finer and less medullated fibers that join the radix mesencephalica V come in part from a ventro-laterally situated tract of the medulla which the writer interprets as the tractus tecto-bulbaris et spinalis (*tbs.*); some come also from more centrally running tracts, possibly the lemniscus system (*lm.*). These finer fibers evidently contribute to the internal part of the radix mesencephalica V and in all probability form the lateral finer-fibered division of the latter (*Vrml.*) to be described later. As in Necturus, so in Siren, a division of the radix into medial and lateral parts occurs, the lateral being finer fibered. The lateral finer-fibered part does not, however, arch up around the lateral lobe of the cerebellum and form a commissure in the dorsal wall of the cerebellum. It divides and one part passes postero-dorsally close to and parallel with the cerebellar commissure for some distance (figs. 22, 24–26). At the point where the trochlearis enters the latter commissure (fig. 27) fibers from the lateral fine fibered part of the radix also enter the commissure, but the greater part of the lateral fine-fibered portion of the radix passes into the posterior dorsal part of the tectum (figs. 20, 24). In some instances this fine-fibered part on one side of the brain joins the cerebellar commissure so closely as to be indistinguishable from it, but on the other side is sharply distinct, contributing to it only a few fibers at the point above described. In Necturus, in the words of Johnston (l.c.),

"as the bundle ascends in the cerebellum it gives off two bundles, one near the base of the tectum and the other near the dorsal surface." In Siren the lateral fine-fibered part of the radix divides into two portions at its separation from the medial coarser fibered part, one of these divisions passing into close relation with the cerebellar fiber tract as above stated, the other extending into the tectum more directly and ventrally. In Siren as in Necturus "the mesial bundle is larger and contains the coarser fibers. It continues forward and upward into the cellular zone of the tectum in which the fibers spread widely and soon lose their sheaths" (fig. 20, *Vrm.*).

The figure given by Johnston ('06, p. 246, fig. 125) of a cross-section of the brain of Necturus through the cerebellum, in which the decussatio veli occurs in the tectum opticum, entirely distinct from the cerebellar commissure, calls for some comment. As far as the writer can learn no such condition is reported for any other Amphibian. A slender band of fibers, constituting a dorsal commissure extending across at the posterior border of the velum medullare anterius, and with which is closely associated the decussation of the trochlear nerve, is characteristic of the Amphibia, and commonly known as the 'decussatio veli.' As to the nature of the commissure there is difference of opinion. It has been generally considered a part of the cerebellum; in fact it is always associated with the latter in whatever degree the latter is developed. Bindewald ('11) states that, in the absence of a cerebellum in Proteus and Hypogeophis, this commissure is wholly concerned with the terminal nuclei of the sensory part of the trigeminal nerve, and he terms it 'commissura intertrigemina.' He asserts that in other Amphibia, while the presence of a cerebellum may involve other fiber constituents in the commissure, the latter is primarily not a cerebellar commissure. According to Johnston ('06, p. 229) the decussatio veli (of Selachians) is a commissure between the secondary gustatory sensory nuclei. In the figure of a cross-section of the brain of Necturus, referred to above, Johnston represents the decussatio veli passing, not through the velum, but through the tectum and connecting the secondary gustatory nuclei. But the cerebellar commissure is represented

as a distinct tract connecting what appear to be the terminal nuclei of the fifth nerve and in addition receiving the radix mesencephalica V. The writer is compelled to challenge the correctness of the figure by Johnston, even though it is avowedly somewhat diagramatic. In the first place the writer is unable to verify the statement (Johnston '05 a, p. 370) that in Necturus the cerebellum "lies forward over the tectum opticum." Kingsbury ('95 a, plate 11, figs. 38–41) shows the cerebellar commissure in Necturus in the characteristic Urodele position, at the posterior border of the midbrain, on the middle line, decussating ventral to the tip of the midbrain. The writer's preparations of adult and larval Necturus brains show a similar position (fig. 19). Moreover this is the only dorsal commissure in the velar region of Necturus. Decussatio veli and dorsal cerebellar commissure are not separate structures in the Urodela. In Siren the writer finds at least three distinct elements in the velar commissure: (1) The trochlear decussation; (2) A small component from the radix mesencephalica V, probably from the tractus tecto-bulbaris et spinalis. Whether this passes across the middle line or not the writer is unable to say. The indications are that it passes to the tectum on the same side on which it enters the commissure; (3) As the commissure on each side passes into the ventro-lateral portion of the auricular lobe (fig. 22) its fibers gradually vanish, presumably terminating in an end nucleus, possibly, as Bindewald suggests, in a terminal trigeminal nucleus.

From the description of Osborn ('88, p. 69) the radix mesencephalica V in Cryptobranchus is very similar to that in Necturus and Siren. He says:

> Opposite the cerebellum it splits into two bundles. One of these passes into the cerebellum, and, without crossing, enters the roof of the optic lobe at one side of the median line. The second bundle passes forward, and scatters into rays over the whole wall of the optic lobe, nearly as far forward as the posterior commissure.

The similar relations of the radix mesencephalica V in these three forms—Necturus, Cryptobranchus and Siren—make it possible to define rather exactly the relations of the tract in the Urodela. From the sensory portion of the trigeminal root the

tract passes antero-medially through the acusticum, first as a rather loose tract or separate bundles, but near the base of the cerebellum collected into a compact bundle. Here it divides into a lateral and a medial portion. The lateral becomes closely associated with the fiber tract of the cerebellum, and gives off to it fibers, that possibly have been derived from the tractus tecto-bulbaris et spinalis. But the most of the lateral fibers are distributed to the posterior part of the tectum. The medial portion of the radix passes to the more anterior part of the tectum.

A definite connection of the radix mesencephalica trigemini of Siren with a nucleus in the tectum was not established. Hence this paper adds little if anything to the information that recently has been summed up by van Valkenburg ('11) regarding such a nucleus.

2. The Gasserian ganglion

From its external origin the trigeminal nerve extends anteriorly into the Gasserian ganglion in the characteristic manner. The ganglion is wholly intra-cranial and occupies a hollow along the medial border of the anterior part of the ear capsule and the anterior cartilaginous extension of the latter, the postorbital process (fig. 12, *gg.*). The ganglion is at first somewhat triangular in cross-section with the nerve root passing along its medial border and soon penetrating it. Not far from its posterior end the Gasserian ganglion is joined on its ventro-lateral border by the geniculate ganglion of the communis component of the facialis nerve (figs. 12, 36, *gen.*), the two sometimes becoming indistinguishably fused, but in some cases remaining clearly distinct from each other. Dorsally the Gasserian ganglion soon comes into contact with the dorsal lateral line ganglion of the seventh nerve (*glld.*) and fuses with it, the two forming in cross-section a somewhat half-lens shaped mass (fig. 12). The sensory fibers spread diffusely through the ganglion in the process of becoming ganglionated, and the main divisions of the trigeminal nerve are soon outlined. The motor component (*md.*) preserves its integrity through the ganglion to the ramus mandibularis, joined by sensory fibers; ventrally in the ganglion fibers become aggregated to form the ramus oph-

thalmicus profundus (*op.*); dorsally a group of fibers (*Vad.VII*) separates to join the dorsal lateral line division of the facial nerve.

3. The ramus mandibularis V

This nerve of motor and general cutaneous fibers passes out through a foramen, common to it and the dorsal lateral line division of the seventh nerve, between the orbito-sphenoid cartilage and the postorbital process of the petrosal cartilage (figs. 10, 11, *md.*). These nerves emerge where the orbito-sphenoid cartilage becomes distinct from the petrosal and the latter is separate from the quadrate cartilage. Figure 10 shows the lateral line trunk passing dorsally out of the interval between the orbito-sphenoid and the petrosal, and the ramus mandibularis turning laterally around the ventral border of the latter. The ramus mandibularis V passes out of the cranium on the dorsal border of that portion of the temporal muscle which has its origin on the orbito-sphenoid cartilage and bone (figs. 10, 11, *tmv.*). Farther anteriorly and laterally it has a position between the temporal and masseter muscles, and farther on passes through the masseter. As it is passing out through the interval between the petrosal and the quadrate it gives off a few minute twigs to the temporal muscle. The first branch of considerable size (fig. 44, *md.1*) is given off from the ventro-medial border and supplies the temporal and pterygoid muscles and also sends a small branch (*mao.*) to two small muscles connected with the antorbital cartilage. A second branch (*md.2*), which is sometimes the first given off, supplies the masseter muscle and sends posteriorly a large general cutaneous branch, ramulus malaris, to the skin overlying the region of the angle of the jaw. A third branch, in figure 44 included in the preceding, passes dorsally between the temporal and masseter muscles and supplies the latter. The main nerve passes antero-ventrally through the masseter muscle to the dorso-lateral border of the lower jaw, where it divides. (a) One branch (*md.5*), of motor and general cutaneous fibers, the ramulus intermandibularis, enters the jaw between Meckel's cartilage and the dentary

bone, passes out almost directly ventrally between the dentary and the gonial[1] bones, and then divides anteriorly and posteriorly, its posterior division innervating the posterior portion of musculus intermandibularis posterior, the anterior part of m. interhyoideus, and the overlying skin, its anterior division supplying the anterior part of m. intermandibularis posterior, and all of m. intermandibularis anterior. It should be said, however, that an anterior intermandibular muscle is not sharply distinguishable. (b) A second branch or group of branches (*md.3*) of general cutaneous fibers, the ramulus labialis, supplies the skin overlying the jaw for some distance anterior and posterior to the level of its origin from the main nerve. (c) The main nerve (*md.4*), ramulus mandibularis externus, passing along the dorso-lateral border of the dentary, gives off numerous branches, some of which, arising not far anterior to rm. labialis, are of considerable size, supplying the skin latero-ventral to the jaw and extending far anteriorly, and also reaching antero-dorsally as far as the upper lip. In some of these dorsal branches are doubtless included portions of ramuli labiales of other Urodela. Two of these posterior branches of the mandibularis externus (*md.4a*, *md.4b*) require more definite description. One of them (*md.4a*) arises on the dorsal side of the mandibularis externus, ascends through the anterior part of the masseter muscle (figs. 8, 9), and divides into two parts, the smaller dorsal division of which passes through the tendon of the median portion of the muscle and is then joined by a small nerve arising from the ventral (*md.4b*) of the two branches from the mandibularis externus. The combined nerves pass posteriorly along the extreme lateral wall of the mouth just beneath the epithelium. The fibers either pass but a short distance or else soon lose their myelinic sheaths. The second division of the dorsal branch (*md.4a*) also enters the tendon of the masseter muscle, but farther anteriorly and close to the jaw. It passes medially through the tendon, over the jaw, and ventrally to join a branch (*alv. 1*) of the ramus alveolaris VII (figs. 8–5). Its subse-

[1] Gaupp ('11 b and c) has proposed the name 'goniale' for the bone which quite generally in the urodelous Amphibia has been designated 'angulare,' pointing out the fact that the latter is an entirely different structure.

quent course will be noticed in connection with the latter nerve. The ventral (*md.4b*) of the two branches from the ramulus mandibularis externus passes along the ventral border of the masseter muscle and gives off a branch which passes almost directly dorsally through the muscle to join the dorsal branch, as above stated, on the dorsal border of the tendon of the masseter muscle. The rest of the branch runs anteriorly along the ventral border of the muscle and anteriorly to the latter, and, passing dorsally around the angle of the mouth, is distributed to the skin of the upper lip (figs. 8–6). The remainder of the ramus mandibularis with its branches supplies the skin of the lower jaw and to some extent the mucous membrane of the mouth.

The two small muscles having their insertion on the antorbital cartilage in Siren appear to correspond to the two muscles in Amphiuma designated by the writer as levator and retractor bulbi. In Amphiuma the movements of the antorbital cartilage, to which the muscles in question are attached, seem to have definite relation to the position of the eyeball. The levator bulbi muscle raises the cartilage, pushing the eyeball dorsally and laterally; the retractor bulbi muscle pulls the cartilage ventrally and posteriorly allowing the eyeball to sink in. In Siren the antorbital cartilage appears to have no direct relation to the movements of the eyeball. It extends laterally from its attachment to the orbito-sphenoid around the posterior border of the internal naris, then curves anteriorly along the lateral border of the opening, tapering into a sharp point (figs. 7–9, 15, *ao.*). One muscle (*rtao.*) which corresponds to the retractor bulbi in Amphiuma, has its origin on the orbito-sphenoid bone (in Amphiuma on the pterygoid cartilage and maxilla) and, running anteriorly, is inserted on the ventro-lateral border of the antorbital cartilage. As in Amphiuma, its action is to pull the cartilage posteriorly and ventrally. This movement, from the relation of the cartilage to the lateral valvular fold of the postnares, will open the nostril. The other muscle (*lvao.*), which has its origin on the side of the orbito-sphenoid (as in Amphiuma) and its insertion on the postero-dorsal part of the antorbital cartilage, by its contraction raises the latter and pulls it somewhat anteriorly, thus closing the in-

ternal nostril. Fischer ('64) and later Wilder ('91) noticed the relation of the posterior of these two small muscles to the lateral valvular fold of the postnaris, but neither detected the other muscle, nor, apparently, determined the insertion of the retractor muscle on the antorbital cartilage. Anton ('11) recognizes in Siren a mechanism for the closing of the choana, but seems to overlook the presence of the antorbital muscles, and any relation of the cartilage to the regulation of the size of the opening.

As the antorbital cartilage in Siren has no close relation to the eyeball it is hardly appropriate to designate its muscles as bulbar muscles. They are here termed retractor and levator antorbitalis muscles, as they should have been designated in Amphiuma. Their origin, insertion and innervation in Siren point to their complete homology with the muscles in Amphiuma termed retractor and levator bulbi. They evidently do not correspond to any of the muscles described by Bruner (01') in the Urodela and Anura, which are concerned in the regulation of the size of the opening of the external nares.

Vaillant ('63) in describing the muscles of the head in Siren mentions "l' abducteur de la machoire superieure," a small muscle inserted in part upon a small bone believed by Cuvier to be a maxilla. The writer has not had access to the paper on Siren by Cuvier, but has consulted the reproduction of his figures by Hoffmann ('78). Fischer and Wilder have not been able to find either the muscle mentioned by Cuvier and Vaillant or the small bone upon which it was said to be inserted. Parker ('82, p. 188) mentions and figures two "small seed-like centers opposite the middle of the premaxillaries" as maxillaries, but he makes no record of muscles connected with them. The writer finds in the position described by Parker a minute ossification on each side (fig. 3, *max.*). This may, however, be larger on one side than on the other; in fact is wanting on one side in some specimens. Its minute size, and possibly complete absence on both sides in some instances, may explain the failure of some investigators to find it. It has no muscles connected with it. It may possibly represent a maxilla as Cuvier, Vaillant and Parker believed.

Huxley ('78) states, and his account is approved by Parker, that from the tip of the postorbital cartilage in Siren "a band of

fibrous tissue passes and encircling the eye, is attached to the antorbital process." If this were true it is easy to see that any movement of the antorbital cartilage might affect the position of the eyeball. The writer finds a ligamentous band extending from the postorbital cartilage over the dorsal border of the eyeball, but not directly attached to the antorbital. It is possible, however, that movements of the antorbital through its attachment to the subdermal connective tissue may modify the position of the eyeball.

That in both Amphiuma and Siren there is an antorbital process, with which are connected two muscles obviously homologous in the two species, both innervated in each species by a twig of the pterygoid branch of the ramus mandibularis V, is a fact of considerable importance. From position and innervation it may be concluded that these antorbital muscles are derivatives of the anterior portion of the pterygoid muscle. That these antorbital muscles in Amphiuma and Siren correspond to the retractor and levator bulbi muscles in other Amphibia is not probable, in the light of present knowledge. In Spelerpes (Bowers) and in Triton (Coghill) the retractor bulbi is innervated by the abducens nerve. In Amblystoma (Coghill) it is innervated by the same nerve and the levator bulbi by fibers derived from the abducens and the ophthalmicus profundus V. In Salamandra (von Plessen and Rabinovicz) the retractor bulbi is said to receive a twig from the oculomotor nerve. In the Anura (frog, Gaupp, '99) the retractor bulbi is innervated by the abducens, and the levator bulbi by a branch of the ramus maxillaris superior.

4. The ramus ophthalmicus profundus V

Wilder's ('91, p. 673) statement that the trigeminal trunks, together with the dorsal lateral line trunk of the facial nerve, after leaving their respective ganglia "pass into the same opening in the cranial wall" is correct but misleading. On leaving the ganglia the nerves in question pass into the narrowed anterior extension of the hollow in which the fused ganglia are situated. From this through a common opening the ramus mandibularis

V and the dorsal lateral line trunk pass out dorso-laterally (figs. 10, 11). The ramus ophthalmicus profundus, however, runs directly anteriorly from the Gasserian ganglion in a gap between the orbito-sphenoid cartilage medially and the base of the petrosal laterally. The coalescence of the two cartilages farther anteriorly converts the gap into a groove, inverted trough-like (fig. 11), but not a canal as described by Wilder. On its complete emergence from the cranium the ramus ophthalmicus profundus lies between the ventral part of the orbito-sphenoid cartilage medially and the origin of the anterior ventral part of the temporal muscle laterally (fig. 10). It maintains this position between the cartilage, and more anteriorly the orbitosphenoid bone, and the temporal muscle, undivided, until the optic foramen is passed. A little anterior to this foramen a large branch (fig. 9, *op.1*), the ramus ophthalmicus profundus minor of Wilder, is given off dorsally, which, with its branches, supplies the skin of the dorsal part of the head for some distance anterior and a little posterior to the eye. The trochlearis comes into close relationship with a posteriorly directed twig of this branch (fig. 8). For some distance, before entering the superior rectus muscle, the dorsal division of the oculomotor nerve runs along in close contact with, or indistinguishably fused with the dorsal border of the ramulus ophthalmicus profundus minor. When the oculomotor branch enters its muscle there is an appearance of other fibers also entering the muscle, but coming from the ramulus ophthalmicus profundus minor, possibly constituting a superior ciliary nerve. But these fibers were not traced into the eyeball.

The ramulus ophthalmicus profundus minor of Siren evidently corresponds to a large branch (*Va.*) in Spelerpes described and figured by Miss Bowers. In Spelerpes the trochlearis nerve unites with this ramulus; also from this branch what is plainly a superior ciliary nerve is given off to the eyeball. Dorsally, as in Siren, the ramulus supplies the skin of the dorsal surface, posterior and anterior to the eye and running anteriorly, parallels in a general way the course of the ramus ophthalmicus superficialis VII. Coghill describes a number of small branches of the ramus ophthalmicus profundus in Amblystoma (*o.p.V.2 and 3*)

which have a similar distribution to that of the ramulus ophthalmicus profundus minor. From one is given off the superior ciliary nerve; with another the trochlearis nerve comes into close association. In Salamandra (von Plessen and Rabinovicz), Plethodon (Dodds, Norris) and Necturus (Norris and Buckley) a ramulus ophthalmicus profundus minor evidently occurs, very similar to that in Siren. In Amphiuma this branch is included in the ramulus nasalis internus V, or is very closely connected with the latter through anastomoses. Dorsally the ramulus nasalis internus of Amphiuma anastomoses with the ramus ophthalmicus superficialis VII and has the same general distribution as the ramulus ophthalmicus profundus minor of Siren and others; ventrally it enters the nasal capsule and has the same relations as the median terminal division of the ramus ophthalmicus profundus V, ramulus nasalis internus proper, of Urodela in general. In Siren the anastomoses between the ramulus ophthalmicus profundus minor and ramus ophthalmicus superficialis VII are insignificant and confined to a few small twigs.

The main profundus nerve now passes, after giving off the ramulus ophthalmicus profundus minor, somewhat more laterally through the temporal muscle with the optic nerve on its ventromedial border. Anterior to the entrance of the optic nerve into the eyeball the ramus ophthalmicus profundus lies between the temporal muscle and the eyeball (fig. 9). While passing the eyeball the main nerve divides into the three terminal branches that seem to be characteristic of the Urodela (figs. 7, 8): (a) A large dorsal division (*op.2*), ramulus nasalis internus (not the r. nas. int. of Wilder), passes anteriorly into the nasal capsule, running along its medial dorsal border and over the olfactory nerve (fig. 6). Near the level of the anterior end of the brain it gives off a small branch (*op.2a*) which passes medially to take a position close to the middle line with its fellow of the opposite side, between the two frontal bones (fig. 5), farther anteriorly, dorsal to the anterior ends of the frontals (figs 3, 4), and is finally distributed to the skin of the extreme antero-dorsal part of the snout. In Amphiuma a small nerve with a similar distribution leaves the nasalis internus just before the latter enters the nasal

capsule. The ramulus nasalis internus emerges from the nasal capsule on the median border of the latter, in the midst of the internasal or Jacobson's gland, between the capsule and the internasal cartilage (fig. 3). Here it breaks up into many small twigs (nervi ophthalmici anteriores of Wilder) which innervate the skin at the side of the snout, anterior to the nasal capsule. (b) A second branch (*op. 3*), ramulus nasalis externus, runs laterally around, pressed close against the anterior wall of the eyeball (figs. 2, 7, 8). This may arise as a single branch which soon divides, or as two branches, distinct from each other from their origin, approximately equal in size, or very unequal. On reaching the posterior border of the nasal capsule the nasalis externus has divided into three or four divisions, one of which enters the capsule and runs along the inner border of the lateral wing, as described by Wilder, later emerging through the cartilage (figs 6, 15) to be distributed with the other branches to the skin, lateral to the nasal capsule. (c) The ventral of the three terminal branches of the ramus ophthalmicus profundus (*op.4*) is the one that anastomoses with the ramus palatinus VII (figs. 7, 8). The ophthalmic-palatine anastomosis in Siren is fundamentally on the same plan as that described in Amblystoma by Coghill ('02, pp. 223, 229) and in Amphiuma by the writer ('08, p. 535). The two nerves, the branch of the ramus ophthalmicus profundus and the ramus palatinus, on approaching each other, divide, each into two branches, which unite in pairs in such a way that the resulting nerves each contain profundus and palatine fibers, One of these two nerves is lateral and runs along the lateral border of the nasal epithelium; the other extends anteriorly, medial to the nasal epithelium.

In Siren the profundus-palatine anastomosis is modified, first by an anastomosis between the profundus constituent (*op.4*) and a branch (*buc.2* + *mx.2*) of the infra-orbital trunk of the facialis of lateralis and general cutaneous composition; second by the fact that the palatine divides into lateral and medial portions far posteriorly, shortly after leaving the trunk common to it and the ramus alveolaris VII, by which it has emerged from the skull; third by the introduction from the infraorbital trunk of maxillaris fibers.

In passing antero-ventrally to the palatine anastomosis the profundus branch fuses for a short distance with a division, composed of lateral line and general cutaneous fibers (*buc.2* +*mx.2*), from the infraorbital trunk of the facial nerve as mentioned above (figs. 8, 7). The lateral line fibers of the anastomosis soon separate as a distinct nerve (*buc.2*) which, running anteriorly along the medial border of the nasal capsule at the lateral edge of the orbito-sphenoid cartilage and, more anteriorly, the internasal cartilage, ventral to the ramus nasalis internus, emerges from the nasal capsule along with the latter nerve, and is distributed to neuromasts, mostly at the side of the tip of the snout (figs. 6–3). This is the nerve termed by Wilder ramus nasalis internus. We see here an illustration of the errors that result from the comparing and homologizing of nerves according to their course and general distribution, regardless of their composition. The ramus nasalis internus V is a general cutaneous branch of the ramus ophthalmicus profundus V; the nerve in Siren, designated by Wilder as nasalis internus, is wholly lateral line, and derived from the ramus buccalis VII. It seems to correspond to a great extent in its terminal distribution to that branch in Amphiuma designated by the writer as *buc.(2)*. The latter, however, runs along the lateral border of the nasal epithelium and comes into relation with a profundus branch that has nothing to do with the palatine anastomosis.

When the lateral line constituent leaves the anastomosis, the general cutaneous portion from the infra-orbital trunk is left behind with the profundus fibers. To form the profundus-palatine anastomosis there is given off from the profundus branch a twig (fig. 7, *op.4l.*) that passes ventro-laterally and finally somewhat posteriorly between the posterior wall of the postnaris and the anterior border of the base of the antorbital cartilage, through a small foramen in the latter, and on the ventro-lateral border of the latter in close relation with the lateral palatine (*pal.2*) divides and extends anteriorly and posteriorly, in part in union with, in part parallel with the lateral palatine. Most of the general cutaneous fibers in the lateral anastomosis appear to run posteriorly. The lateral palatine can be traced but a com-

paratively short distance anterior to the anastomosis. The remaining general cutaneous fibers, profundus and maxillaris, unite with the medial palatine (*pal.1*) in two or three anastomoses. As the medial palatine has already divided it is only with the lateral of its two divisions that these unions take place. The exact composition of the general cutaneous constituents of the profundus-palatine anastomosis usually cannot be determined with accuracy, but in one instance where the lateral profundus constituent arose from the ramulus nasalis externus it was possible to see that the maxillaris element from the infra-orbital trunk passed into both the lateral and the medial resulting nerves. This exceptional origin of the lateral profundus constituent from the ramulus nasalis externus is reported by Coghill ('06, p. 254) in Triton.

An anastomosing of branches of the ramus buccalis VII and the ramus ophthalmicus profundus V is not uncommon, it would seem, in the Urodela. In Amphiuma, as shown by Wilder and the writer, the buccalis anastomoses with two branches of the ramus ophthalmicus profundus, (*op.(4)* and *op.(5)*) which may be considered as collectively representing the ramulus nasalis externus V. In Cryptobranchus (Menopoma) alleghaniensis Wilder describes similar anastomoses which are evidently between the buccalis and branches of the ophthalmicus profundus, similar to those in Amphiuma. In C. japonicus (Osawa '02) the anastomoses appear to be almost identical with those in C. alleghaniensis. It is possible that this particular kind of anastomosing may be peculiar to the derotreme Urodela, for in Siren and in Triton the relations are somewhat different. A study should be made of both species of Cryptobranchus from the standpoint of nerve components.

5. Trigeminal fibers entering the facial nerve

Groups of fibers (*V ad VII*), after becoming ganglionated in the Gasserian ganglion, may be traced dorsally into the great lateral line trunks which pass anteriorly out of the dorsal lateral line ganglion (fig. 12). Their subsequent course will be noticed in the description of the rami of the facial nerve.

Although the cranial nerve components have been worked out in but few Urodele amphibians, yet the uniformity in the branching of the peripheral portions of the trigeminal nerve makes it possible in our present state of knowledge to represent this arrangement by a definite scheme.

Nervus trigeminus

A. Radices:
  I. Radix spinalis
  II. Radix mesencephalica
  III. Radix motor
B. Ganglia:
  Ganglion gasseri (cum g. trigemini)
C. Rami:
  I. Ramus ophthalmicus profundus
    1. Ramulus ophthalmicus profundus minor
    2. Ramulus nasalis internus
    3. Ramulus nasalis externus
    4. Ramulus palatinus profundus
    5. Ramuli ciliares
  II. Ramus mandibularis
    1. Ramuli musculares
    2. Ramulus malaris
    3. Ramulus labialis
    4. Ramulus mandibularis externus
      4a. Rm. alveolaris
    5. Ramulus intermandibularis
  III. Ramus maxillaris (with r. buccalis VII forming truncus infraorbitalis)
  IV. Ramus oticus (?) (with r. ophthalmicus superficialis VII forming truncus supraorbitalis)

THE FACIAL AND AUDITORY NERVES

1. Roots of the facial and auditory nerves

The fibers of this complex arise by two groups of rootlets. The more dorsal group, (figs. 21, 22, 30, 31, *VII ll.*), which comprises the lateral line fibers of the seventh nerve, arises by three rootlets. The dorsal of these three rootlets enters what may be termed the lateral line lobe (*ll.*, figs. 20, 21) of the medulla oblongata. Its fibers, on entering, lose their sheaths so soon that their destination can be surmised only. They appear to end almost directly opposite the point of entrance, turning slightly posteriorly. The other two rootlets enter the brain somewhat

posterior to the first, and ventral to it, into that part of the brain lying between the two fiber tracts termed 'a' and 'b' by Kingsbury in Necturus. On entering the brain the rootlets turn posteriorly, to be traced a short distance only.

In the second and ventral group of rootlets are the acoustic and the motor and communis facial fibers, together with a rootlet considered by the writer as general cutaneous.

The origin of the motor component may be described in the words of Kingsbury ('95 a, p. 182) for Necturus:

> At about the exit of the tenth nerve, myelinic fibers begin to appear in the cinerea dorsad of a nidus of large cells in the ventro-lateral portion of the floor. From here to slightly cephalad of IX[1] [*Xrll.*, Siren], fibers spring continuously from this region and unite to form a close bundle which passes mesad to lie dorsad of the posterior longitudinal bundle. . . . .In this position they run cephalad to just caudad of the exit of the eighth nerve where they turn laterad and ventrad in two (or three) bundles, to leave the oblongata as the motor roots of the seventh nerve.

In Siren there are commonly three rootlets, one (*VIIm.3*) emerging through the roots of the eighth nerve, or through the communis component of the seventh nerve, a second (*VIIm.2*) through the spinal V tract, and a third larger one (*VIIm.1*) ventral to the spinal V tract (figs. 28–31). At the point where these motor tracts turn laterally from the posterior longitudinal columns, Mauthner's (*mth.*) fibers decussate and, running along the ventral border of the motor fibers, give an appearance of passing out into the seventh nerve (figs 30, 31). They are not to be traced farther than the immediate vicinity of certain giant cells (*mthc.*) lying on the ventral border of the gray matter in this region.

The motor root of the facialis is the one described by Osborn ('88, p. 66) in Cryptobranchus and Siren as the third and fourth roots of the eighth nerve. According to him it arises in both cases from the "posterior longitudinal fasciculus," which statement is, of course, an error.

The communis component of the facialis (*VIIc.*), entering and composing the fasciculus communis (*fc.*) at this level, is finer fibered than the other constituents of the VII–VIII complex, but densely medullated.

In Amphiuma the writer ('08, p. 537) described four groups of auditory fibers:

> (1) Medium and large fibers that pass posteriorly into the spinal VIII tract; (2) medium fibers that pass anteriorly into the so-called (incorrectly) 'descending VIII' tract; (3) medium and small fibers that pass into 'tract b'; (4) large fibers forming a tract at first distinct from (1) but posteriorly passing into the spinal VIII tract or into very close proximity to it.

In Necturus Kingsbury (l. c., p. 181) recognized three groups of fibers: two corresponding to (1) and (2) in Amphiuma and a third group ending almost immediately on entering the brain in close proximity to certain large cells. In Cryptobranchus Osborn (l. c.) mentions four roots of the eighth nerve, but, as Kingsbury has pointed out, only one of these, VIII,[2] contains acoustic fibers, and is really the auditory nerve.

In Siren the auditory nerve consists of two groups of fibers: (1) a large fibered anterior ventral tract turning posteriorly into the spinal VIII tract (figs. 21, 28–30, *VIIIa.*); (2) a tract of medium sized fibers, dorsal and posterior (*VIIIp.*), which passes anteriorly within the brain (fig. 21). These two groups of fibers correspond to (1) and (2) in Amphiuma. A group of fibers passing into 'tract' b does not appear distinct from (2) in Siren. Group (4) of Amphiuma is evidently contained in (1) of Siren.

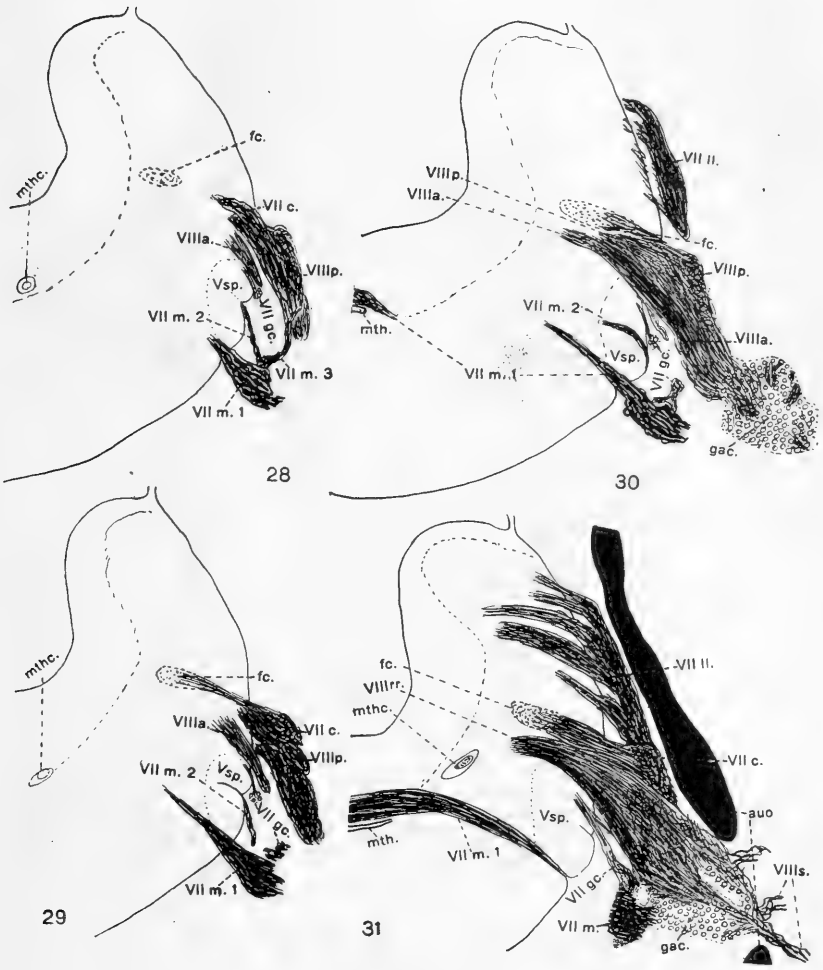
2. The general cutaneous component of the facial nerve

The presence of general cutaneous fibers in the seventh nerve of Amphibia has been commonly recognized, but no distinct root of the facialis entering the spinal V tract has been found. It is generally assumed by students of nerve components that any and all such fibers find their way into the seventh nerve from the tenth nerve through the ramus communicans cum faciali. To the writer's knowledge no one has found general cutaneous fibers in the roots of the facial nerve. Any suggestion, therefore, of the occurrence of such fibers challenges contradiction and calls for a most critical examination of the so-called evidence.

Drüner ('04, p. 661) has called attention to the small size of the ramus communicans in Siren. The writer has found in one

instance that on one side it contains approximately fifteen fibers, and on the other side about thirty fibers. In both cases the corresponding ramus jugularis VII contained many more general cutaneous fibers. The conclusion follows: either the contributed fibers increase in number through division or there is some additional source for them other than the ramus communicans. In the specimen above mentioned on the side where the ramus communicans contains only fifteen fibers the ramus jugularis VII receives a general cutaneous anastomosis from the ramulus malaris of the ramus mandibularis V (fig. 13, *md.2*), but on the other side no such anastomosis occurs. In one specimen the ramus communicans is wanting on one side, yet general cutaneous fibers in characteristic abundance occur in the ramus jugularis VII, without any anastomosing with the ramus mandibularis V.

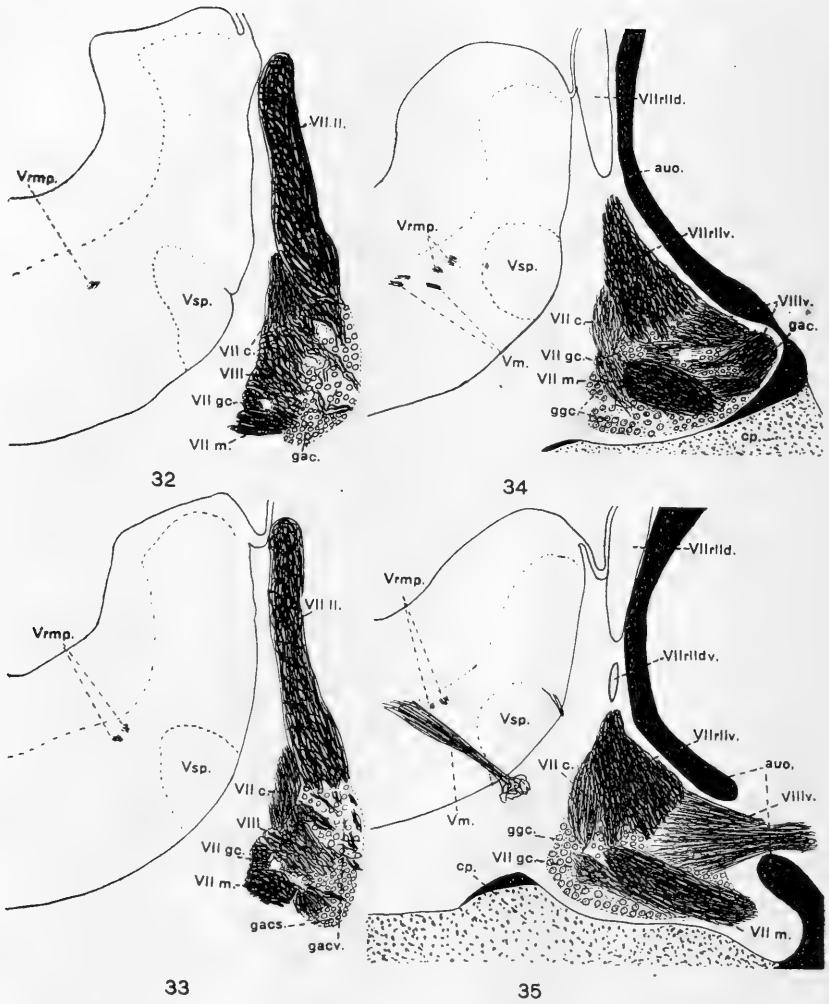
In specimens where there is just the right degree of staining attained, so that the motor, general cutaneous, communis and lateral line components are differentiated by their differences in color intensity, there is seen in the ramus jugularis VII, along with the very dark motor fibers, two lighter colored bands, one of which may be traced into the ramus communicans and the other into the main trunk of the facial nerve. At the brain wall this lighter colored band occupies a position dorsal to the motor portion of the nerve (figs. 30-33, *VIIgc.*). As has been stated the motor portion of the seventh nerve emerges from the brain in (usually) three rootlets (figs. 28–31). There is a large rootlet passing out ventral to the spinal V tract; a smaller one coming out through the same tract; and a third which passes out in the auditory (or communis) rootlets. The two smaller rootlets combine to form (apparently) a part of the lighter mass of fibers. At the point where the second rootlet (*VIIm.2*) emerges from the brain (or in some cases near it but sharply distinct from it) a band of fibers from this lighter area enters the spinal V tract (figs. 28–31, *VIIgc.*). In some instances it is impossible to differentiate between the motor and general cutaneous fibers. Tracing the tract in question externally from the brain wall, as it passes anteriorly and peripherally it shifts medially and ventrally



Figs. 28 to 31 Cross sections through the origin and roots of the acusticofacial complex. ×50. Compare with figures 32 to 35.

Fig. 28 Section through the posterior part of the motor root of the facial nerve and the posterior auditory root; shows the three motor rootlets and the general cutaneous rootlet of the facial (*VII gc.*).

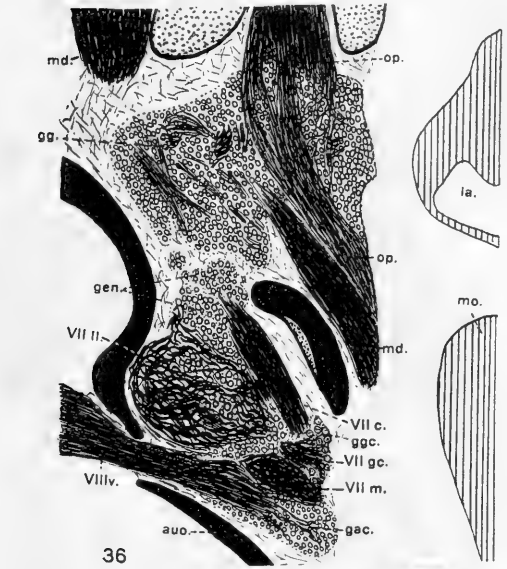
Figs. 29 to 31 The same, one, two and four sections respectively anterior to that shown in figure 28.



Figs. 32 to 35 Cross-sections through the origin and roots of the acusticofacial complex. ×50. Compare with figures 28 to 31.

Figs. 32 and 33 Eleven and fifteen sections respectively anterior to that shown in figure 28. The VII-VIII nerve roots and posterior part of the auditory ganglion are seen compacted together, but most of the components are easily distinguishable. In these and the two following figures the posterior continuation of the radix mesencephalica V (*Vrmp.*) is shown.

Figs. 34 and 35 The twenty-fourth and twenty-seventh sections respectively anterior to that shown in figure 28. The ganglion belonging to the general cutaneous component (*ggc.*) is shown; the vestibular portion of the auditory nerve enters the ear capsule.



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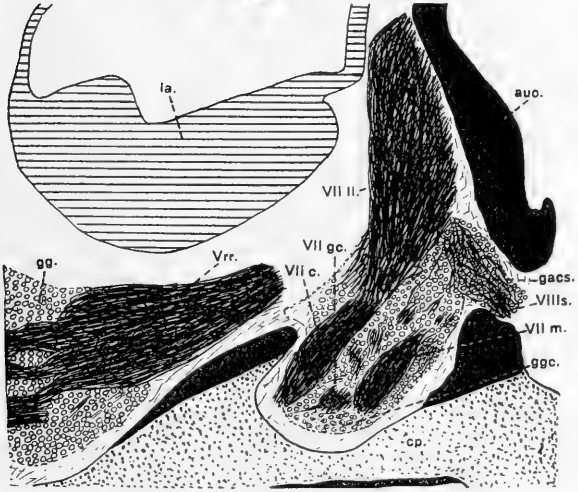

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Fig. 36 Horizontal section through the V-VII-VIII ganglionic mass. To show the relative situation of the respective ganglia. The general cutaneous component and ganglion of the facial nerve are shown. ×50.

Fig. 37 Sagittal section through the V-VII-VIII ganglionic mass. ×50.

in reference to the motor root, until it lies on the ventral border of the latter (figs. 34, 35). At this point there is a small mass of ganglion cells with which these lighter colored fibers appear to be related (*VIIggc.*). These ganglion cells are very sharply distinguishable from the neighboring auditory ganglion cells in size and nuclear characteristics. With van Giesen's picro-fuchsin they stain a deeper ruddy color than the other ganglion cells. They are grouped together in a peculiar fashion, different from the other ganglion cells of the geniculate, Gasserian or lateral line ganglion. They disappear as soon as the lighter tract closely joins the other fibers to enter the facial canal. Figures 36 and 37 show some of the relations of this tract to other portions of the facial nerve roots and ganglion as seen in horizontal and sagittal sections.

3. The ganglia of the VII–VIII complex

From the points of exit of its fibers from the brain, the root of the lateral line portion of the seventh nerve extends anteriorly as a flattened band closely compressed between the brain and the ear capsule (figs. 13, 30–35). The exact destination of the fibers of the several rootlets could not be determined with the accuracy that was possible in Amphiuma. The root divides into a dorsal portion (*VII rlld.*), continuing anteriorly into the dorsal lateral line ganglion, and a ventral portion (*VII rllv.*) which descends to join the auditory and ventral facial roots. From this ventral lateral line portion, shortly after the division, one or two small bands of fibers ascend to join the dorsal division (fig. 35, *VII rlldv.*). The dorsal lateral line ganglion (*glld.*), as previously stated, is situated dorsal to and confluent with the Gasserian ganglion. The auditory ganglion (*gac.*) has the shape and relationships which seem characteristic of Urodela. There is a posterior, somewhat cylindrical part (figs. 30, 31, 37) which passes into the ear capsule, supplying the sacculus, posterior semicircular canal, lagena and the macula neglecta. The fibers of the posterior dorsal rootlet, (2) above, of the auditory nerve seem to belong chiefly to this part of the ganglion. The anterior

portion of the auditory ganglion lies within the skull (figs. 32–36). The cells of the posterior part are small and of one size; the cells of the anterior part are of two kinds, one kind appearing identical with those of the posterior part and situated ventrally, the other larger-celled portion being dorsal. These two kinds of cells in the anterior part of the ganglion evidently correspond to the medium and large sized fibers which innervate the utriculus and the anterior and horizontal canals. The fibers of this anterior part of the ganglion seem to correspond to the anterior ventral rootlet of the auditory nerve.

The ventral division of the lateral line fibers of the facial nerve, after giving off the small constituent which rejoins the dorsal division, as described above, descends to the dorsal border of the anterior portion of the auditory ganglion, and at the anterior end of the latter, as the anterior division of the auditory nerve enters the ear capsule, joins the motor and general cutaneous constituents of the facial nerve and with the latter, entering the facial canal in the petrosal cartilage, forms the truncus hyomandibularis VII, and passes into the ventral lateral line ganglion (fig.13). Anteriorly the auditory ganglion becomes confluent with the ventral lateral line ganglion. The latter (*gllv.*) is an elongate, almost cylindrical mass of cells on the truncus hyomandibularis, and is confined to the peculiar canal in the petrosal through which the truncus emerges from the skull.

The geniculate ganglion (*gen.*) extends anteriorly from the medial anterior end of the ventral lateral line ganglion in an extension of the facial canal which re-enters the cranial cavity where the geniculate ganglion becomes confluent with the Gasserian ganglion, as already described, although usually the two ganglia are easily differentiated from each other (fig. 36).

These six ganglia, dorsal lateral line, Gasserian, geniculate, general cutaneous VII, ventral lateral line and auditory, make a continuous ganglionic mass in which often there is difficulty in distinguishing the individual ganglia. The geniculate ganglion in Siren is much more distinct than in other Urodela hitherto described.

4. The truncus supraorbitalis

Passing anteriorly from the dorsal lateral line ganglion the lateral line fibers, already separated into a dorsal and a ventral group (fig. 11), are joined by general cutaneous fibers from the Gasserian ganglion (the third group of fibers mentioned under the head of the trigeminal nerve). It is questionable whether any general cutaneous fibers should be considered as a constituent part of the dorsal or supraorbital division in Siren. The general cutaneous branches which appear to spring from this nerve, in reality come from the fibers associated with the ventral or infraorbital trunk, or perhaps it were better stated that they pass directly from the Gasserian ganglion dorsally, right and left, around the lateral line trunks, some of them associating with lateral line branches of the latter nerves. Close to the ganglion all the tracts of fibers and nerves are so closely associated that only by most careful examination and comparison of the condition in different individuals can the true relations be determined. Three or four branches containing general cutaneous fibers arise seemingly from the supraorbital trunk in the vicinity of the ganglion. In Amphiuma similar branches of general cutaneous and lateral line composition, arising from the base of the supraorbital trunk, were considered by the writer as equivalent to the ramus oticus of fishes. In both species the lateral line constituent of these branches supplies the posterior part of the supraorbital series of neuromasts, and in Amphiuma the extreme dorsal end of the infraorbital series. In Siren few if any of the infraorbital series of neuromasts are innervated from this group of nerves. The general cutaneous portions of these nerves supply the skin in the regions of the neuromasts innervated by their lateral line components. The exact origin of the small nerves from the base of the supraorbital trunk, and from the infraorbital as well, is extremely variable. Anteriorly from this region of the supraorbital near the ganglion, where these small nerves already mentioned are given off, the nerve is exclusively lateral line in composition, and therefore represents the ramus ophthalmicus superficialis VII (*os.*). It continues anteriorly between the pterygoid and masseter muscles

at the inner dorsal border of the anterior extension of the petrosal cartilage, the postorbital process (fig. 10), thence at the lateral border of the temporal muscle, dorso-medial to the eyeball and dorsal to the ramus ophthalmicus profundus V (figs. 9, 8, 7). Reaching the nasal capsule it runs along its dorsal border (figs. 6–3), and breaks up into numerous small branches in the snout, throughout its entire length supplying the supraorbital series of neuromasts.

5. The truncus infraorbitalis

Of the general cutaneous fibers from the Gasserian ganglion that ally themselves with the dorsal lateral line nerves the greater part become associated with the infraorbital trunk, if, indeed, we may not regard them all as primarily belonging to this nerve. As stated above, there arise from the bases of the supraorbital and infraorbital trunks in variable fashion three or four groups of small nerves of general cutaneous fibers, commonly associated with lateral line fibers which supply the posterior part of the supraorbital series of neuromasts together with the skin of the same region. From this region, near the ganglion, the infraorbital trunk runs anteriorly, at first ventral to and close to the supraorbital, giving off only one more branch, which however, follows and remains pressed closely against the main nerve, until a region a short distance posterior to the eye is reached. From this point to the posterior border of the eyeball numerous general cutaneous and lateral line branches are given off, including the one just mentioned which has arisen far posteriorly. One of these branches, larger than the others, consisting of lateral line and general cutaneous fibers (*buc.2* + *mx.2*), and forming the anastomosis, already described, with the ophthalmicus profundus component (*op.4*) of the palatine anastomosis, passes into the nasal capsule, ventral to the ramulus nasalis internus V (figs 9–7), and, after giving off the general cutaneous constituent, supplies the infraorbital series of neuromasts at the sides of the tip of the snout (figs 6–3, *buc.2*). This anastomosing branch sometimes arises as two (fig. 8).

The main trunk passes around the ventro-lateral border of the eyeball, exhibiting three main branches: (1) the ramus buccalis proper (*buc. 1*) which extends antero-ventrally, ventral to and grazing the extreme posterior tip of the lateral wing of the nasal capsule, thence along the ventro-lateral border of the upper lip, ending just ventral to the nostril, supplying the infraorbital series of neuromasts along the upper lip as far as the prenaris (figs. 9–3); (2) more or less distinct from (1) and running parallel with it, sometimes dorsal and in others cases ventral to it, nearly to its extreme anterior end, is a general cutaneous branch (*mx.1*), the ramus maxillaris V, which innervates the skin of the upper lip and that ventral to the eye; (3) a smaller nerve of general cutaneous and lateral line composition (*buc.3* + *mx.3*), running around and close against the eyeball, passes nearly straight anteriorly lateral to the lower edge of the wing of the nasal capsule, ending immediately dorsal to the nostril. Its lateral line fibers innervate neuromasts of the infraorbital series which extend from the ventral border of the eye anteriorly, ending just over the nostril.

6. The ramus mentalis internus VII

The hyomandibular trunk (of lateral line, general cutaneous and visceral motor fibers) on emerging from the facial canal immediately divides into two main portions, a posterior ramus jugularis and an anterior lateral line ramus mentalis. The latter almost immediately divides into the two characteristic rami, mentalis internus and externus. The ramus mentalis internus passes posteriorly, laterally and ventrally around the lateral border of the depressor mandibulae muscle to the lower medial border of the lower jaw, thence anteriorly, at first ventral to the insertion of the pterygoid muscle, soon dividing into two portions, the medial of which shifts medially to the ventral border of the interhyoideus muscle, while the lateral division runs along the ventral medial border of the pterygoid muscle both finally passing along the ventral surface of the intermandibularis muscle (figs. 13, 11, 8, 6). The ramus mentalis internus innervates the neuromasts which seem to correspond to the typical amphibian gular series as described by Kingsbury ('95 b), situated on the side and ventral

surface of the head. Posteriorly these neuromasts in a surface view are not distinguishable from the postorbital series, but from their innervation it is seen that the two series overlap. Wilder seems to have overlooked this ramus.

7. Ramus mentalis externus VII

From the point of separation of the rami mentalis internus and externus the latter passes anteriorly laterally and ventrally over the lateral border of the quadrate cartilage and squamosal bone to the lower jaw, along the ventro-lateral border of which it runs to its final termination (figs. 12, 11, 8, 4). It innervates the oral series of neuromasts. From the main trunk of the mentalis and from the base of the ramus mentalis externus there arise five or six small nerves which supply neuromasts widely scattered over the side of the head, posteriorly from a short distance anterior to the gills nearly to the eye anteriorly, encroaching dorsally upon the territory of the occipital, supraorbital and infraorbital series, and ventrally upon the gular series. This poorly defined group of neuromasts—in fact differentiated from other fields solely by its innervation—appears to be the postorbital series, although it does not correspond exactly to the series so designated by Kingsbury for Amphibia in general. In Amphiuma the writer found a small number of nerves arising from the hyomandibular trunk and from the base of the ramus mentalis externus innervating a widely scattered series of neuromasts, considered by him as the postorbital series, although not coinciding with the series so named by Kingsbury. It is possible that one or two of the small nerves arising from the base of the mentalis internus should be reckoned with this postorbital innervation. Kingsbury describes a small series of neuromasts, the angular, situated at the angle of the mouth and extending upon the upper lip. The writer has shown that in Amphiuma these neuromasts, about six in number, are supplied by two small branches of the mentalis externus. When we seek for corresponding neuromasts in Siren, taking as criteria the situation upon the side of the head and extension anteriorly upon the upper lip, and the innervation by small branches of the mentalis externus given off near the base of the same, we find

about thirty neuromasts which may be termed an angular series. But between these and the oral series there seems no natural demarcation; in fact, it is doubtfully allowable to distinguish between them in any urodele amphibian. The ramus mentalis externus was overlooked by Fischer.

8. The ramus jugularis VII

From the point of its separation from the lateral line components of the truncus hyomandibularis the ramus jugularis passes at first anteriorly around the anterior dorsal border of the depressor mandibulae muscle, and thence ventrally, laterally and posteriorly around the lateral border of the same muscle. As it leaves the hyomandibular trunk it gives off posteriorly a small motor branch (*dma.* + *lhy.*) to the anterior division of the depressor mandibulae and to the levator hyoidei muscles. Near the same point it receives the ramus communicans X ad VII of general cutaneous fibers. As the ramus jugularis passes postero-ventrally around the muscle it gives off a number of small branches from its posterior border. Two of these branches run to the posterior division of the depressor mandibulae muscle (*dmp.*). In one specimen the jugularis of the left side was found to receive a large anastomosis of general cutaneous fibers from the mandibularis V (fig. 13, *md.2*), but on the other side no trace of such a union could be found. Other branches given off from the posterior border of the nerve supply the cerato-hyoideus externus and interbranchialis 1 muscles and furnish general cutaneous elements to the skin overlying these muscles (*che.*+*ib.1*). The ramus jugularis toward the lower border of the depressor mandibulae, curves anteriorly and innervates the interhyoideus muscle (*ih.*). Wilder, following Fischer, incorrectly ascribes the innervation of the m. ceratohyoideus externus to the ninth nerve.

9. The lateral line anastomosis with the vagus nerve

At the dorso-lateral border of the dorsal lateral line ganglion, a little posterior to the emergence of the truncus supraorbitalis from the ganglion, there is a small tract of lateral line fibers (fig. 12, *VII ad X*). These pass anteriorly at the edge of the ganglion

and may emerge from the skull by a special foramen in the petrosal, or, running more anteriorly, they may pass out with the truncus supraorbitalis (fig. 11). This small group of lateral line fibers may or may not be accompanied by general cutaneous fibers. On emerging from the skull the nerve turns sharply posteriorly and runs a short distance along the dorsal border of the lateral wing of the parietal bone (figs. 12, 13), then passes dorsally through the masseter muscle to take a position just beneath the skin on the side of the head at about the level of the dorsal border of the skull (fig. 14). Its course is thence posteriorly to an anastomosis with the rami supratemporalis et auricularis X. Just what neuromasts of the occipital series are supplied by this branch it does not seem possible to determine. In some instances a branch of the nerve leaves the main portion at the emergence from the skull and supplies some neuromasts of the supraorbital series. Between the supraorbital series of neuromasts and the occipital series there seems to be no dividing line externally, a condition fully borne out in the innervation. It seems however, that most of the neuromasts supplied by this union of the seventh nerve constituent with the rami supratemporalis et auricularis X belong to the occipital series, although it is reasonable to suppose that a few of the more anterior ones are supraorbital. This anastomosis seems to be peculiar to Siren among the amphibians. Johnston ('05 b) shows in Petromyzon a lateral line anastomosis between the seventh and the ninth-tenth nerves, and in Lampetra between the seventh and tenth nerves. He suggests that this anastomosing branch of the seventh nerve with its neuromasts may represent the ramus oticus of fishes and the organs supplied by it. Such an explanation for the condition in Siren seems plausible.

10. The ramus alveolaris VII

From the extreme anterior end of the geniculate ganglion the communis fibers of the facialis emerge and pass ventrally through the posterior portion of an elongate slit-like opening between the orbitosphenoid (petrosal) cartilage medially and the quadrate cartilage laterally (fig. 12, *alv.-pal.*). Immediately after passing

through the cranial wall the nerve trunk divides into a medial ramus palatinus and a lateral ramus alveolaris. This common origin of the two rami from the ganglion has been noted by Fischer ('64) and Wilder ('91). The following statement by Drüner ('04. p. 660) is, therefore, surprising: "Dadurch wie auch für den N. alveolaris eine besondere Austrittsöffnung weiter medial und ventral geschaffen, die wiederum von der des R. palatinus geschieden ist. Wir haben hier dadurch die Anfänge der Bildung eines Fallopi'schen Canals mit 3 Austrittsöffnungen vor uns, eine für den R. palatinus, eine für den N. alveolaris und eine für die äussern Aeste."

On separating from the ramus palatinus the ramus alveolaris passes anteriorly, laterally and ventrally, at first between the pterygoid muscle and the quadrate cartilage (fig. 11), then between the pterygoid and temporal muscles, then through the pterygoid muscle (fig. 10), emerging from the latter at the medial dorsal border of the lower jaw. It then passes along the inner border of the gonial bone, taking a position between the pterygoid muscle and the insertion of the tendon of the temporal muscle, just dorsal to the origin of the intermandibular muscle. Some distance anterior to the point where the intermandibular branch of the ramus mandibularis V passes through the lower jaw the alveolaris divides into a smaller dorsal and a larger ventral branch. The dorsal branch, passing around the anterior edge of the tendon of the temporal muscle, ascends to the dorsal medial border of the gonial bone and in close contact with it, giving off a few small branches to the mucous membrane at the lateral border of the pharynx. As the dorsal wing of the gonial gradually disappears the nerve comes to lie at the medial border of Meckel's cartilage (fig. 8, *alv.1*). When the opercular bone (a thin scale-like tooth-bearing ossicle) is reached the nerve takes a position in a somewhat groove-like space between the opercular bone medially and Meckel's cartilage laterally (figs. 8–6), but the bone is never developed enough to enclose the nerve in a canal. As the nerve runs along the dorsal border of the operculare it is joined by a small branch of the mandibularis V (*md.4a*), which passes from the lateral border of the jaw through the tendon of the masseter muscle to the

medial border of Meckel's cartilage (figs. 8–6). The two nerves fuse into one in some cases, in others they merely come into contact without losing their individuality. Even where a fusion occurs there soon results a separation into two branches which run along the dorso-medial border of Meckel's cartilage, supplying the overlying mucous membrane and presumably the opercular teeth. The larger ventral division of the main alveolaris stem (*alv.2*) passes anteriorly just dorsal to the origin of the intermandibular muscle on the gonial bone, at the extreme lateral border of the mouth just beneath the mucous membrane, and medial to the gonial bone and more anteriorly medial to Meckel's cartilage (figs. 8–5). It supplies the mucous membrane of the sides and more anteriorly of the floor of the mouth.

The accounts given of the distribution and relationships of the alveolaris in various Urodela differ very widely in detail. In Amblystoma (Coghill, 1902) the alveolaris enters a canal in the lower jaw, divides within the canal and one of its branches unites with a branch of the mandibularis V. Sometimes it gives off a branch before entering the jaw, but in that case the branch enters a special canal of its own in the jaw. Apparently all of the alveolaris in Amblystoma enters a canal in the jaw. In Spelerpes Miss Bowers ('00) finds no branch of the alveolaris entering the jaw, but in this she is plainly mistaken, for preparations of Spelerpes in the possession of the writer show unmistakably that the alveolaris divides, one branch entering the jaw and fusing with a branch of the mandibularis V, the other branch running along the inner border of the jaw as described by Miss Bowers. In Amphiuma (Norris '08) the nerve divides into a number of terminal branches, one of which enters a canal in the jaw and anastomoses with a branch of the mandibularis V. In Plethodon (Norris '09) the condition is almost identical with that in Spelerpes. In larval Triton (Drüner '01) the alveolaris gives off a large branch before entering the jaw. In the adult Triton the chief part of the nerve does not enter the jaw. In Salamandra (Drüner, l. c.) the alveolaris enters a canal in the jaw and fuses with a branch of the mandibularis V. In Cryptobranchus alleghaniensis and C. japonicus (Drüner '04) the alveolaris enters a

canal in the jaw, and in C. japonicus, according to Osawa ('02), fuses with a branch of the mandibularis V. In Proteus, Necturus (Drüner '01) and Siren (Drüner '04, Wilder '91) the alveolaris does not enter a canal in the jaw. In Necturus Norris and Buckley ('11) find that the alveolaris, on reaching the lower jaw, divides into two branches, one of which runs far anteriorly and comes into close association with a branch of the ramus mandibularis V.

From the facts above stated there may be deduced two characteristics of the ramus alveolaris in urodele amphibians: (1) a division into two (or more) branches, one of which enters a canal in the lower jaw; (2) fusion within this canal with a branch of the ramus mandibularis V. The peculiar condition in Proteus, Necturus and Siren may be explained as due to the imperfect development of the opercular bone, it being too rudimentary to form a canal. In consequence of not being confined in a limited space, the alveolar and mandibular branches do not fuse completely. It is evident that the smaller dorsal branch of the alveolaris in Siren is the one which corresponds to the branch entering the canal in the jaw in most Urodela. This condition in Proteus Necturus and Siren can hardly be regarded as primitive, but accords very well with the view of Boas cited by Drüner ('04, p. 361) "dass Siren, Menobranchus und Proteus Larvenformen seien."

11. The ramus palatinus VII

This nerve runs anteriorly, at first along the lateral border of the parasphenoid bone (figs. 11, 10, *pal.*), and farther anteriorly, ventral to its lateral border. Along its course many small twigs are given off to the dorsal wall of the pharynx and mouth. Not far anteriorly from its origin the nerve gives off a lateral branch (*pal.2*) which runs for some distance parallel to the main nerve (*pal.1*), along and in the dorso-medial border of the pterygoid muscle, but gradually shifting laterally, gives off small twigs to the dorso-lateral wall of the pharynx, and, as a very small nerve, reaches the lateral border of the antorbital cartilage (figs. 9, 8), where it receives the anastomosing branch from the ophthalmicus

profundus and maxillaris V, as previously described (p. 281). The main palatine nerve, which has at this level a position ventral to the lateral wing of the parasphenoid, divides near the transverse level of the anterior wall of the eyeball, one division (*pal.1a*) continuing anteriorly, ventral to the parasphenoid, approaching nearer and nearer to the middle line (figs. 6, 5) until it meets its fellow of the other side, when a fusion takes place. From this union two nerves arise, one dorsal and the other ventral, which pass anteriorly in the middle line, supplying chiefly blood vessels in the roof of the mouth. The other larger division (*pal.1*), shifting laterally, forms with the profundus and maxillary constituents the medial portion of the profundus-palatine anastomosis. The character of this anastomosis has been described on page 282.

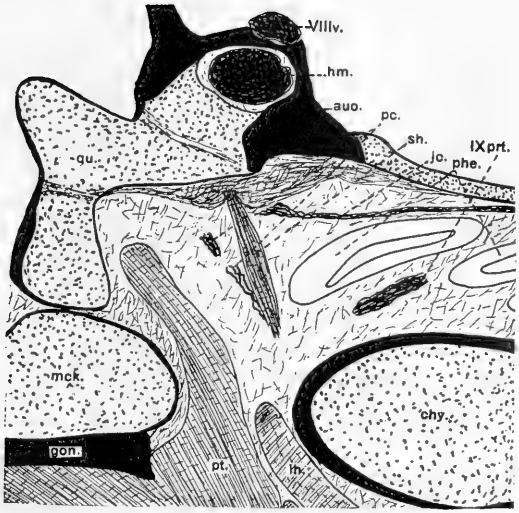
The palatine nerve is thus seen to have divided into a lateral branch (*pal.2*) which runs into the lateral part of the nasal capsule, sharing in the lateral portion of the anastomosis as above described, and into a medial portion (*pal.1*) which contributes to the medial part of the anastomosis and runs anteriorly along the medial wall of the nasal capsule. From the anastomosis the medial combined nerve extends along the lateral border of the internasal cartilage and ventral to the lateral line nerve which has come from the buccalis-profundus anastomosis. In the anterior nasal region it divides into two branches, medial and lateral, which are distributed to the medial and lateral dorsal walls of the mouth (fig. 4).

12. Palatinus caudalis

From the main communis trunk as it leaves the skull (fig. 12) there is given off posteriorly a small nerve, the posterior palatine of Wilder, which may be joined by a small posterior branch of the ramus palatinus (or ramus alveolaris), although there may be no anastomosis of the two. In addition a small nerve may leave the geniculate ganglion a little posterior to the point where the main communis trunk emerges, which passing out through its own foramen in the petrosal cartilage, runs posteriorly into the vicinity of the two nerves mentioned above. From these nerves

minute twigs supply chiefly the walls of blood vessels in the dorso-lateral pharyngeal wall. The posterior palatine (*pc.*) contains some deeply medullated fibers. Most of these pass into a branch which terminates in a small vestigial muscle (figs. 13, 38, *sh.*) which has its origin on the fascia between the quadrate and the lateral edge of the parasphenoid and its insertion on the lateral border of the ceratohyal cartilage. That motor fibers should occur in a branch of the palatine and alveolar rami seems so improbable that the writer ventures little more than a bare statement of fact. Wilder says ('91, p. 663) that a few of the anterior fibers of the cerato-hyoideus externus muscle are innervated by the posterior palatine nerve, but this vestigial muscle is certainly no part of the ceratohyoideus externus muscle. The vestigial muscle is uniformly present, but, like most rudimentary structures, varies greatly in the degree of its development. With the giving off of the branch to the muscle the posterior palatine becomes much attenuated and passes posteriorly (*jc.*) into the ramus pretrematicus IX, uniting with it in two anastomoses, one with the main pretrematic trunk, the other with a small branch which arises far posteriorly near where the pretrematic leaves the glossopharyngeal ganglion. Whatever may be the significance of the branch terminating in the rudimentary muscle it is seen that the posterior palatine in Siren is in part a Jacobson's anastomosis, for, while the latter typically unites with the palatinus, in Siren it joins the common trunk from which the palatine and alveolar rami arise.

A search through the literature on the subject reveals no mention of a muscle in the other Urodela similar to this rudimentary one in Siren. Schulze ('92, p. 21) describes in the larva of the anurous Pelobates fuscus a muscle, m. suspensorio-hyoideus, which has its origin "von der lateralen Randparthie der Unterseite des Corpus suspensorii und des dicht hinter dem Corpus suspensorii folgenden Theiles des Suspensoriums," and is inserted on the processus lateralis of the ceratohyal. In the larval condition of Rana pipiens and R. catesbiana the writer finds a similar muscle innervated by a branch of the truncus hyomandibularis VII.



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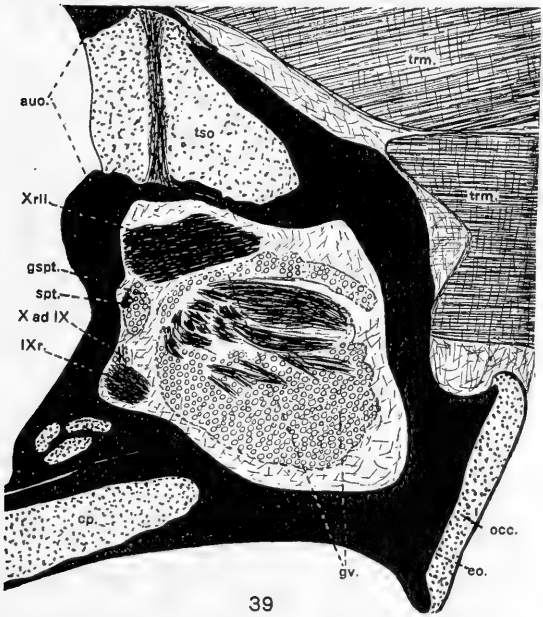

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Fig. 38 Sagittal section through the ventral wall of the ear capsule and facial canal, showing the vestigial muscle (*sh.*) which has its origin on the fascia ventral to the quadrate (*qu.*) and its insertion on the ceratohyal cartilage (*chy.*). Its innervation by a branch of the palatinus caudalis (*pc.*) is shown. ×30.

Fig. 39 Sagittal section through the posterior border of the left ear capsule and the vagus ganglion. To show the ganglion of the supratemporal root of the vagus nerve. ×50.

Nervus facialis (Urodela)

Portio dorsalis:
- Radix lineae lateralis
- Ganglion lineae lateralis dorsale
- Rami:
  - Truncus supraorbitalis
    - Ramus ophthalmicus superficialis
    - Ramus oticus(?), cum r. otico V
  - Truncus infraorbitalis
    - Ramus buccalis, cum r. maxillari V

Portio ventralis:
- Radix communis (fasc. communis)
- Radix motor
- (Radix spinalis, Siren)
- Ganglion geniculi
- Ganglion lineae lateralis ventrale
- (Ganglion spinale, Siren)
- Rami:
  - Truncus hyomandibularis
    - Ramus mentalis
      - Ramus mentalis externus
      - Ramus mentalis internus
      - Rami postorbitales
    - Ramus jugularis
    - Ramus alveolaris (cum r. palatino, Siren)
  - Ramus palatinus
    - Ramulus palatinus caudalis

THE GLOSSOPHARYNGEAL AND VAGUS NERVES

1. The roots of the IX–X complex

Four groups of rootlets may be recognized in the IX–X complex of the Urodela: (1) lateral line fibers of the vagus; (2) communis and motor fibers constituting the glossopharyngeus root; (3) a group of communis, general cutaneous and visceral motor fibers forming the vagus proper; (4) motor fibers arising by a variable number of rootlets, but which may be traced posteriorly as a compact tract of coarse fibers in the lateral columns of the medulla oblongata passing into the spinal cord. This may be termed a motor accessory tract.

The first of these groups in Siren (*X rll.*) enters the brain in the usual manner, by two rootlets (figs. 42–44). On examination

of the dorsal part of the medulla between the origins of the seventh and the ninth-tenth nerves it will be seen that the lateral line lobe ('dorsal island' of Kingsbury), into which the dorsal of the three lateral line rootlets of the facial nerve enters, has disappeared at the level of the origin of the IX–X nerves. The two lateral line rootlets of the vagus nerve, therefore, enter that part of the brain wall (acusticum) that corresponds to the part entered by the two ventral of the lateral line rootlets of the seventh nerve. The lateral line component of the vagus nerve supplies all the lateral line fibers of the IX–X group, except those that may enter by way of the anastomosis between the dorsal lateral line facialis ganglion and the supratemporalis-auricularis X nerves. The lateral line rootlets, on combining into a flattened band at the side of the medulla, pass postero-ventrally into the vagus ganglion.

The glossopharyngeal group of rootlets arises ventral and slightly posterior to the preceding, composed of a dorsal rootlet of communis fibers and a ventral motor rootlet. From their connections with the brain the lateral line and glossopharyngeal roots pass posteriorly parallel with, but distinct from each other until their respective ganglia are reached, except that a small band of fibers (fig. 14, *Xrspt.*) descends from the lateralis root and enters the extreme antero-dorsal part of the IXth ganglion, later emerging from the vagus ganglion as the ramus supratemporalis X. A few sensory fibers of a different character often, perhaps always, descend with this small band of lateral line fibers. Their origin and occurrence is usually obscured by the dense medullation of the lateral line fibers. They appear to come from the communis portion of the third group of rootlets, at a point where the latter comes in contact with the lateral line root. They separate from the lateral line tract, as the latter passes out of the skull, and pass into the IXth ganglion.

The third group of rootlets (*Xr.2*) in Siren consists of two communis, two general cutaneous and a variable number of motor rootlets, the latter sometimes as many as ten in number. The motor rootlets evidently come from cells situated opposite or nearly opposite their point of exit from the brain. The com-

bined rootlets of this third group form, at the median ventral border of the lateral line root a band which, running posteriorly parallel with the latter (fig. 14), is joined just before the two roots enter the ganglion by the fourth group of rootlets (*Xr.3*). The loose distribution of the numerous small motor rootlets makes it possible to distinguish them by their color only. Thus three fiber groups enter the IX–X ganglionic mass (1) the lateral line root; (2) the glossopharyngeal root, communis and motor fibers, to which has been joined the small lateral line contingent from the preceding, with its accompanying communis (?) fibers from the third group; (3) general cutaneous, communis and motor fibers of the third and fourth groups of rootlets.

Wiedersheim ('77, p. 17) states that Siren is the only urodele in which the ninth nerve has a foramen of exit distinct from that of the vagus. Parker ('82, p. 194) says that "the glossopharyngeal and vagus pass out of a common passage in the exoccipital." The writer finds this latter statement confirmed by the condition in young individuals.

2. The IX–X ganglionic mass

The elongate glossopharyngeal-vagus ganglionic mass, as in all Urodela, has its anterior glossopharyngeal end wedged under the posterior part of the ear capsule. The glossopharyngeal portion (*ggl.*) is more distinct from the vagal (*gv.*) than it is in most other Amphibia. In some instances a separating line is distinguishable throughout between the two. The IXth root enters the dorso-medial portion of the sub-capsular part of the ganglionic mass. Slightly dorsal to the IXth root the lateral line root of the ramus supratemporalis X enters the ganglion; or in some instances it has a small distinct ganglion of its own at the antero-dorsal border of the IXth ganglion (fig. 39, *gspt.*). The other sensory fibers (X *ad IX*) entering along with the lateral line elements, sometimes, if not always, possess a distinct ganglion at the medial border of the IXth ganglion. Of the peripheral destination of these latter sensory elements little more can be said than that they apparently enter the ramus posttrematicus IX. Whether they are general cutaneous or communis fibers

it has not been possible to determine with certainty, but they have the histological characteristics of general cutaneous fibers, although apparently coming from the communis portion of the third group of rootlets as mentioned above. No general cutaneous fibers, however, have been identified in the ramus posttrematicus IX. The dorsal part of the vagus portion of the ganglionic mass is composed of the large lateral line ganglion cells. The ganglion cells of the general cutaneous constituent are situated for the most part anteriorly, postero-dorsal to the IXth portion of the ganglionic mass. The ventral part of the ganglion is composed mostly of communis ganglion cells. Within the ganglionic mass it is impossible to follow and differentiate the constituents of the roots already mentioned with very much exactness. The fibers of the IXth root all pass into the glossopharyngeal nerve. General cutaneous fibers from the vagal roots enter the base of the pretrematicus IX on their way to the ramus communicans cum faciali. The writer finds no evidence of general cutaneous fibers in the peripheral portion of the ninth nerve. The constituents of the third and fourth groups of rootlets are so much mixed that the sensory components are with difficulty differentiated in the ganglion itself. The motor fibers of the fourth group of rootlets appear to enter mostly, if not exclusively, the ramus intestino-accessorius X.

3. The ramus communicans cum faciali

Drüner considers this ramus in Siren, as well as in other Urodela examined by him, except Siredon (Amblystoma), to be exclusively motor. He bases his opinion largely upon theoretical grounds: that muscles belonging primarily to the glossopharyngeal territory have come to be innervated by the jugularis VII through fibers which are in reality (in the opinion of Drüner) furnished by the ramus communicans. In Amblystoma Coghill has shown that the ramus communicans is composed of general cutaneous and communis fibers. Drüner himself admits that there are communis fibers in the communicans of Siredon. Miss Bowers describes the same nerve in Spelerpes as general cutaneous

exclusively, but the writer has shown ('11) that there is a communis constituent also in Spelerpes. In Amphiuma he has found the ramus communicans to be mostly if not wholly general cutaneous. In Necturus Norris and Buckley find the conditions similar to those in Amblystoma and Spelerpes. In Triton Coghill finds no motor fibers in the ramus communicans.

The ramus communicans of Siren arises in different ways. It may be found entering the ganglion along the dorsal border of the ramus posttrematicus IX, becoming ganglionated at a point where the anterior border of the third (*Xr.2*) IX–Xth group of rootlets enters the ganglion. It is clear that no motor fibers enter the nerve, as all its fibers become interrupted, that is, become ganglionated, in their course through the ganglion, in marked contrast to the neighboring motor tract of the glossopharyngeal root. Their continuation into the root of the vagus is in that portion where most if not all of the general cutaneous fibers are situated. The communicans may enter the ramus posttrematicus IX near the exit of the latter from the ganglion. In that case, incorporated in the nerve, its course through the ganglion is not so easily followed. In one specimen the nerve enters the ganglion on one side as a single nerve; on the other side it divides into three parts, one portion entering the ganglion in the typical way, a second joining the posttrematicus IX, and the third passing posteriorly into the ramus auricularis X. In specimens, where the fiber differentiation is such that communis and general cutaneous components can be differentiated from each other, the communicans can be traced beyond question into the general cutaneous constituent of the vagus roots. In one specimen the communicans of the left side is large and general cutaneous fibers can be traced from the vagal root through the ganglion and base of the ramus pretrematicus IX into the ramus communicans; on the right side the communicans is totally lacking and no general cutaneous constituent enters the glossopharyngeal region of the ganglionic mass.

Drüner and Fischer have called attention to the small size of the communicans in Siren. The writer finds that the number of fibers in the ramus is wholly inadequate to furnish all the gen-

eral cutaneous fibers of the ramus jugularis VII. This inadequacy led to a search which resulted in the discovery in the roots of the facial nerve of what the writer has interpreted as a general cutaneous component of that nerve.

From its passage through the IXth ganglion the peripheral course of the ramus communicans is around the posterior wall of the ear capsule, over the stilus of the columella and along the dorsal border of the latter, thence along the lateral wall of the ear capsule, medial to the inner division of the depressor mandibulae muscle, to its junction with the ramus jugularis VII. It joins the jugularis immediately after the latter emerges from the facial canal. The lighter colored fibers of the communicans may be traced some distance peripherally in the jugularis before being lost in the larger mass of motor fibers. Fischer ('64, p. 147) calls the communicans in Siren "das Kopftheil des Sympathicus," believing that it passes from the IX–Xth ganglion into the VIIth ganglion, rather than into the jugularis VII.

4. The first branchial nerve

The glossopharyngeal or first branchial nerve divides as it emerges from its ganglion into two distinct trunks, the ramus pretrematicus and the ramus posttrematicus which pass out over the anterior end of the subvertebral rectus muscles. The ramus pretrematicus, wholly communis in constitution, passes posteriorly and laterally to the medial border of the ceratohyal bar, thence anteriorly and ventrally, ventral to the hyo-columellar ligament and lateral to the columella (fig. 14,*prt. IX*), along the dorsal border of the ceratohyal and at the dorso-lateral border of the pharynx and mouth (fig. 13). At about the level of the posterior end of the jaw it divides into two main nerves (fig. 11), one of which passes along the lateral border of the ceratohyal, supplying the lateral wall of the mouth and part of the tongue; the other is distributed to the floor of the mouth and the dorsal part of the tongue.

As the ramus pretrematicus, on emerging from the ganglion, is shifting from a posteriorly to an anteriorly directed course, it gives off a number of small pharyngeal branches, some passing

anteriorly and other posteriorly in distribution to the dorsal and lateral walls of the pharynx. Between some of them and similar branches of the ramus posttrematicus there may occur anastomoses. One of these small branches of the ramus pretrematicus (or one given off more posteriorly, or two anastomosing branches) connects with the so-called posterior palatine branch of the alveolar-palatine trunk of the facial nerve, thus forming a Jacobson's

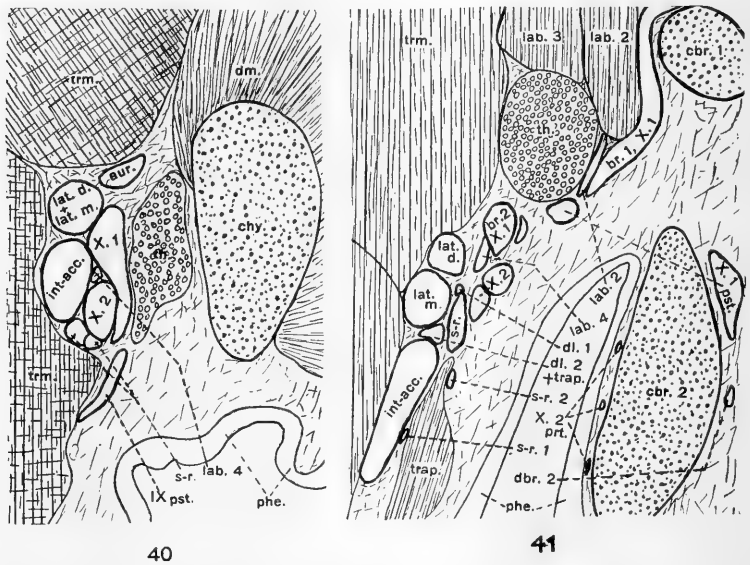

Fig. 40 Cross-section of the great bundle of nerves passing posteriorly from the vagus ganglion. Section 535. ×50.

Fig. 41 The same, but farther posteriorly where the dispersal of the individual nerves is taking place. Section 613. ×50.

anastomosis (*jc.*). The middle portion of the anastomosis is very much attenuated, but it is larger at the ends, showing that it is made up largely of pharyngeal fibers of the ninth and seventh nerves, and only a small part of it extends from one nerve to the other. In some instances it is double in character almost its entire length.

Anastomoses of communis fibers between the ninth and seventh nerves seem to be common if not universal in the Urodela. In

Amphiuma the writer finds that the ramus pretrematicus IX anastomoses with the ramus palatinus VII and the ramus alveolaris VII; two anastomoses with the latter. Sometimes Jacobson's anastomosis is double. In one instance there was an anastomosis of the ramus pretrematicus IX with the hyomandibular trunk of the facialis. In some cases there developed out of the anastomoses a definite plexus between the IXth and VIIth nerves. In Amphiuma the ramus communicans appears to have no communis fibers. In Siren the interchange of fibers has become reduced, and, since the palatine and alveolar nerves issue from the skull in a common trunk, Jacobson's anastomosis connects with this trunk, thus combining in one anastomosis all the different connecting communis branches in Amphiuma. In Amblystoma Coghill finds the ninth nerve anastomosing with the ramus palatinus VII (palatinus caudalis), forming Jacobson's anastomosis, and sometimes also the ramus pretrematicus IX with the ramus alveolaris VII. In the ramus communicans he finds a communis constituent that joins the ramus alveolaris VII. In Triton he finds a Jacobson's anastomosis, but no glossopharyngeal anastomosis with the ramus alveolaris VII, and apparently the ramus communicans consists of general cutaneous fibers only. In Spelerpes Miss Bowers does not find a Jacobson's anastomosis. The writer confirms this to the extent that there occur anastomoses between the smaller branches only of ramus pharyngeus IX and ramus palatinus VII. Miss Bowers recognizes only general cutaneous fibers in the ramus communicans of Spelerpes, but the writer finds a large communis constituent that joins the alveolaris as in Amblystoma. The anastomoses in Necturus seem to be about identical with those in Spelerpes. In Plethodon there is evidently a definite Jacobson's anastomosis, and the ramus communicans has a communis element that joins the ramus alveolaris.

Thus it is seen that there are three possible communis anastomoses between the glossopharyngeal and the facial nerves: (1) a communis component may occur in the ramus communicans, connecting the glossopharyngeal ganglion and the ramus alveolaris VII (Amblystoma, Spelerpes, Plethodon, Necturus); (2) Jacobson's anastomosis between the ramus pharyngeus IX and

the ramus palatinus VII (all Urodela); (3) an anastomosis between the ramus pretrematicus (or pharyngeus) IX and the ramus alveolaris VII (Amphiuma, Amblystoma).

The ramus posttrematicus IX (*IX. pst.*), or ramus lingualis (figs. 42, 44), of communis and motor fibers, passes directly posteriorly from its emergence from the ganglion, along the dorso-lateral border of the anterior part of the sub-vertebral rectus muscle, accompanied by small pharyngeal branches of the ramus pretrematicus. It gives off a number of very small pharyngeal branches to the dorso-lateral pharynx wall. At about the level of the roots of the second spinal nerve it begins to ascend rapidly in a postero-dorsal direction, turns sharply antero-dorsally and laterally until it reaches the lateral border of the dorsal tip of the ceratohyal. There, after giving off one or two branches to the levator muscle of the first branchial arch, (*lab.1*), it turns sharply again, but in a postero-ventral direction; then curving antero-ventrally reaches the first ceratobranchial along whose lateral border it passes obliquely across to its antero-ventral edge. As the nerve is passing along the lateral border of the ceratobranchial it gives off all its communis branches in a number of small nerves (*IX.pst. ph.*), most of which pass around the dorsal border of the branchial arch to be distributed to the ventro-lateral pharyngeal epithelium. At the extreme ventral border of the ceratobranchial 1 the motor portion of the ramus posttrematicus unites with a motor branch of the ramus posttrematicus of the second branchial nerve (*X1.pst.*), the combined nerves innervating the ceratohyoideus internus muscle.

5. The rami supratemporalis et auricularis X

As previously noted, as the lateral line root of the tenth nerve passes posteriorly towards its ganglion there is given off from it a small tract (fig. 14, *Xrspt.*) which enters the dorsal border of the IXth ganglion. It appears to become ganglionated at once, its cells occupying the antero-dorsal part of the ganglion, on the border between the vagus and the glossopharyngeal portions (fig. 39). The fibers emerge from the vagus ganglion a little dorsal and posterior to the exit of the ramus posttrematicus IX as a small nerve of lateralis composition that is unquestionably

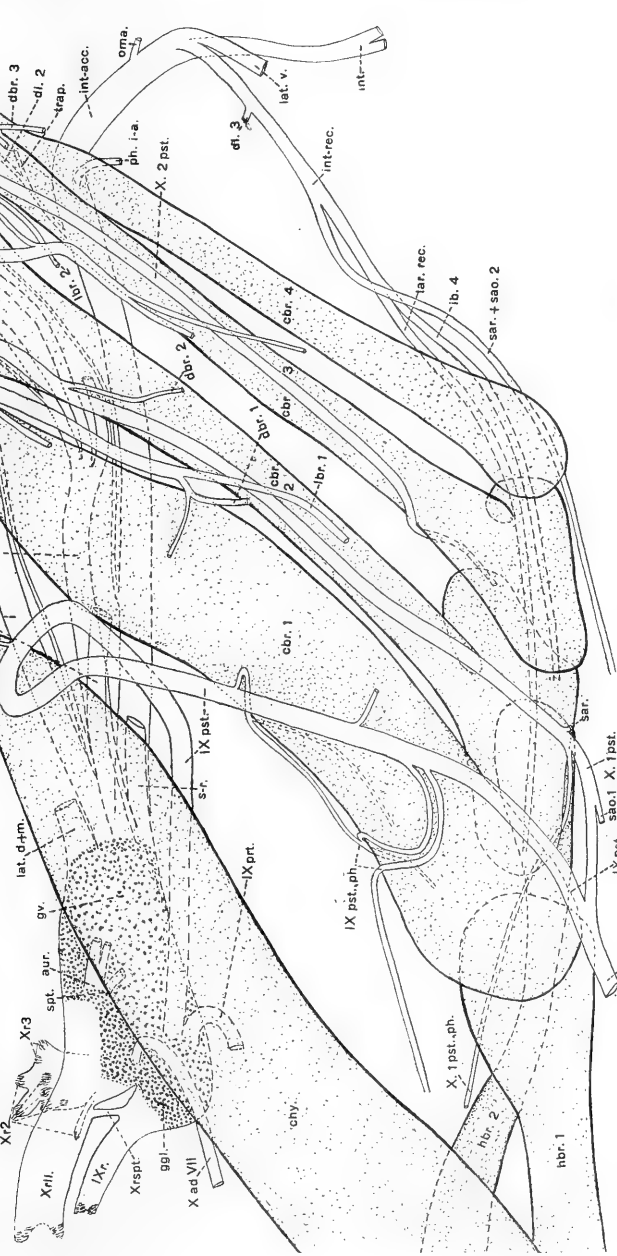

Fig. 42 A projection upon the sagittal plane of the branchial nerves and the branchial arches. To show the relations of the nerves to the arches, especially of the posttrematic rami. ×30.

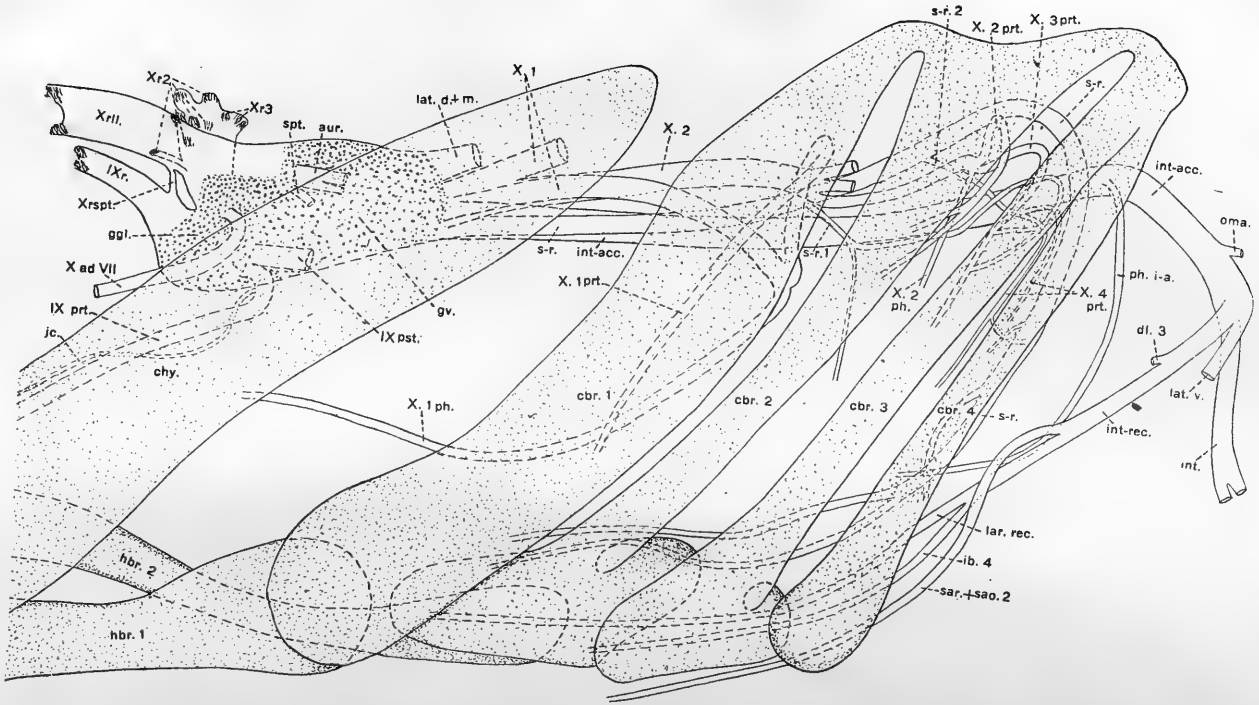

Fig. 43 A projection upon the sagittal plane of the branchial nerves and the branchial arches. To show the relations of the pretrematic and pharyngeal rami to the arches. ×30.

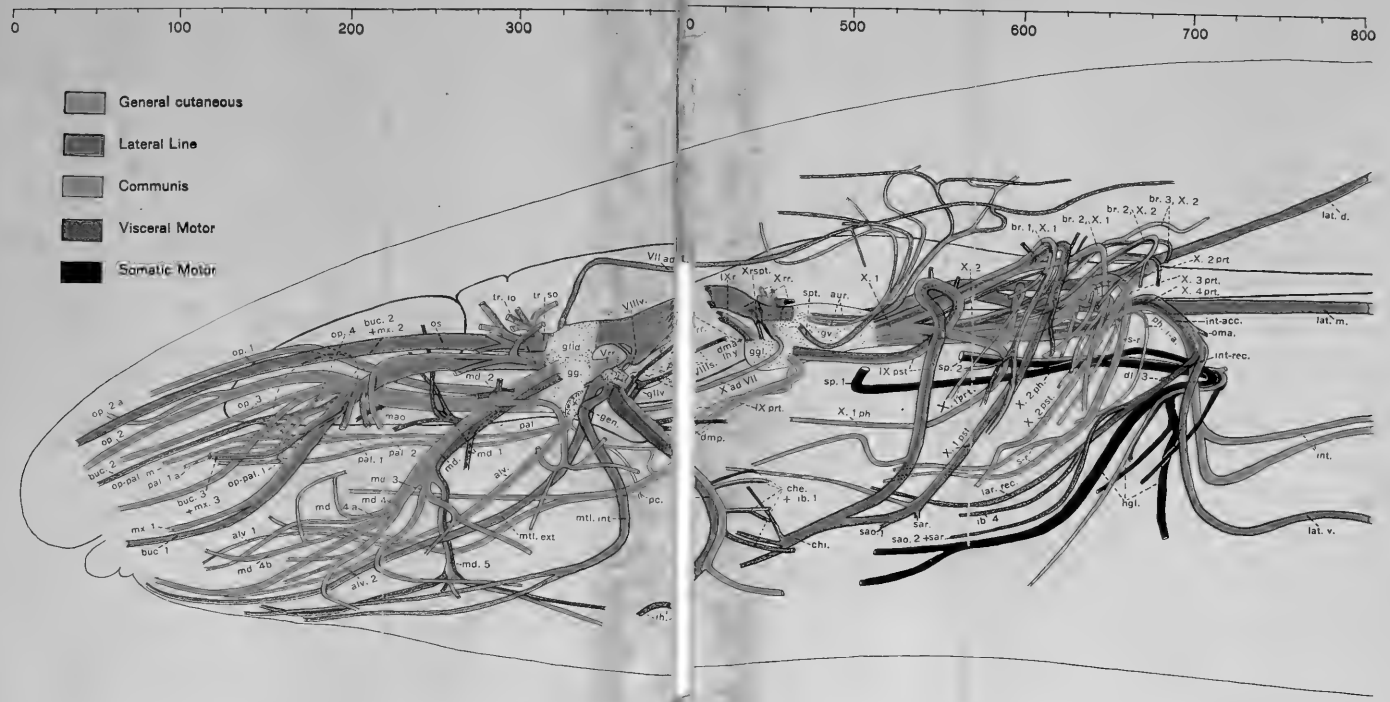

Fig. 44 A projection upon the sagittal plane of the V, VII, VIII, IX, and [illegible] cranial nerves, and parts of the first and second spinal nerves, of Siren lacertina. The scale above the figure indicates the serial number of the transverse sectio[illegible] employed in the reconstruction, the sections being 20μ thick. $\times$16.

the ramus supratemporalis X. Passing posteriorly and dorsally, it anastomoses with another vagus branch of lateralis and general cutaneous fibers which has arisen almost directly dorsal to the exit of the ramus supratemporalis. The two nerves combined innervate the neuromasts of the occipital series, receiving the anastomosis from the facial dorsal lateral line ganglion. The general cutaneous fibers of the auricularis supply the skin in the occipital region.

6. The second branchial nerve

The nerves taking their origin from the posterior part of the vagus ganglion, as Drüner has observed, are bound into a compact bundle which passes posteriorly as such, before any important branches are given off, as far as the anterior border of the anterior thymus gland. Examination of cross-sections of this nerve shows that the characteristic vagus rami, although in close juxtaposition, are distinct throughout (figs. 40, 41). In figures 42 to 44 these nerves are represented as somewhat displaced vertically in order to show the component parts. The writer is unable to confirm Drüners' statement that all three lateral line nerves are bound into a truncus lateralis, nor that the third branchial nerve is bound into a single stem with the rudiments of the following branchial nerves. These nerves are all distinct from each other.

The second branchial nerve (*X.1*) leaves the postero-lateral border of the vagus ganglion, and soon divides into a dorsal and a ventral division, the latter being the ramus pharyngeus. The dorsal division passes postero-dorsally, to the extreme dorsal tip of the first branchial arch. There it divides, or has already divided, into three main divisions: (1) A branch of motor, general cutaneous and communis composition (figs. 42, 44, *br.1*,*X.1*) curves over the top of ceratobranchial 1 and descends on its lateral border to the first gill and the levator (*lbr.1*) and depressor (*dbr.1*) muscles of the same. (2) A little posterior to the preceding a second branch, of motor and communis fibers, curves over the top of ceratobranchial 2 and descends along its lateral border as the ramus posttrematicus (*X.1*,*pst.*). It gives off a motor branch to the depressor muscle of the second gill (*dbr.2*). Thence pass-

ing antero-ventrally, after giving off its communis fibers chiefly to the ventral wall of the pharynx (*X.1,pst.ph.*), and some small motor branches to mm. subarcualis rectus (*sar.*) and subarcualis obliquus 1 (*sao.1*), its main motor portion joins the ramus posttrematicus IX to innervate the ceratohyoideus internus muscle. Drüner says that musculus subarcualis obliquus 2 is innervated by a branch of this nerve, but the writer has not confirmed this with certainty. (3) A third branch of general cutaneous and communis fibers (*br.2,X.1*) turns out over the top of ceratobranchial 2 and, after receiving a small anastomosis from the third branchial nerve, is distributed to the second gill. Fischer considered the second branchial nerve as a part of the glossopharyngeus. Wilder (l. c., p. 668) says: "the entire 2nd. external gill, including its muscles, is supplied by a branch of the glossopharyngeus."

The pharyngeal branch of the second branchial nerve (figs. 43, 44, *X.1,ph.*), after running posteriorly some distance, turns upon itself and passes anteriorly and ventrally along the dorsal wall of the pharynx. At the point where it turns anteriorly it gives off a ramus pretrematicus (*X.1,prt.*) which ascends around the dorso-lateral angle of the pharynx to reach the medial border of the first branchial arch (ceratobranchial 1), along which it passes antero-ventrally. Other small pharyngeal branches may be distributed to the dorsal pharynx wall immediately posterior to the origin of the ramus pretrematicus.

7. The third branchial nerve

This nerve (figs. 42–44, *X.2*) leaves the ganglion at the medial border of, and slightly posterior to the origin of the second branchial nerve. Very early a differentiation of a ventral pharyngeal branch from a more dorsal portion is indicated, although the actual separation may be deferred until well back in the branchial region. The dorsal portion of the nerve divides into two parts, one of which, the more ventral, passes posteriorly between the levator arcus branchialis 2 and levator arcus branchialis 3 muscles, more posteriorly between the latter muscle and the second ceratobranchial cartilage, and divides into two branches which curve

over the extreme posterior dorsal tip of ceratobranchial 2. One of these two branches, of communis and motor fibers (*X.2 pst.*), running antero-ventrally along the lateral border of ceratobranchial 3 as the ramus posttrematicus, gives off a branch (*dbr.3*) to the depressor muscle of the third gill and communis (and possibly general cutaneous) fibers to the same gill. The other branch (*br.2,X.2*) of motor, general cutaneous and communis fibers, supplies the levator muscle of the second gill (*lbr.2*) and the region of the second (and third?) gill. It also sends an anastomosing branch to the main sensory nerve of the second gill. The second division (*br.3,X.2*) of the dorsal portion of the third branchial nerve, composed of motor, general cutaneous and communis fibers, passes posteriorly between the levator arcus branchialis 3 and levator arcus branchialis 4 muscles, gives off a branch to the levator muscle of the third branchial arch (*lab.3*), divides into two parts and curves laterally over the top of the third ceratobranchial a little posterior to the first division of the nerve. Of its two parts, the one more ventral (*lbr.3*), of motor and general cutaneous fibers, supplies the levator muscle of the third gill and sends general cutaneous fibers to the same gill. The nerve for the depressor branchialis 3 muscle sometimes comes from this branch. The second more dorsal part of general cutaneous and communis fibers supplies the third gill. After giving off the branch to the depressor branchialis 3 muscle, the ramus posttrematicus of the third branchial nerve consists of communis fibers only. It passes along the lateral border of the third ceratobranchial arch to about the ventral fourth of its length and then shifts dorsally around to the median side of the arch, where it supplies the mucous epithelium of the arch, apparently replacing functionally the ramus pretrematicus of the dorsal part of the arch.

The ramus pharyngeus of the third branchial nerve (*X.2,ph.*) divides into three parts: a pharyngeal branch to the dorsal pharynx wall; a ramus pretrematicus (*X.2,prt.*) which curves over the dorso-lateral extension of the pharyngeal cavity to the medial border of the second branchial arch, dividing into a number of small branches while in that position; a ramus pretrematicus (*X.3,prt.*) which passes back, dorsally and laterally, in a

manner similar to the preceding, to take a position on the medial border of the third branchial arch. As described in other Urodela the ramus pretrematicus of the third arch arises from the truncus intestino-accessorius X.

8. The truncus intestino-accessorius X

In the great mass of nerves passing posteriorly from the vagus ganglion (fig. 40) the truncus intestino-accessorius is the largest. It occupies a ventro-medial position in the bundle. At the posterior edge of the posterior thymus gland (fig. 41) the dispersal of these vagal derivatives begins to become noticeable, and a little posterior to this the intestino-accessorius begins to curve dorsally. At the posterior border of the dorso-laryngeus muscle it occupies a position at the ventral border of the scapula and at this point it begins to recurve upon itself, passing ventrally and laterally, and, at the lateral angle of the esophagus, dividing into three divisions (figs. 42–44). At this point where the intestino-accessorius begins to recurve upon itself there occur, in some specimens, numerous ganglion cells, situated on the dorsal border of the nerve and evidently belonging to the lateral line constituent. In one instance there were about seventy-five of these ganglion cells, in another only one ganglion cell was found; in most specimens none whatever. These cells are clearly of no especial significance, but are merely cells which have wandered out from the dorsal-lateral line part of the vagus ganglion. About half-way back from the ganglion to the point where the nerve divides there is given off from its dorsal border a small motor nerve (*dl.2 + trap.*) which supplies the trapezius muscle and a part of the dorsal portion of the dorso-laryngeus muscle. In figure 42, for the sake of clearness, the origin of this small branch is placed farther posteriorly. The nerve supplying the levator muscle of the fourth branchial arch (*lab.4*) arises in other Urodela from the ramus intestino-accessorius, usually far posterior on the latter. In Siren it may arise from the third branchial nerve near the ganglion, or from the intestino-accessorius near the point where the latter leaves the ganglion. In any case it is an independent nerve nearly to the ganglion. Another motor branch to the dorso-

laryngeus muscle (*dl.1*) may arise from the intestino-accessorius farther anteriorly than the one above mentioned, or it may spring from the ramus sensitivus recurrens to be described later. From the intestino-accessorius near where it begins to curve dorsally there may arise a pharyngeal branch (figs. 42–44, *ph.i-a.*) of communis fibers which supplies the dorsal pharynx wall posterior and medial to the third and fourth branchial arches.

The three main divisions of the truncus intestino-accessorius are: (1) The ramus lateralis ventralis (*lat.V*), of lateral line fibers, which passes antero-ventrally and then posteriorly to innervate the ventral series of neuromasts of the trunk. (2) The ramus intestinalis (*int.*) of communis fibers, which, after division into two branches, passes postero-ventrally into the visceral region. (3) The ramus intestinalis recurrens, (*int.rec.*), in Siren exclusively motor, which runs antero-ventrally into the ventral branchial and laryngeal region. It divides into three branches, one supplying the subarcualis rectus and subarcualis obliquus 2 muscles (*sar.* + *sao.2*), a second innervating the interbranchialis 4 muscle (*ib.4*) and the third branch, which may be termed the laryngeus recurrens (*lar.rec.*), after passing along the medial border of the laryngeal portion of the dorso-laryngeal muscle, supplies the laryngeal muscles: laryngo-trachealis ventralis, laryngeus ventralis, laryngeus dorsalis, and constrictor laryngeus. Fischer describes and figures in Siren two branches of the laryngeus recurrens as passing across the middle line and forming two loops out of which no fibers pass. Drüner mentions a commissure between the two laryngeus recurrens nerves. The writer finds the two branches mentioned by Fischer. The posterior one unites in the middle line with the one from the other side, forming a nerve which, running anteriorly in the middle line between the two laryngo-trachealis ventralis muscles, is distributed to the latter. The anterior branch apparently passes in a commissure wholly across the middle line to the muscle of the opposite side. Wilder describes and figures (l. c., p. 670) the interbranchialis 4 (hyotrachealis) muscle as innervated "by a recurrens nerve which branches from one of the vagus twigs supplying the external gills." As the intestino-recurrens nerve is leaving the other elements of

the intestino-accessorius it gives off from its posterior border a nerve to the omo-arcualis muscle (*oma.*), and a little anterior, after the division, it supplies the dorso-laryngeus muscle with a number of small twigs (*dl. 3*). Wilder states that the omo-arcualis (procoraco-branchialis) is innervated by the ramus lateralis ventralis.

9. The ramus recurrens sensitivus X

Leaving the lateral border of the extreme posterior end of the vagus ganglion, in close association with the ramus intestino-accessorius in origin, is a nerve, of communis composition, although it may have a few motor fibers (*dl.1*), which form a second small nerve running to the dorsal portion of the dorso-laryngeus muscle. It runs on the ventral border of the great bundle of nerves issuing from the posterior end of the vagus ganglion (fig. 40, *s-r.*). It may be a single trunk, or its main branches may arise separately from the ganglion. At about the level where the second branchial pretrematic ramus (*X.1 prt.*) is formed there is given off a nerve (fig. 43, *s-r.1*), which sends one or more branches along the dorsal pharyngeal epithelium. An anastomosis may be formed with the pretrematic ramus of the fourth branchial nerve (*X. 3 prt.*). At about the level of the formation of the third branchial pretrematic ramus (*X.2,prt.*) another branch (*s-r.2*) arises which divides into two nerves, both passing posteriorly and ventrally between the two levator muscles of the third and fourth branchial arches, to take a position on the medial border of the fourth ceratobranchial, following it antero-ventrally, thus constituting a fifth branchial pretrematic ramus (*X.4,prt.*). The main trunk of the nerve, which at first runs at the ventral border of the great nerve bundle, farther posteriorly begins to pass dorsally along the dorsal border of the trapezius muscle, later passing between the latter and the levator muscle of the fourth branchial arch. At the anterior border of the dorso-laryngeus muscle the nerve passes between this muscle and the levator arcus branchialis 4 muscle, then, curving ventrally between the same two muscles, it turns anteriorly into the ventral branchial region, its later course for some distance paralleling approximately that of the

ramus intestino recurrens situated more ventrally. It divides into two chief branches distributed to the floor of the pharynx lateral to the larynx. As it is recurving upon itself from the dorsal to the ventral position it gives off a small anastomosis to one of the fifth branchial pretrematic divisions. It is this 'recurrens nerve' that Wilder (l. c., p. 670) believed to innervate musculus interbranchialis 4. As previously noted, motor fibers may occur in this nerve for some distance posterior to the vagus ganglion, these fibers being destined for the dorsal portion of the dorso-laryngeus muscle. They sometimes arise from the ramus intestino-accessorius near the ganglion, sometimes independently from the ganglion directly.

As to the nature of this communis recurrent nerve, peculiar to Siren, Drüner suggests that it represents a fifth branchial nerve, whose sensory part has become somewhat hypertrophied. This is doubtless true to some extent, for it produces a pretrematic ramus on the fourth branchial arch. In the opinion of the writer it represents as a whole the sensory component of the ramus intestinalis recurrens of other Urodela. This latter nerve, it has been noted already, is exclusively motor in Siren. This sensory recurrent nerve innervates the regions that in other urodelous amphibians are supplied by the sensory constituent of the ramus intestino-recurrens. The motor nerves to the dorsal portion of the dorso-laryngeus muscle, which in other Urodela arise from the dorsal border of the ramus intestino-accessorius, may arise from this sensory recurrent nerve. In Amblystoma the fourth branchial pretrematic (*X.3,prt.*) and in Amphiuma the fourth and fifth branchial pretrematic rami (*X.3* and *X.4,prt.*) arise from the ramus intestino-accessorius. In Siren the fourth branchial pretrematic arises with the third from the third branchial nerve (*X.2*), but the fifth branchial pretrematic arises from this sensory recurrent ramus. Also, between this nerve and the fourth branchial pretrematic (*X.3,prt.*), anastomoses may occur. In its origin from the ganglion it comes from and is associated with the same region as the ramus intestino-accessorius. The writer fails to confirm Drüner's statement that it is united with the third branchial nerve (*X.2*) in origin, but there is no improb-

ability in its so occurring in some instances. For some distance its course is along the medial border of the fourth branchial arch to which it contributes pretrematic branches.

10. The fourth and fifth branchial nerves

On the view that Siren is not a primitive form, but a permanent larva, we see an explanation of the fact that in this species, as in the larval stages of caducibranchiate Urodela in general, great reduction has occurred in the posterior branchial nerves. A typical branchial nerve of the Urodela, as outlined by Wiedersheim ('77) and by Drüner ('03), may be described in the terms of the nerve-component theory as follows: on leaving the ganglion the nerve trunk divides into (1) a ramus posttrematicus of motor, communis and general cutaneous fibers, which runs, posterior to its gill slit, along the lateral border of its corresponding branchial arch; (2) a ramus pretrematicus of communis fibers only, which runs, anterior to its gill-slit, along the median border of the next anterior branchial arch; (3) a ramus pharyngeus, at first united with the ramus pretrematicus, of communis fibers only, which is distributed to the dorsal pharyngeal wall. In the larval stages of most of the existing Urodela are found four well developed branchial arches, but the corresponding branchial nerves are not equally well developed. The first (*IX*), second (*X.1*) and third (*X.2*), as in Siren, show the characteristic rami, but in the third there is commencing a reduction in the ventral motor constituent ramus. In the other, more posterior, branchial nerves there is a complete loss of the ventral portions of the ramus posttrematicus, or of the entire ramus, or a great transformation which obscures the original condition. As noted by Drüner ('04, p. 425), the nerves are the most conservative structures of the branchial region. They will therefore constitute more reliable guides in the search for the primitive relations in this region than will the branchial arches themselves.

Drüner finds in Siredon a fourth branchial nerve with a distinct posttrematic ramus. From this nerve a branch is given off which bears such a relation to the rudiment of a fifth gill-slit

that he interprets it as a posttrematic ramus of a fifth branchial nerve. There is also found a possible representative of a sixth posttrematic ramus. In Siredon, as in other Urodela, the truncus intestino-accessorius X is plainly a nerve of multiple origin. Excluding the accessorius, lateral line, and posterior intestinal constituents, there is left a ramus intestinalis recurrens of motor and communis composition which represents parts of one or more posterior branchial nerves, that in certain respects have become much hypertrophied. If we consider the laryngeal cartilages as representatives of posterior branchial arches of urodelan ancestors, then the muscles connected with these cartilages and innervated by the ramus intestino-recurrens: musculi dorsolaryngeus, laryngeus ventralis, and so-forth, must be regarded as modified branchial muscles. There is beginning, or has already taken place, a considerable usurpation by the ramus intestino-recurrens, of territory of the ventral branchial region belonging orginally to the posttrematic rami of the primitive branchial nerves. The musculi subarcuales, and interbranchialis 4, and to some extent the ceratohyoideus internus muscle, innervated by the ramus intestino-recurrens, are muscles which originally had no relation to that nerve, but have been appropriated to some extent by it. In consequence of this usurpation the ramus recurrens has been disproportionately enlarged as a branchial nerve, and the third to fifth or sixth branchial nerves have undergone a variable amount of atrophy. Drüner believes that, in the common progenitors of the Selachians and the Urodele Amphibians, there must have been present at least seven branchial arches between the hyoid arch and the shoulder girdle.

In Siren the fourth branchial nerve is represented by a ramus pretrematicus, (*X.3,prt.*), which takes its origin from the pharyngeal division (*X.2,ph.*) of the third branchial nerve, rather than from the ramus intestino-accessorius X as in most Urodela. With this r. pretrematicus there may anastomose a pharyngeal branch (fig. 43, *s-r.1*) of the ramus recurrens sensitivus X. The representative of a ramus posttrematicus is seen in the nerve which innervates the levator muscle of the fourth branchial arch (*lab.4*).

This arises, not as in the other Urodela from the ramus intestino-accessorius X along with the ramus pretrematicus, but independently from the ramus intestino-accessorius near the vagus ganglion or directly from the ganglion.

A fifth branchial nerve is seen in a ramus pretrematicus (*X.4, prt.*) on the inner border of the fourth branchial arch, derived from a second pharyngeal branch (*s-r.2*) of the ramus recurrens sensivitus X. As has been noted, this pretrematic ramus is double. There is possibly to be seen here in this double condition a remnant of a sixth branchial pretrematic ramus. With one of these pretrematic nerves on the fourth branchial arch occurs an anastomosis from the descending portion of the main trunk of the ramus recurrens sensitivus. In Siredon Drüner describes a communis anastomosis between the ramus intestino-recurrens X and the remnant of the fifth branchial nerve. In the Urodela in general, as far as investigated, the fifth branchial pretrematic arises with the fourth from the ramus intestino-accessorius. From the ramus intestino-accessorius X of Siren, about opposite the fourth branchial arch, a large pharyngeal branch (*ph.i-a.*), arises, which possibly represents combined pharyngeal rami of the fourth and fifth branchial nerves. As noted, Drüner interprets the ramus recurrens sensitivus X, and, in part at least, correctly, as a representative of a possible fifth branchial nerve. Its position and relations justify such an assumption, but to the writer, as previously stated, it seems more reasonable to regard it as the communis portion of the ramus intestino-recurrens. In so far as the latter represents posterior branchial nerves the ramus recurrens sensitivus is also to be included in the same category.

11. The rami laterales dorsalis et medius

These two nerves have the same general course and distribution as in the Urodela in general, supplying the dorsal and medial series of neuromasts of the trunk. The statement of Drüner that all three main lateral line nerves leave the ganglion in a common trunk has not been verified.

Nervus glossopharyngeus (Urodela)[2]

Radix lineae lateralis (supratemporalis), cum radice lineae lateralis X
Radix motor
Radix communis (fasciculus communis)
Ganglion glossopharyngeum (ganglion petrosum?)
Ganglion lineae lateralis (supratemporale)
Rami:
- 1a. Ramus posttrematicus
- 1b. Ramus pretrematicus
- 1c. Ramus pharyngeus (anastomosis Jacobsoni)

} Nervus branchialis primus

- 2. Ramus communicans cum faciali (portio communis)
- 3. Ramus supratemporalis

Nervus vagus (Urodela)

Radix lineae lateralis
Radix spinalis
Radix communis (fasciculus communis)
Radix motor
Radix accessorius (motor)
Ganglion vagi:
- Portio lineae lateralis
- Portio communis (ganglion nodosum?)
- Portio spinalis (ganglion jugulare?)

Rami:
- 1. Ramus communicans cum faciali (portio cutaneus)
- 2a. Ramus posttrematicus X. 1
- 2b. Ramus pretrematicus X. 1
- 2c. Ramus pharyngeus X. 1

} Nervus branchialis secundus

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[2] Since the manuscript of this paper was sent to the publishers there has appeared a paper by Landacre and McLellan: "The cerebral ganglia of the embryo of Rana pipiens" (Jour. Comp. Neur., vol. 22, no. 5, 1912), in which it is shown that the ganglion of the ramus supratemporalis X should be classed with the glossopharyngeal rather than with the vagus nerve. This is in full agreement with the adult condition in Siren. Preparations of the embryo of Amblystoma tigrinum in the possession of the writer show beyond question that the ramus supratemporalis and its ganglion belong with the ninth nerve, the ganglion being widely separated in early stages from all other lateral line ganglia and closely joined to the glossopharyngeal ganglion. It is also worthy of notice that in Amblystoma embryos there is a distinct lateral line ganglion of the ramus auricularis X.

It is clear that the schematic analysis of the IX–Xth complex given above is very incomplete as far as the ganglia are concerned. Careful investigation of conditions in urodele embryos will doubtless dispel much of this uncertainty.

3a. Ramus posttrematicus X. 2
3b. Ramus pretrematicus X. 2 } Nervus branchialis tertius
3c. Ramus pharyngeus X. 2
4a. (Ramus posttrematicus X. 3)
4b. Ramus pretrematicus X. 3 } Nervus branchialis quartus
4c. (Ramus pharyngeus X. 3)
5a. (Ramus posttrematicus X. 4)
5b. Ramus pretrematicus X. 4 } Nervus branchialis quintus
5c. (Ramus pharyngeus X. 4)
6. Ramus auricularis
7. Rami laterales dorsalis et medius
8. Truncus intestino-accessorius
Rami intestinales
Ramus lateralis ventralis
Ramus intestinalis recurrens
Ramulus laryngeus recurrens
Ramus accessorius (ad musculum trapezium)

THE FIRST AND SECOND SPINAL NERVES

The first spinal nerve arises by two ventral roots and is exclusively motor. It is not certain that the double nature of its roots signifies that it is a compound nerve, for the second, third and fourth and presumably other spinal nerves have from two to four ventral roots or rootlets. It must be noted that the roots of the first nerve are more distinct from each other than are those of the other nerves. The main trunk of the first spinal nerve soon divides into a dorsal and a ventral ramus. The latter runs ventrally a short distance, turns sharply anteriorly and then posteriorly, and, giving off a few small branches to the neighboring muscles, passes postero-ventrally in the inscriptio tendinea between the first and second segments of the sub-vertebral hypaxial musculature until it reaches the dorsal wall of the pharynx. Passing posteriorly along the latter it is joined by the ventral ramus of the second spinal nerve. According to Drüner the two rami of the first spinal nerve emerge from the vertebral canal through separate foramina in the first vertebra. The writer finds that the main nerve divides while passing through the foramen, but there is no cartilaginous bridge between the two rami such as Drüner describes. This difference in description, however, may be due to the fact that the specimens studied by the writer were more immature than those to which Drüner had access.

The second spinal nerve arises by a single dorsal and two to three ventral roots. It possesses a small ganglion and divides into characteristic dorsal and ventral rami of mixed constitution. The ventral ramus passes posteriorly through the longitudinal musculature dorsal to the pharynx, comes into contact with the ventral ramus of the first spinal nerve as mentioned above, but does not unite with it until a level is reached a little posterior to the point where the ramus intestino-accessorius X breaks up into its larger divisions. There the two spinal nerves curve sharply laterally and ventrally around the lateral border of the pharynx and, running anteriorly at the dorso-lateral border of the hypobranchial musculature, unite into a common trunk. Drüner states that the two rami unite shortly after they emerge from the spinal column, a little posterior to the transverse process of the first vertebra. This may occur in exceptional cases but the writer has seen no indications of it. Rather the fusion takes place in the mode characteristic of the Urodela.

The nerve resulting from this fusion is the hypobranchialis of mixed constitution (fig. 44, *hgl.*). Its general cutaneous fibers are given off shortly after the formation of the nerve. The nerve runs at first at the dorso-lateral border of the hypobranchial musculature, but more anteriorly sinks ventrally until it lies at the ventro-lateral edge. Posteriorly, the nerve supplies the three anterior segments of the abdomino-hyoideus muscle, the sterno-hyoideus and omo-hyoideus muscles, and anteriorly the genio-hyoideus muscle only, the genio-glossus muscle, as Drüner has observed, being absent in Siren. As the two rami which form the hypobranchialis are passing posteriorly from their emergence from the spinal column they give off a few small branches to the longitudinal musculature, musculi intertransversales, through which they run. Shortly before they recurve to their point of union they give off a few small dorsal branches, some of which anastomose with the branch of the ramus intestino-accessorius X supplying the trapezius muscle, and others innervate the basi-scapularis muscle. From the ventral ramus of the second spinal nerve a branch is contributed to the brachial plexus. Otherwise the latter is formed from the ventral rami of the third and fourth spinal nerves.

SUMMARY

A study of the cranial nerves of Siren lends little if any support to the view that it is a primitive form. Rather, the opinion based upon general considerations of comparative anatomy (Drüner '04; Kingsbury '05; Emerson '05; Norris '11) that the perennibranch Urodela are permanent larvae of forms that once had a complete metamorphosis, is confirmed. This view has been set forth with great clearness by Drüner. The branchial nerves and musculature of Siren have an arrangement which can be explained satisfactorily only on the hypothesis that it is the result of general reduction processes, more or less modified, to be sure, by local and restricted specialization. The popular view (Holmes '06, p. 3) that "the Proteidae constitute the most primitive of the Urodeles" becomes absolutely untenable from the standpoint of comparative anatomy. Primitive amphibian characters are not to be sought in larval stages only, temporary or permanent, for the larval condition itself is to be looked upon as an amphibian acquirement and not an ancestral pre-amphibian character. In short, the amphibian larval characters are fish-like only by analogy. For a correct interpretation of the anatomy of Siren we should ignore its especial larval characters only as we compare them with corresponding features of the larvae of other Urodela. Though Siren may be the permanent larva of a form by no means primitive, yet among its specialized structures and retrograde developments, it may be possible, nevertheless, to distinguish very significant relationships and perhaps even primitive characteristics.

The olfactory nerve in Siren is more distinctly double in origin and distribution than in any other urodele amphibian.

The nervus terminalis seems to have the relations characteristic of the Urodela.

The eye-muscle nerves have the typical arrangement, but this may be due largely to their imperfect development.

The levator and retractor antorbital muscles, having their insertion on the antorbital cartilage, and their innervation by a branch of the ramus mandibularis V, have been described in but

two amphibians, Amphiuma and Siren. The occurrence in these two species of such peculiar structures has some definite significance. They suggest a closer relationship between these forms than has been suspected hitherto.

The facial nerve of Siren exhibits a number of peculiar and somewhat puzzling characteristics. Siren is the only urodele in which is found a levator muscle of the hyoid arch. This is innervated by a branch of the ramus jugularis VII. As Drüner suggests, this may have no especial significance, but be merely one of the peculiarities in structure of this form.

The lateral line anastomosis of the seventh nerve with the tenth nerve (*VII ad X*) may be interpreted as the persistence of a ramus oticus of fish-like ancestors, or it may be looked upon as incidental.

The contribution of maxillaris and buccalis fibers to the profundus-palatine anastomosis has such a closely corresponding arrangement in Triton (Coghill) and also in Salamandra, if von Plessen and Rabinovicz's figures be correct, that it can hardly be explained as incidental. Excluding the maxillaris and buccalis fibers, the profundus-palatine anastomosis is of such a character as to make it certain that Coghill discovered in Amblystoma its typical urodelan relations.

The ramus alveolaris VII in Siren is not, as has been assumed by some, essentially different from the corresponding nerve in the Urodela in general, but, as in Necturus, owing to the imperfect development of the opercular (splenial) ossicle, the alveolar branch proper is not confined to a canal in the jaw and in consequence does not form a definite anastomosis with an alveolar branch of the ramus mandibularis V. Yet both the trigeminal and the facial alveolar branches are present and in such a position that if the operculare were in its fully developed urodele condition, they would be enclosed by it. Bender ('06) undoubtedly interprets correctly the origin of the anastomosis between these two branches as due to the development of membrane bones around Meckel's cartilage and the confining of the nerves in a canal, for the primitive arrangement is doubtless that found in the lower selachians where the two nerves are free and without anastomoses.

But the condition in Necturus, and in Siren, is to be regarded as secondary rather than primitive.

The origin of the palatine and alveolar rami of the facial nerve by a common trunk is a condition so unique that we seek in vain elsewhere in the vertebrate series for a corresponding formation. It is certainly far removed from any primitive condition.

The presence of motor fibers in the palatinus caudalis, apparently innervating a rudimentary ceratohyoidean muscle, is so unusual as to provoke little but skeptical comment. Until more and very careful comparative studies are made it seems useless to speculate regarding the significance of such fibers and their vestigial muscle.

The apparent occurrence of a general cutaneous constituent in the facial nerve roots also suggests that it is possibly more generally present in other Urodela, but because of its minuteness and close association with other components has hitherto escaped notice. Its presence is best explained on the ground of a persistence of a primitive and at one time more fully developed characteristic of the facial nerve.

The peculiar relations of the ramus supratemporalis X (IX?) to the glossopharyngeal ganglion, and its development of a distinct ganglionic mass of its own suggest that we have here in Siren a condition closely bordering on that seen in selachians and ganoids where the ninth nerve is described as having a lateral line constituent. If it be permissible to speak of a lateral line portion of the facial nerve, and of the vagus nerve, rather than of a lateral line complex the parts of which are distributed peripherally with these nerves, then we may consider the ramus supratemporalis as a part of the ninth nerve in Siren. But, as Allis ('97) has shown in Amia, the ramus supratemporalis IX arises internally from the main lateral line tract of the trunk series of neuromasts. In the text of this paper the writer has treated the supratemporalis in Siren as a ramus of the vagus nerve.

The structure and connections of the ramus communicans vagi cum faciali in Siren are such as to warrant the conclusion previously expressed by the writer regarding this nerve in Amphiuma,

that the ramus is exclusively sensory and primarily general cutaneous.

In comparing the branchial region in Siren with that of other Urodela we must consider the larval stages of the latter. We then see that in Siren, as stated in foregoing sections, there has occurred a considerable modification in the posterior branchial region. The fourth and fifth branchial nerves have largely disappeared, and to a less degree the third, and to a great extent have been replaced functionally by the ramus intestinalis recurrens X, which may be interpreted as the much hypertrophied ventral portion of still more posterior branchial nerves.

We may hope to appreciate the real significance of the seeming peculiarities in nervous and other structures in Siren only as we work out more exactly their comparative anatomy. Before generalizations can be made safely there must be carried on investigations of the cranial nerves and associated structures of other Urodela, such as Cryptobranchus, Necturus, Salamandra, etc. Studies upon these and upon the Caecilia will doubtless throw considerable light upon the problems connected with the peripheral distribution of the cranial nerves of the Urodela. A study of the cranial nerves of Siren confirms the conclusion drawn from a consideration of its general structure that it, like Necturus, is a specialized rather than a primitive form, but that, nevertheless, there may be recognized in it structures and characters that suggest persistences and survivals from an ancestral condition. Drüner would derive the Urodela from a pre-selachian stock; Bender sees in the selachians, through the crossopterygians and dipnoans, the urodelan phylogeny. Obviously a study of Siren can aid but little in such theoretical considerations.

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THE DEVELOPMENT OF THE BLOOD AND THE TRANSFORMATION OF SOME OF THE EARLY VITELLINE VESSELS IN AMPHIBIA

WILLIAM A. HILTON

Department of Histology and Embryology, Cornell University, and the Institute of Anatomy, University of Minnesota

FORTY-FOUR FIGURES

INTRODUCTION

While collecting embryonic amphibian material several summers ago, I became much interested in the yolk circulation of living Desmognathus embryos. In the early stages these animals are entirely without pigment and the formation of vascular areas shows beautifully on the surface of the pure white yolk. It was possible, in the living embryos, to trace the development of the blood and blood vessels from the time of their first appearance until they were well formed in late larval stages. At first clear areas made their appearance on the surface of the yolk mass; later these became more extensive and ran together. Still later a gradually increasing network of clear lines became evident. There were blind ends and disconnected parts in early stages; these were afterward seen to form themselves into a closed system which became connected with the heart. As somatic vessels were formed in the extensions of the body-wall over the yolk, complex changes in the capillaries gave an excellent opportunity to observe their methods of growth and their transformations.

The first observations were made on Desmognathus, but other amphibian embryos were studies for comparison. In this paper the results from the study of two species of Urodela are given. Each of these offered some advantages; Amblystoma, with its smaller yolk mass was investigated by means of a large and

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complete collection of serial sections loaned me by Professor Gage; Desmognathus, because of its lack of pigment in early stages, was especially valuable for surface studies of living embryos. The difference in the size of the eggs and the differences in their early environment made these very interesting for comparison. The eggs of Amblystoma are smaller and laid in water. Those of Desmognathus are larger and are deposited on land in moist or nearly dry situations. A few series of frog embryos were studied but no particular attention was given to any part of development except the early formation of the blood.

The blood and blood vessels were considered to be derived from mesoderm, but a detailed cytological study of these cells was not followed out. This would be necessary in a consideration of the question of an angioblastic layer. The development of the heart and later blood vessels are not included in this study.

There are many opinions as to the origin of the vascular system in Amphibia. Probably no greater differences of interpretation are encountered in the literature than those dealing with the germ layers involved in various parts of the system. Among investigators favoring the entodermal origin of the heart endothelium were: Reichert ('40), Remak ('50), Goette ('75), Rabl ('87), Marshall and Bles ('90), Schwink ('91), Houssay ('93), Nusbaum ('94), Brachet ('98). Most of these also considered the vascular endothelium in general as entodermal. Some of those who believed in a mesodermal origin for the heart endothelium were: Van Bambeke ('67), Salensky ('95), Brachet ('03), Johnston ('03), Muthmann ('04), Mollier ('06), Greil ('08). The vascular endothelium was considered to be of mesodermal origin by Marshall and Bles ('90), Brachet '03), Mollier ('06), Greil ('08).

In respect to the blood there were also the two views as to its origin. Some of those favoring the entodermal origin were: Goette ('75), Schwink ('91), Houssay ('93), Brachet ('98). Those who gave it a mesodermal origin were: Marshall and Bles ('90), Brachet ('03) in Anura, Mollier ('06), Greil ('08), Mietens ('09).

Many older workers were of the opinion that the heart endothelium had an unpaired origin, but Schwink ('91), Houssay ('93), Mollier ('06) and Greil ('08), believe in a paired anlage.

Rabl ('87) described the development of the endothelium of some of the vessels as outgrowths from cardiac endothelium and a number of other observers, as Brachet ('98), and Mollier ('06) to some degree, favored this view of the origin of some of the vessels. Greil ('08) believes the vascular endothelium has a similar origin to that of the heart and that it arises at about the same time.

The origin of blood vessels independently of the blood, seems to have been shown in a number of cases. Schwink ('91), Houssay ('93), and Brachet ('98) were among the first to distinguish between blood-forming cells and the cells which develop into vascular endothelium

All the earlier writers, up to the time of Mollier ('06), recognized the general ventral region of the embryo which gave origin to the blood. Some considered it to be mesodermal, others entodermal. Molier traced this back to early stages and interpreted the material as arising from the mesoderm of the ventral lip of the blastopore; the vascular and cardiac endothelium arising in part from the splanchnic mesoderm. Greil ('08) traced the mesoderm still farther back in Amphibia and other vertebrates and recognized special areas of cells on the surfaces of blastula stages which afterwards gave rise to the blood and a large part of the vascular system. He also recognized some of the vascular cells as having a segmental origin, from somites. Houssay, at an earlier time ('93), believed in a segmental origin of the vascular system, but considered it to be from entoderm.

The way in which the capillaries and early blood vessels develop cannot be easily learned from the literature. Some of the early investigators believed than many of the first vessels were simply spaces in connective tissue, the endothelial walls being formed later from neighboring or surrounding mesenchymal cells. Others described the spaces, but believed that more or less isolated cells migrated in and gradually formed endothelium. Such was the idea of Goette ('75), and of Mollier ('06) in more recent times. Whether or not there was a circulation of blood before all the endothelium was formed, was not determined in many cases, but, judging from the vitelline circulation in some teleosts and from the descriptions and figures of Mollier, there seems to be some

evidence of it. The development of blood vessel endothelium from isolated cells as described by Mollier ('06), Greil ('08) and others, seems to be somewhat different from the growth of lymphatic vessels from sprouts as described by Clark ('09), in the tail of a frog tadpole. The development of blood vessels from capillary networks as described by Evans ('09) in certain vertebrate embryos, may not seem, at first sight, to be in accord with other work on Amphibia; but this lack of agreement may be explained by supposing that the early circulation was almost of an invertebrate type, merely in spaces before the capillary walls were developed, and that later certain of these channels were selected for the main blood vessels and gradually developed a more complete endothelium.

REVIEW OF LITERATURE RELATING TO THE EARLY ORIGIN OF THE BLOOD AND VASCULAR SYSTEM OF AMPHIBIA

Reichert ('40) believed that the heart in Amphibia developed from part of the original head yolk-mass and was at first solid. Remak ('50) made similar observations on the development of the heart and he probably recognized the fold cavity into which it develops. Van Bambeke ('67) considered the heart to come from the visceral layer of the mesoderm in Pelobates. Oellacher ('71) believed that the heart endothelium came from a fold from the splanchnic mesoderm, a line of cells being formed which folded off into a delicate tube.

Goette ('75) thought that the endothelium of the heart of Bombinator igenus came from a loose cell group detached from the ventral wall of the fore-gut. The blood vessels, to some extent, develop independently of the heart and blood, as spaces in mesodermic connective tissue, the walls being formed by cells migrating from the early yolk-formed blood masses. The corpuscles, according to him, arose from an unpaired blood island which forked in front at the level of the liver. Some of the vascular endothelium arises from this area, which also furnishes blood corpuscles.

Rabl ('87) describes the heart in Amphibia as arising between the mouth and liver and probably coming from entoderm. In

Salamandra, he considers the first aortic arches to be formed from the endothelium of the heart sac and suggested that perhaps all the endothelium of the vessels comes from it. Marshall and Bles ('90), were of the opinion that the heart endothelium of the frog was from entoderm at first, but later became united with the mesodermic cells and lost its original connection. The blood vessels are from mesoderm. In most parts of the body they appear as irregular spaces or lacunae. They are at first independent of each other, but soon extend so as to meet and open into one another as irregular channels. The cells surrounding these channels assume a more definite arrangement and convert them into blood vessels. The blood corpuscles are either cells which are inclosed within the lacunar spaces from the first, or cells budded off from the walls of the vessels into their cavities at a later stage.

Schwink ('91), working with Salamandra, Triton, Rana and Bufo, describes the heart endothelium as derived from paired masses of cells on the surface of the yolk and states that cells from these areas migrate forward. The blood vessel cells he considered as arising from a single primary budding mass, lying somewhat caudally on the yolk, and from the entoderm. The origin of the blood islands he conceived to be on the border line between yolk entoderm and mesoderm, if one consider the mesoderm to be formed by delamination. He comes to this idea through observations on Urodela, where he found as good evidence for the formation of blood from the entoderm as from the mesoderm, but he did not come to a definite decision, although he did not think it probable that two germ layers entered into the formation.

Houssay ('93), working with axolotl, believed that the whole vascular endothelium had a segmental origin from the entoderm. He called it 'angiotome' or 'parablast.' He thought that the blood originated from the central cells of the first solid anlage of the subintestinal vein, while the peripheral cells formed the vascular walls; the subintestinal vein being formed out of the long, compact mass of a single segmental derivative of the entoderm.

Nusbaum ('94), studying the Anura, came to conclusions similar to those of Schwink with regard to the vascular endothelium. He

also believed that cells migrated forward from these first masses. He believed that the blood and vascular endothelium were entirely from yolk entoderm.

Salensky ('95), regards the endothelium of the heart of frog as derived from the middle germ layer, and arising from mesoderm on either side of the heart area.

Morgan ('97), does not particularly favor either the mesodermic or entodermic origin of the heart endothelium. He makes the statement however, that if the cells forming this endothelium arise from the ventral wall of the archenteron, as has been described by some, they have a different origin from other parts of the heart. He describe the first blood vessels as lacunae in connective tissue which are lined by mesodermic cells. The vessels arise in part as outgrowths of already existing vessels and in part as isolated lacunae in the mesoderm. The walls in either case are from the same germ layer.

Brachet ('98), found in Triton an unpaired median mass of tissue between the mouth and the liver, arising from the gut-wall and forming the walls of the primitive heart cavity. He considered the cranial yolk vein to be partly developed from sprouts from the heart anlage and considered the general view of Rabl quite probable. He believed in an entirely entodermic origin for the blood in Urodela and clearly distinguished between blood cells and blood vessel cells. In 1903 Brachet extended his work to Anura but considered the heart endothelium of frogs to be derived from mesoderm and not entoderm as he had found in Triton. He recognized very clearly in the frog the same area of blood-formation as the earlier writers, Goette and Schwink, and considered it to be from mesoderm. Although he reviews his work on Triton, he finds nothing to change in his earlier paper, but a short study of axolotl convinced him that it is not possible entirely to exclude the ventral mesoderm from the formation of the blood-vascular apparatus.

Johnston ('03), found the heart endothelium of an unknown species of amphibian to be strictly mesodermic, although not at any time identified with the undifferentiated mesoderm. That is, it was split off from the entoderm in the same manner as the

rest of the mesoderm, only somewhat later and separately. This view of the origin of the endothelium might well be applied to other cases in which an entodermic origin of the heart has been given.

Muthmann ('04), does not agree with Brachet's account of the origin of the urodele heart from the entoderm, and he is of the opinion that Brachet confused the early stage of the thyreoid which is from entoderm, with the heart which is from mesodermal cells. These cells separate on the middle line and fill in a little cavity under the entoderm and between it and the ectoderm, just in front of the liver.

Mollier ('06), in his article in Hertwig's Handbuch, shows in Triton a little more clearly the origin of the heart from splanchnic mesodermal cells in either side of the body. These cells penetrate into the fold cavity described by Muthmann and form the endothelium. In Bufo he agrees in the main with Brachet, but finds no median anlage. He believes the cardiac endothelium arises from paired groups of cells derived from the visceral pericardial plate.

In Triton embryos of twelve somites Mollier recognizes early blood-vessel cells which separate more or less dorsally from the mesoderm on the surface of the yolk. Later there are developed in connection with these, or independently of them, lacunae on the yolk just under the splanchnic mesoderm, and the vascular cells penetrate into these and form the endothelial lining. In Bufo a similar development is shown, although the vascular cells are not so unquestionably of mesodermal origin. According to Mollier, Maurer ('92) saw in a Siredon embryo with fifteen somites two groups of cells, one of which he called connective tissue, the other, a ventral group, which he described as coming from entoderm and giving rise to the subintestinal vein. Mollier, however, believes the upper of these were the blood vessel cells similar to those which he describes, and the lower, heart forming cells. Houssay ('93) saw this ventral group and regarded it as the anlage of the subintestinal vein.

In Triton, Mollier traces the history of the mesoderm which arises from the ventral lip of the blastopore up to a time when it

becomes thickened and may be followed forward to the region of the liver. This forms the blood and becomes transformed into the vitelline vein in later stages. Although most of the blood would thus be of mesodermic origin, it was impossible to be sure that all of the early cells came from a thickening of this germ layer, because a certain small number of them seemed to be added from the entoderm. In Bufo the blood seems to come almost entirely from cells of the yolk mass. However he regards these, in a way, as mesodermic cells which have not yet separated from the others.

Marcinowski ('06), studied Bufo and Siredon. The results of his work in some respects resemble those of Muthmann, Mollier, and Greil. The heart develops from cells which migrate from the mesoderm into a little space just in front of the liver. These come from the mesoderm on each side of the middle line and not from a mid-ventral thickening. In one of his figures he shows the forming heart endothelium at an early stage, with two little cavities in the mass. He recognizes the ventral keel of entoderm which so many observers apparently have confused with the heart anlage, and shows very clearly in his diagrams how the confusion could have arisen. He, like Muthmann ('04), regards this as a part of the thyreoid anlage. The vascular endothelium he regards as formed from secondary mesenchyme. The ectoderm is shown to furnish some of the primary mesenchyme. Wandering cells which give rise to endothelium become localized in various places. All the larger vessels are developed as isolated areas which secondarily come into union. Vascular tissues are developed from the sclerotomes and mid-ventral mesoderm. This last area gives rise to the blood. The first blood vessels are solid and may or may not communicate with the spaces in the mesenchyme or those between connective tissue cells. The endothelium is continuous with the spaces at the time the blood corpuscles come into the circulation.

Greil ('08), traces the origin of the blood and vascular endothelium in Ceratodus, Amphibia and other vertebrates. He recognizes in early stages, at least approximately, the cell areas which

give rise to the vascular system. He traces the peristomial mesoderm back to blastula or early gastrula stages. Some of these peristomial cells extend forward to join with and form a part of the axial mesoderm; the cells which form mesoderm in the ventral lip of the blastopore are continuous with the others. The ventral part of the mesoderm becomes thickened and forms blood. The vascular endothelium of the heart and blood vessels come from isolated cells which migrate from the thickened masses, or from the more ventral axial mesoderm which is partly formed from peristomial cells as mentioned above. The ventral mesoderm giving rise to vascular endothelium and blood is given the name of 'angiohaemoblast.' Cells separate from the somites in the body region and differentiate from a corresponding part of the mesoderm in the head region. Those cells which are more or less segmental in origin soon lose such an arrangement and, with others, migrate to form part of the heart and vascular endothelium. As these vascular cells are associated with the sclerotomes they constitute the 'angiosclerotome.'

Greil and Mollier differ in a number of points: First, they do not agree as to the separation of dorsal from ventral mesoderm. Mollier, to some degree, takes as a basis for such a separation, the splitting of the more dorsal mesoderm into two layers, but Greil believes that this has no true significance in this connection. According to Greil's interpretation, it would be very hard to distinguish accurately the boundary lines between mesodermal cells of the dorsal lip and those from the ventral lip of the blastopore.

Mollier is in doubt as to how much of the ventral mesoderm in early stages is derived from yolk cells or entoderm, but Greil has an entirely different idea of these as he considers this mesoderm to be from 'ectoderm,' or cells on the outside in blastula stages and not from the primitive entoderm.

In a consideration of the question whether the thickened masses of mesoderm which go to form blood are derived from local thickening by multiplications of cells or derived from migrations from the region of the blastopore, Greil decides for the former.

He believes Mollier has no good evidence from cell division figures to show that the splanchnopleure contributes to the formation of blood vessel cells.

As to the question whether or not some cells are added to the blood from the yolk, about which Mollier was not willing to make positive assertions, Greil believed from his evidence from Ceratodus, that the yolk cells have no part in the formation of the blood.

Mollier believed the blood vessel strand, that is to say the subintestinal vein, was connected with the heart from the beginning. Mollier, Rabl and others think of the heart endothelium as taking part in the development of the vessels in connection with it. Greil believes that the heart is only one part of the general vascular endothelium and does not initiate the development of the rest. Greil agrees with Mollier as to the paired origin of the heart although he does not believe that the cells come from the splanchnic mesoderm as does Mollier.

Mietens ('09) traces the history of the blood in Bufo vulgaris. At an early stage the mesoderm is free from the yolk in the forward part of the embryo; it is not distinct from it farther back. The middle germ layer is continuous with the yolk cells ventrally. Later the ventral mesoderm splits off from the yolk It does not increase by a multiplication of cells from the lip of the blastopore, but by a separation from yolk cells and multiplications in situ. If the ventral lip of the blastopore does contribute mesoderm, only a little of the caudal portion is formed in this way and the distinction between peristomial and axial mesoderm has little significance here. In a later stage there is a secondary ventral fusion with entoderm. Mietens believes that Mollier saw only such later stages in his work on Bufo and that other early investigators in this and later stages considered the blood as arising from yolk cells. The blood develops from the ventral mesoderm after this fusion with the yolk. Wandering cells arise chiefly from the sclerotomes and more dorsal portions of the mesodermal sheet; possibly also some of the cells where the blood is formed give rise to wandering cells. The parenchyma of the liver forms blood and endothelial cells at an early stage.

EARLY DEVELOPMENT OF BLOOD AND VASCULAR SYSTEM IN AMBLYSTOMA PUNCTATUM

In specimens of about 2.8 to 3 mm. in length with six primitive segments, the mesoderm has extended well about the yolk just under the ectoderm. Back some distance from the region which will form the liver it is a continuous band, completely encircling the yolk cells; forward, in the liver region, it does not meet in the middle line and so forms a lateral band on each side. At this stage there is no indication of ventral thickenings and there were no blood vessel cells found such as Mollier describes for Triton (fig. 1). It is also impossible at this stage to be sure just how much of this mesoderm was formed from the ventral lip of the blastopore, although in a general way some of this caudo-ventral part may correspond.

In specimens of about 4 mm. length with 8 to 13 somites there is a decided ventral thickening of the mesoderm, both on the caudal fused part and the cephalic lateral portions (figs. 2 and 3). There is also a more decided indication of the little fold cavity just anterior to the liver, with a few cells which seem to have come into it from either side. These may be the cells of cardiac endothelium such as Mollier describes (fig. 4). There are also a few cells on the surface of the yolk, between it and the mesoderm, which are in the same position as the blood vessel cells recognized by Mollier and Greil and may correspond to them. These stain like other mesodermic elements and seem to have been derived from the splanchnic layer. They are much smaller than any of the yolk cells.

The ventral thickenings of mesoderm are the first indications of blood and there is no difficulty in tracing them through various stages into blood corpuscles and vitelline vessels. In this species they are at all times well differentiated from yolk and ectodermal cells. From the stages I have studied I am not at all inclined to regard the early mesoderm as added to by cells from the yolk; the earlier cells of the middle germ layer merely multiply to form the thickened masses.

In a specimen of 4.5 mm. or 17 somites, the heart is a mass of loose cells between the two pericardial chambers formed by the

separation of the two mesodermic layers in this region (fig. 5). This group seems to have been added to from the visceral layer, especially in several specimens in stages between this and the last one described. At this time, as in one earlier specimen (figs. 2 and 5), a few rather small isolated cells on the surface of the yolk in the dorsal and lateral regions begin to look more like early blood vessel cells, while the ventral thickening of mesoderm has become much more marked and, in the regions where blood will be formed, the cells are much smaller than adjoining yolk cells, and they begin to look more like blood corpuscles. The mass is median behind, and forked and lateral in front. It really is the thickened ventral mesoderm, and in later stages passes directly into the vitelline vessels.

In a specimen 5.5 mm. in length, with 19 somites, the heart is a solid mass of cells, and the pericardial cavity well formed. There are as yet no blood vessels and the ventral portion of the mesoderm is thick, while laterally it has become reduced to a thin line of cells (fig. 6).

Fig. 1 Section of an embryo Amblystoma, 2.8 mm. long, with six somites, showing *m*, mesoderm as yet not thickened. × 25.

Fig. 2 Section of embryo Amblystoma 3.5 mm. with 8 somites. The section is through the liver and far enough forward to show the mesoderm separated on each side. The mesoderm is thickened ventrally and some early vascular cells, *v*, on the left between mesoderm and yolk. × 25.

Fig. 3 Section through the same embryo as figure 2, only farther towards the tail end. It shows the beginning of the thickening of ventral mesoderm, *n*. × 25.

Fig. 4 Section through an Amblystoma, 3.5 mm. with eight somites, showing *h*, the early heart-forming cells. × 25.

Fig. 5 Section through the heart region of an Amblystoma 4.5 mm., showing also some vascular cells (*v*) on the right. × 25.

Fig. 6 Section of Amblystoma, 5.5 mm. long, 19 somites, mesoderm thickened below. × 25.

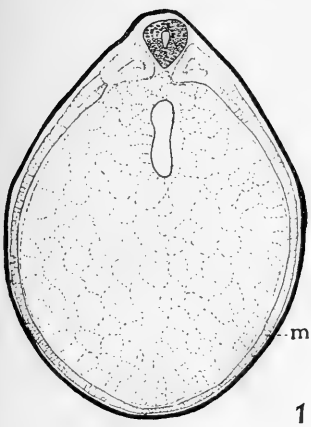
Fig. 7 Section through an embryo of 6 mm. or 22 somites, blood formed of ventral mesoderm. × 25.

Fig. 8 Section through an embryo 7 mm. long, showing two blood vessels, *b*. × 25.

Fig. 9 Section through an embryo about like figure 8, with blood spaces and blood corpuscles on the surface of the yolk. × 25.

Fig. 10 Sections through an embryo which shows lateral cutaneous vessels, *l*. The yolk vessels at this stage are lined with endothelium. × 25.

Fig. 11 Section of late larva showing lateral cutaneous vessels, and the ventral abdominal vein, *a*. × 25.



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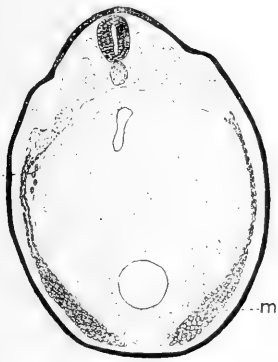

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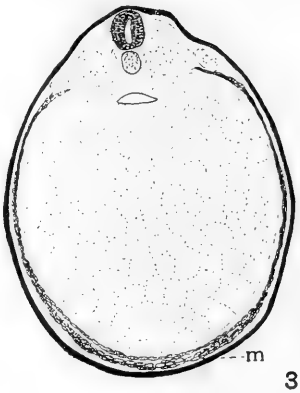

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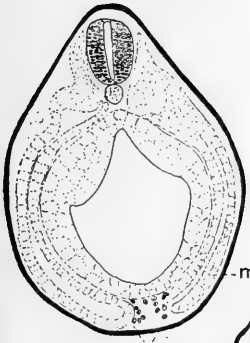

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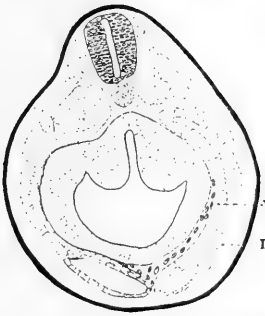

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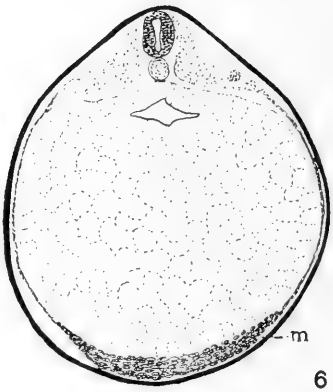

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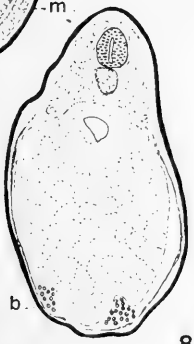

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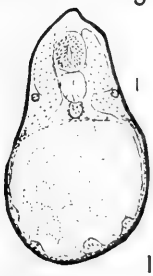

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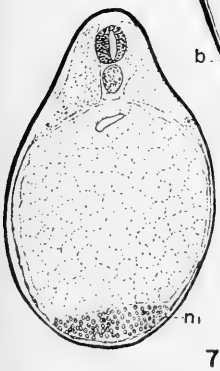

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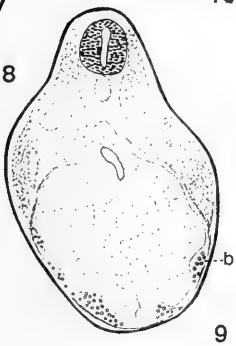

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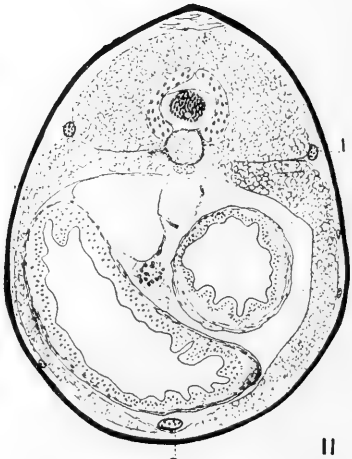

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In a specimen 6 mm. in length and of 22 somites, spaces are evident between cells of the connective tissue; whether lined by these or others I did not determine. In some specimens, apparently younger than this, spaces are found to some degree which do not seem lined with cells, but in one individual of this length many of the vessels within the body of the embryo have a decided lining. In the ventral head region two vessels lead into the heart, the aortic arches. Dorsal to the alimentary canal in the cephalic region two sets of vessels are developed, appearing as spaces in the mesenchyme. There is one each side of the nervous system and one on each side of the notochord just above the alimentary canal. The more dorsal of these divides a number of times until there is again a single pair above and below. The vessels of the more ventral pair migrate towards the middle line and nearly disappear, but fuse to form the dorsal aorta. Lateral vessels from the others are continued down into the somotopleure as spaces between cells and, like the heart and aorta, are without blood corpuscles. In the caudal region of the heart a large sinus venosus is found; continuing into this from both right and left are spaces or vessels in the somatopleure. Farther down, the sinus is divided into a right and a left portion, the right, the smaller becomes reduced and disappears first; these are parts of the right and left vitelline veins. Besides the connections with the somatic vessels at the sinus, there are indications of vessels out towards the blood masses of the yolk ventrally, but in this stage there is no communication. Beyond this, there are lateral vessels in the somatopleure, showing as wide spaces. Below this region in the splanchnopleure, spaces are developed on each side between yolk and mesoderm which, although not yet filled with blood, communicate with the lateral blood masses. These last appear, as in earlier stages, like thickenings of the mesoderm, but with a stronger suggestion of blood because of the small size of the cells and their prominent nuclei. Farther down the two latero-ventral blood masses become fused into a large one which becomes smaller as the caudal end is approached (fig. 7). Branches from the dorsal aorta are seen here and there, some of them seem to communicate with the vascular spaces on the surface of the yolk.

A wax reconstruction was made of the vessels and heart of a 22 somite embryo, 6 mm. in length (figs. 12 and 13). There were some difficulties in determining the exact extent of the blood spaces and the size of some parts may be exaggerated, especially some of the somatic vessels. The heart is shown as a twisted tube connected with a single aortic arch, from the forward part of which only two small branches arise. The aorta runs back some distance, with lateral branches, the vitelline arteries, communicating with the yolk spaces. Only one pair of these is shown. Just how many of these arteries there were was hard to determine.

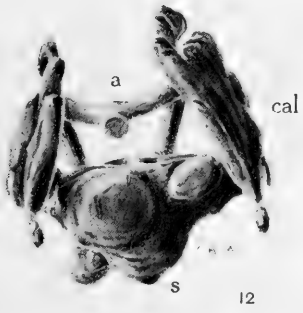


Fig. 12 Caudal view of a model of the vascular system of Amblystoma punctatum 6 mm. long, 22 somites. The caudal end of the heart or sinus venosus (*s*) is shown composed of a larger left side and a smaller right. Lateral to the heart the lateral cutaneous vessels are shown (*cal*). The cut aorta (*a*) is shown in the middle line. × 100.

The veins of the head region branch to some degree and run well back to unite with the broad spaces or veins in the body-wall. These lateral vessels communicate on each side rather broadly with the large sinus.

Spaces on the yolk, most of which are mere lacunae as yet, communicate with each other and come near the sinus on each side, but do not unite with it. These lacunae and beginning vessels also communicate at various levels with the lateral and ventral thickenings of the mesoderm and probably also with branches of the dorsal aorta in a more caudal region.

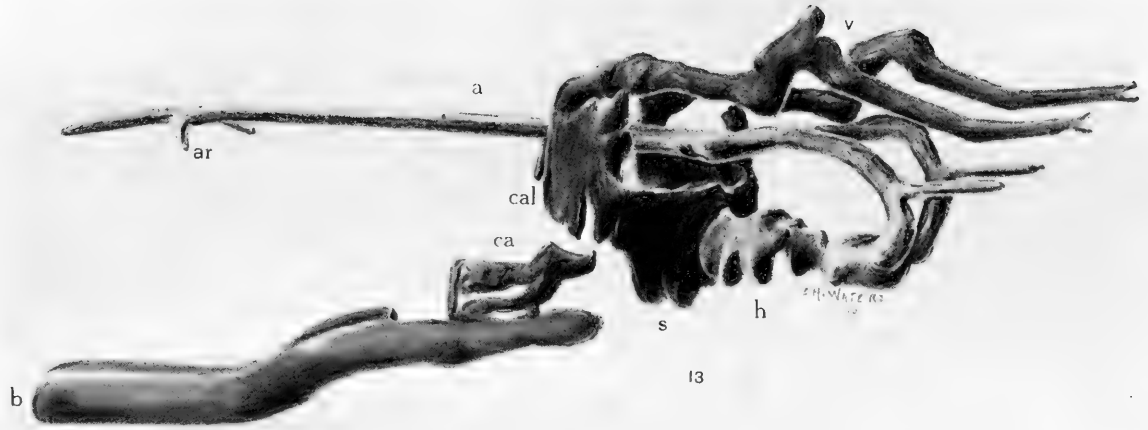


Fig. 13 Lateral view of a model of the vascular system of Amblystoma. The arteries are shown by a little lighter tone and from the aorta (*a*) only two of the vitelline arteries are shown (*ar*). The heart (*h*) is twisted. The spaces in the somatopleure (*cal*) communicates with the veins (*v*) and with the sinus (*s*). To the left the vitelline system is (*ca*) not yet in communication with the sinus. The solid mass, the fork of which is shown cut off on one side, indicates the ventral mesodermic thickening which will form blood and part of the vitelline vessel (*b*) while, extending from this up towards the sinus, are a few of the spaces or lacunae on the yolk surface (*ca*). × 100.

The large sinus venosus, just back of its connection with the veins on either side and back of its partial connection with the yolk spaces, is divided into two parts, a right and a left; the right is smaller and looses its cavity sooner than the left. Probably a great part of the caudal portion of the sinus may represent right and left vitelline veins.

In a specimen of about 22 somites and 6.5 mm. length, the heart leads into a large sinus venosus but not so large as earlier; it has a few blood corpuscles in it and in cross section is just over the backward extension of the liver. Farther down, the sinus becomes divided into two portions—a narrow left and a larger right vitelline vessel; the left soon has communication with the vascular spaces over the surface of the yolk as well as those up in the body-wall. On the right side the vessel remains large for a time, with little or no communication with spaces on the yolk, although it has broad and extensive connections with vessels which appear as large spaces in the body-wall. Farther down, beyond the disappearance of the portion just followed in the right, a yolk space comes into view, and on both sides the yolk vessels or spaces may be followed. In some places there are depressions on the surface of the yolk under the thin mesodermal sheet and these appear without lining and with few or no blood corpuscles, yet the yolk cells seem crowded in as though some fluid had pressed against them. Others of these spaces come to be lined by cells with large nuclei but very flat cell bodies, these cells are perhaps derived from isolated ones described earlier.

Farther down, the two blood masses occur in depressions on the surface of the yolk, communicating broadly with submesodermal spaces on it. The two ventral blood masses have become more like vessels and continue back on the yolk, separated farther from each other than in earlier stages. Only a small mid-ventral vessel some distance back remains of the median fused mass.

In another similar stage, the left vitelline part of the sinus venosus is short, but it has communications with somatic and yolk vessels. The right remains longer and evidently communicates with body-wall vessels and then disappears. At about this level a mass of blood occurs ventrally on the right and left sides.

Farther down, right and left blood masses are still definite; they are rather large and are not separated as far as in the specimen previously described. They fuse into a median mass at a more cephalic level; farther forward this becomes smaller and disappears. Numbers of well marked vitelline arteries communicate with the yolk lacunae, especially in the caudal region.

In another specimen of the same length the caudal part of the sinus venosus leads into a right and a left portion; the left or vitelline vein early receives branches from the dorsal body-wall veins or spaces and has some communication with yolk spaces and then disappears. The right communicates with veins of the body-wall with little or no connection with the yolk vessels.

In another specimen, apparently younger, the left vitelline disappears first, the right grows larger farther down and seems to have some communication both with vitelline and somatic vessels, the latter being best marked. The two lateral ventral blood masses are not greatly differentiated, but may be followed downward a considerable distance as two distinct groups until they approach each other and fuse in one at the middle line; towards the caudal end this becomes small and disappears.

In a specimen 7 mm. long, the right and left branches from the sinus are marked; the right is smaller and communicates in its upper region with somatic vessels, and farther down seems to run into a small right yolk channel. The left in a similar way communicates with both of these sets of vessels.

Blood may be found in the sinus venosus in all of these later stages in considerable quantities; it probably comes to it from its vitelline connections, and in these early stages cannot be forced out of the heart into the small and as yet only partially developed arteries.

In another specimen 7 mm. long the right branch from the sinus venosus is much smaller than the left, which is soon brought into communication with the large viteline spaces on the left side as well as the somatic vessels. The right in this specimen gives little indication of connections with yolk vessels, but is broadly connected with somatic. Farther towards the tail however, after

its communication with somatic vessels, it may be followed into a right yolk vessel and this may be considered to be either a continuation of the right vitelline or the anastomosis of the somatic with the yolk system. In this specimen blood has penetrated into the aorta but not into the somatic veins.

In another specimen of the same length the left vitelline branch of the sinus venosus is very large, with both somatic and visceral connections marked, while the right is small and with much less evident communications. In this specimen it is hard to trace the early blood masses because there are now a number of lacunae on the surface of the yolk, more or less connected with the earlier ones, and the corpuscles from the original blood masses have largely escaped from the earlier spaces and are found in some of the other channels. These cavities are not yet lined with endothelium to any great extent; that is, the blood is not inclosed in vessels and the corpuscles are free on the surface of the yolk (figs. 8 and 9). A few cavities alone show the beginnings of the formation of capillary walls, either from some of the earlier cells or from some of the cells of the blood masses. Individual cells change their bodies into thin plates while the nuclei remain large. In this specimen blood can be seen in the gills.

In a specimen 7.5 mm. long the right vitelline connection has been lost, but branches from the somatopleure communicate on the right side with the sinus venosus. On the left the vitelline vein connects with the blood spaces on the yolk and the sinus is in communication with the lateral vessels of the somatopleure. Farther along a vitelline vein may be seen on the right side, communicating with the vessels on the yolk, not with the sinus. Vessels over the surface of the yolk are prominent, with blood in them; a right and left ventral may be seen, these are united farther back and then lost, although there may be others more caudad. Down near the sinus there is a single small ventral vessel and back of it a small cavity with no blood. Other specimens of the same length showed practically the same condition.

In a number of specimens 8 mm. long, with 27 or more somites, the liver encroaches upon the sinus venosus and forms cords of

cells. The blood corpuscles are abundant in the sinus and rest on the liver cords in some places, while in others there is the beginning of a development of an endothelial covering formed by expanded cells, some of them perhaps derived from the blood mass. The lateral veins on each side come down but communicate with the sinus over the liver in its caudal regions, virtually with the liver itself after the sinus is passed. The vitelline veins are now represented by the left only, which comes into the left side of the liver. There are a number of vessels in the body-wall as described in earlier stages, while in the connective tissue at the sides of the body the lateral cutaneous vessels are first seen as small rather isolated tubes just under the skin. Through part of its length in the caudal region a connection with some of the lateral vessels was seen. The yolk vessels are very numerous, and many of them, especially the smaller, have endothelial linings. In the caudal regions of the aorta especially, there are connections with the yolk vessels, which have here, as farther towards the head, penetrated well under the body of the embryo. Many of the blood corpuscles in this stage are undergoing division, especially within the cavities of the liver. These and others are now almost without yolk granules, the protoplasm of the cell varying from a light to a dark pink with eosin stain. Within the cytoplasm of some of the larger cells, lighter spaces seem to indicate where yolk granules have been absorbed or have dropped out. In the liver and in some of the vessels, the corpuscles are crowded together in masses and blood is found in the gills.

In a specimen 9 mm. long the liver is much larger; into it or into the ducts of Cuvier, which may be recognized on its dorsal and cephalic surface, empty the two large lateral head or somatic veins. These are continued down a short distance on each side as large lateral somatic vessels and have branches communicating with the lateral cutaneous. The connections with the head vessels were higher up. The lateral cutaneous becomes larger as it runs down, or at least it has more blood, and beyond the forelimbs, branches from it may be seen in the body-wall. In this stage, as in several earlier ones, a vestige of the right vitelline vein remains, situated somewhat more dorsally than the left.

Branches from the left vitelline vein are given off to the liver, in this and later stages to form the hepatic veins; that portion of the vitelline vein which is back of these vessels might be called the subintestinal vein.

In a specimen 10 mm. long, about the same conditions were observed as in the last. There is a similar development of the lateral cutaneous vessel (fig. 10). The yolk vessels are small, but definite and lined with endothelium, and there is a larger central vessel on the ventral side of the yolk. The lateral cutaneous veins are similar and with no very extensive branches. The blood corpuscles are now well formed and much like those of the adult; both red and white may be distinguished.

In a specimen 12 mm. long, the intestine is well formed and we may recognize the hepatic-portal vein in connection with it. The lower portion has been formed from the left vitelline or the subintestinal vein. According to Hochstetter ('87), in Salamandra maculosa the vitelline vein retains its subintestinal relation throughout its entire length while in S. atra, Triton and Pleurodeles, the subintestinal vein passes around upon the dorsal side of the intestine and opens into a trunk of the portal vein. In this specimen of a 12 mm. Amblystoma the left vitelline has become somewhat changed in its cephalic portion, with the decrease in yolk and increase in differentiation of the intestine, while its upper portion has been developed into a more dorsal vessel. Hochstetter ('87) and Kellicott ('05) in Ceratodus, apparently in some forms at least, consider this more cephalic portion of the hepatic-portal to be a new formation. From what I have seen of Amblystoma I believe it is not entirely new, but rather a transformed portion of the left yolk vessel. The lateral cutaneous vein is large and branches from it extend down close to the epidermis. The ventral extent of these is least in the cephalic region. They extend about half of the way down in the middle portion of the body and two thirds of the way in the caudal region.

It is not until the larvae are quite large that a ventral abdominal is developed (fig. 11), formed, I believe, by the growing down of cutaneous vessels. It arises from some of these cutaneous ves-

sels in the mid-ventral line and ends in the liver; that is, it retains some of the original connections of the somatic vessels which arise in early stages in connection with the sinus venosus.

The development of the blood and early blood vessels of Amblystoma may be summarized as follows:

1. Cells, which apparently arose from mesoderm from both sides of the body, are found in the fold cavity recognized by Muthmann just in front of the liver. These were the earliest indications of cardiac endothelium and were recognized in a large and complete series of early stages cut in all planes. These seem to correspond to similar cells recognized by Mollier.

2. Mesodermal cells next to the yolk were recognized in a similar position to those described by Mollier, and may correspond in part to some of the blood vessel cells.

3. There comes to be a median ventral thickening of the mesoderm in the caudal region of embryos of about eight somites. In the cephalic region, where mesoderm does not unite across the middle line, this mass is continued on each side as a lateral thickening. The larger portion of the mass becomes transformed into blood corpuscles, but some of the cells develop into endothelium of blood vessels.

4. This area becomes more prominent in later stages and the cells are formed into blood corpuscles and they sink into the yolk-masses, but at all times they are easily distinguished from the larger yolk cells. As the embryo grows in length the fused caudal end of this area becomes farther removed from the heart and, as other vessels are developed, it comes to be much less evident.

5. All along the sides of the body under the mesodermal covering and on the surface of the yolk, spaces begin to be formed, distinct from the blood masses at first, later in communication with them. Somewhat later, mesodermal cells form endothelial linings for these spaces. In early stages the cephalic portions of the vitelline vessels seem to have somatic connections.

6. At about the time of the development of blood spaces on the surface of the yolk, vessels are developed within the body of the embryo. The heart has been formed into an endothelial tube and is in communication with the vessels of the body. Cau-

dally the heart leads into a large sinus, which in early stages has a duct of Cuvier connected with it on each side. Veins empty into these from somatic vessels and also a little later from yolk vessels. The caudal region of the sinus is divided into a right and a left part and thus becomes connected with right and left vitellines so that these veins have their origin from the lower part of the sinus, and from vessels which develop on the surface of the yolk, including the region of the blood masses. As the right vitelline loses its yolk connections, the left vessel drains the yolk. With the development of the liver, the forward end of this vessel comes to run into it. As the intestine develops and changes its position the cephalic portion of the portal comes to be situated more dorsally. The caudal portion forms the subintestinal, while branches are given off from the cephalic end in the liver to form hepatic veins.

7. Well marked cutaneous vessels are developed in rather late embryos; branches from these extend down into the body-wall and the veins retain their early connections with the region of the duct of Cuvier. Branches grow down, and developed from some of these or in connection with them, a mid-ventral vessel, the ventral abdominal, is formed in the somatopleure. This arises rather late and is connected with the lateral cutaneous vein and with the liver.

DEVELOPMENT OF THE BLOOD AND VITELLINE CIRCULATION IN DESMOGNATHUS

The first external indication of the development of blood is found in embryos some little time before hatching. At such a time the outline of the body is well shown above the surface of the yolk, the head end is well differentiated and limb buds are formed, but no pigment has yet made its appearance. The earliest blood stage recognized was in an embryo of about thirteen somites (fig. 14). On the dorso-lateral surface of the yolk sac of such an embryo, a series of rather small, clear specks of blood islands made their appearance. These were more or less isolated from each other and appeared as clearer areas on the surface of the large white yolk mass (fig. 14). In a little later stage, such as shown in figure

21, there is some evidence of anastomosis between these clear areas. Very soon after the first anastomosing vessels are formed, others are added with great rapidity and soon clear lines run over most parts of the yolk. In some embryos the pattern is of one sort, in others it has a slightly different character, but all show at an early stage certain vessels which are larger and clearer than the rest and some of these indicate where the earlier vessels were. Parts of these earlier blood islands and early vessels may remain for a time not completely connected with the rapidly forming network (figs. 22–25). Very soon the first vessels extend ventrally and laterally in every direction and in still later stages the yolk is covered by only a thin layer of ectoderm and a thin sheet of mesoderm, and the body-wall has not begun to grow over it to any degree.

After the formation of a network of clear lines, and about the time of the first development of pigment in the embryo, a slight pink color appears in the larger vessels and soon the heart begins to beat slowly, before there is much color in the blood and before there is any circulation.

At first there are two general systems of capillary network over the surface of the yolk, corresponding roughly with the two blood areas on the right and left sides, but as circulation becomes established these are all fused into one system, with a varying number of branches having many anastomoses, which reaches across the yolk and underneath the body of the embryo. Branches penetrate the body a little before the blood becomes red.

Fig. 14 Desmognathus embryo showing first indication of blood islands. × 10.

Fig. 15 Desmognathus embryo just beginning to have pigment. Side-view showing a vitelline circulation. × 12.

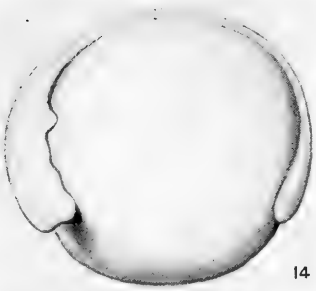
Fig. 16 Later stage, Desmognathus embryo, showing yolk vessels. × 12.

Fig. 17 Embryo of Desmognathus before hatching, showing lateral cutaneous and yolk vessels. × 5.

Fig. 18 Embryo of Desmognathus just before hatching; two parallel vessels have been formed in the body-wall. × 5.

Fig. 19 Larva of Desmognathus just after hatching, with three parallel vessels; the yolk vessel is reduced. × 5.

Fig. 20 Larva of Desmognathus some time after hatching but still with the mother. This shows a ventral abdominal vessel and cutaneous vessels connected with it, the last shown only on the left side. × 5.



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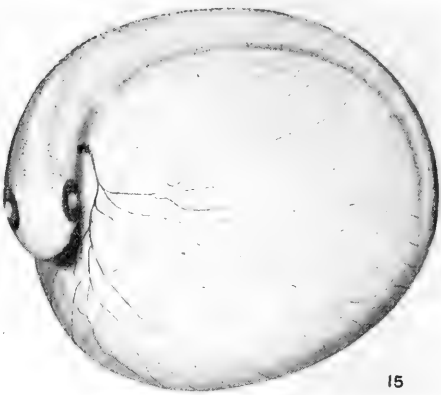

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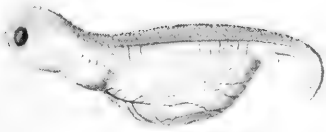

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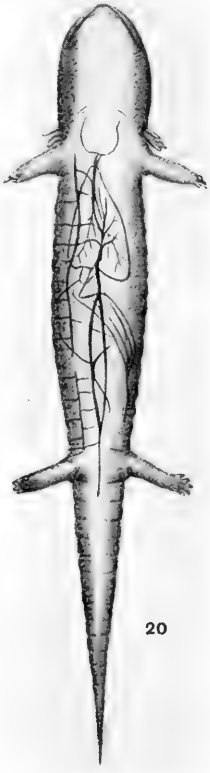

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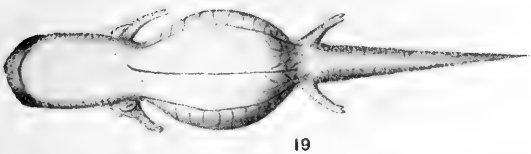

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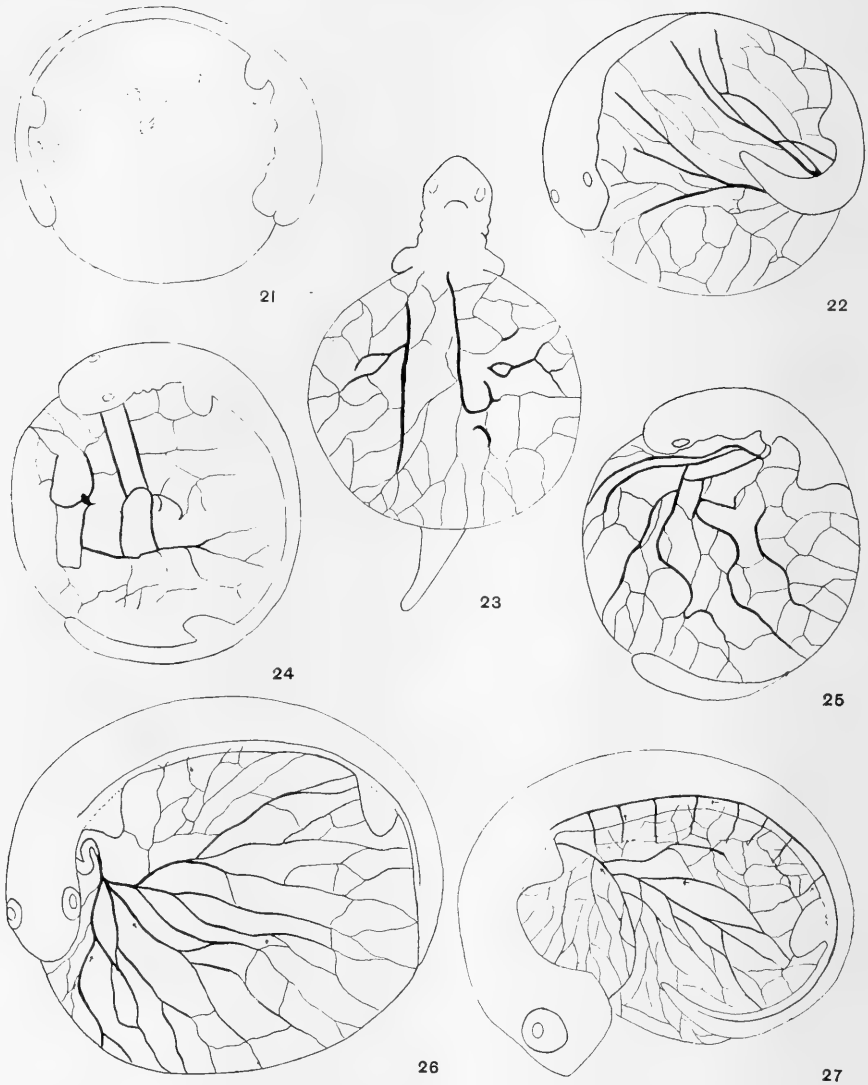


Fig. 21 Outline of early embryo of Desmognathus before pigmentation, showing early blood islands. × 10.

Fig. 22 Early embryo of Desmognathus, showing vessels and channels partly extending over the yolk, but with no circulation and no color in the blood. × 10.

Fig. 23 Early embryo of Desmognathus, the vessels beginning to form appear as clear lines on the white yolk. × 10.

Fig. 24 Side view of another early stage of Desmognathus. × 10.

Fig. 25 Later stage, Desmognathus fusca embryo, showing early vessels; there is yet no color in the blood; the heart is beginning to beat. × 10.

Fig. 26 Stage in which the lateral cutaneous vessel shows blood colored and a complete vitelline circulation. × 12.

Fig. 27 Later stage of Desmognathus, twisted on the yolk; it shows vitelline circulation, lateral cutaneous and the beginning of the first parallel vessel. × 12.

As the circulation becomes stronger and as more and more pigment develops in the embryo and in the blood, the current in all the blood vessels on the surface is towards the head, and these vessels unite into one which joins the heart (figs. 15 and 16). Just at the edge of the body of the embryo the rather large lateral cutaneous vein may be seen with its blood flowing towards the head end. This may be found in a specimen such as shown in figures 17, 26 and 27, at a time when the embryo has begun to become twisted on the yolk. The blood on the surface is in the splanchnopleure, that in the lateral cutaneous vein is in the somatopleure. The steady stream in the lateral cutaneous vein in slightly later stages speaks of an abundant supply and its seems quite probable that some of its blood is obtained from the yolk surface at an early stage if not in a later one, but no clear indication of an anastomosis was found. In later stages the yolk vessels become more and more prominent, the blood is bright red and the flow in the larger vessel more vigorous.

As the embryo coils more about the yolk, the body-wall or somatopleure grows down, and at an early stage, before this has been continued very far and before there is much pigment in the embryo, a series of vessels on each side enters the lateral cutaneous vein from the edges of the body-wall. There are at first about eight of these on a side, more or less equidistant from each other. They are parallel and all are perpendicular to the lateral cutaneous (figs. 17 and 27). In some specimens their lower ends seem at first to be in *slight* communication with the yolk vessels, but as the body-wall continues farther over the yolk these possible connections are lost and a line of anastomoses, parallel with the cutaneous, connects a number of the ends of the perpendicular vessels, so that a more or less closed somatic system (fig. 27) is formed. The blood may be seen running under the somatic vessels and into the larger vitelline, while the blood of the somatic parallel may flow either from the cephalic or from the caudal end into the perpendiculars. When the first perpendicular somatic vessels are developed in connection with the lateral cutaneous they do not seem to be connected to each other. There is no circulation in them and the movement in the lateral cutaneous is slow and jerky towards the head end. Later, about the time a parallel

vessel begins to develop, the ends of the perpendiculars seem to become rather irregular and one or two fine capillary vessels may be seen to connect adjacent lines before circulation. In embryos of about 12 mm. total length when stretched out free from the yolk and at a time when there is considerable pigment developed in the body, a vessel parallel to the lateral cutaneous connects the ventral ends of the perpendicular somatic capillaries which are somewhat variable in number, in part probably due to the stage of development. An early number of these is six and in larger embryos ten to eleven such vessels are found on each side (fig. 28). In some of these larger embryos there may be ten on one side and eleven on the other. Sometimes vessels connecting these perpendiculars may run the whole length of the yolk without a break, but more usually, after about four of these near the head end, there is an interruption with no connection across the lower ends of two adjoining perpendiculars. The blood in all of these runs into the lateral cutaneous, but in the head end the current in the first part of the parallel is towards the tail, the flow coming from the region of the liver, while in the caudal segment of the parallel, the current is towards the head end. The distance between the perpendiculars is somewhat variable.

In all stages up to 12 mm., while these vessels are gradually being formed, the blood of the vitelline system is about as in younger specimens; all portions of the yolk mass, even under the embryo, are drained by an extensive capillary network with frequent anastomoses, and the blood flows with great rapidity in the large and small vessels which are mostly on the ventral part of the yolk.

In larger embryos some vessels seem to a slight degree to extend down on the yolk beyond the limits of the body-wall, but I could not completely satisfy myself, even in early stages, that there was an anostomosis between somatopleuric and splachnopleuric vessels. I believe that if there is any such anastomosis it is chiefly at first, and is not extensive.

At a stage of about 12 mm. total length and a day or so before hatching, but at a later stage than the above, the pigment in the body is much more abundant and has extended down so far into

the body-wall that the lateral cutaneous vein is shut off from view. Indications of other perpendicular vessels begin to show themselves in the lower extension of the body-wall, and soon, in a similar manner, a second series of perpendicular branches and a second parallel is developed (figs. 18, 29). I have no clear indication that this second set is formed from the vitelline vein at an early stage before circulation in the new vessels is perfectly established, as it is difficult to make out all of the vessel ends before there is blood in them. Usually there are from eight to eleven perpendiculars in this second set, but their number and distribution, like those of the first, are somewhat variable. This second body-wall parallel may be interrupted in its course, some times in its central part, or near the head or tail end, and not always symmetrically on the two sides. The blood runs from the head region towards the tail in the cephalic part of the system and from the tail region cephalad in the caudal segments. Although I could not trace it very clearly, I am sure that the first and second parallel and perpendicular sets of somatic vessels change somewhat by the time the next series is developed.

At about 14 mm. length (figs. 19, 30, 31 and 32) a third, more ventral, somatic parallel is formed, while by this time the first is more or less covered by pigment. This third, in some specimens at least, seemed rather smaller than the others and with only a few communications with the lateral. Parts may not all be connected with each other and the current of the blood is from the cephalic region. There are at first however, only a few cephalic connections. The vitelline veins, which have been large up to this time, are now reduced to one main ventral trunk with fewer and less marked branches. It seems that some of the functions of the visceral circulation were taken over by the progressively greater growth of the somatic system. The body-wall is now well down on the reduced yolk sac.

In larvae of 20 mm. (figs. 20 and 33), taken with the female although well able to swim, there is some indication in the yellow yolk sac on part of the intestine that the yolk is not yet all absorbed. The vessel from this and from the intestine is now clearly a part of the portal vein and the body-wall is completed below it.

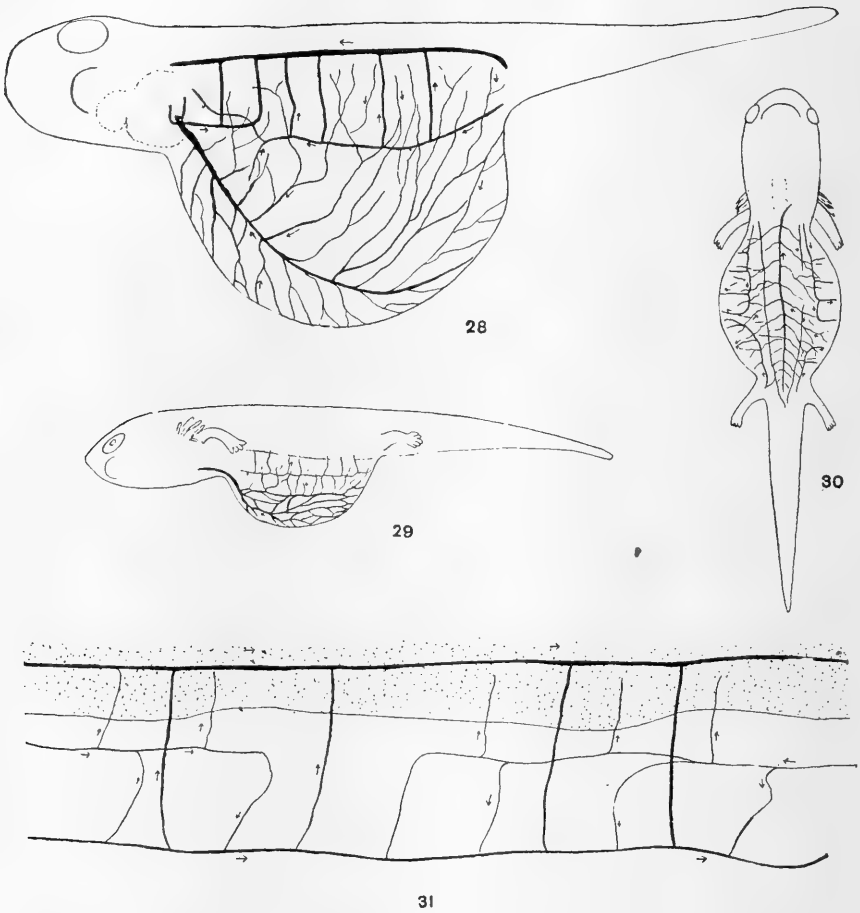
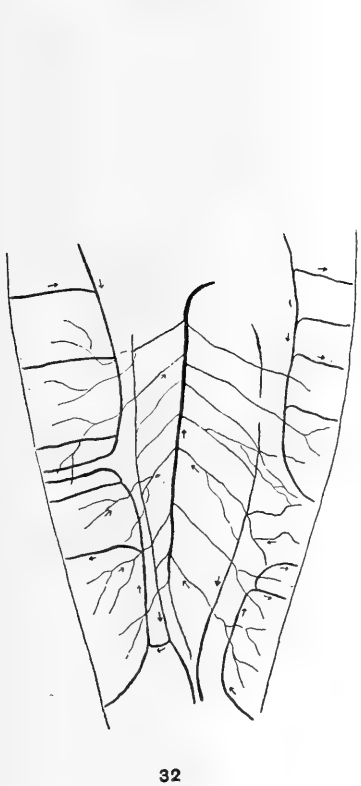


Fig. 28 Diagram of the circulation of the blood in a Desmognathus about 12 mm. long, and before hatching.

Fig. 29 Sketch of a Desmognathus embryo just before hatching, showing the direction of flow in the blood vessels at a time when the first parallel has been formed. × 5.

Fig. 30 Sketch of vessels in a larva of Desmognathus just after hatching, having three parallel systems of vessels and a central yolk vessel. × 5.

Fig. 31 Diagram of the flow of blood in the central portion of the cutaneous system of a Desmognathus larva 14 mm. long. The head end is at the right. The heavy vessel is a part of the lateral cutaneous. The dotted area is the pigmented edge of the body-wall. Two parallel vessels with their perpendiculars are shown.



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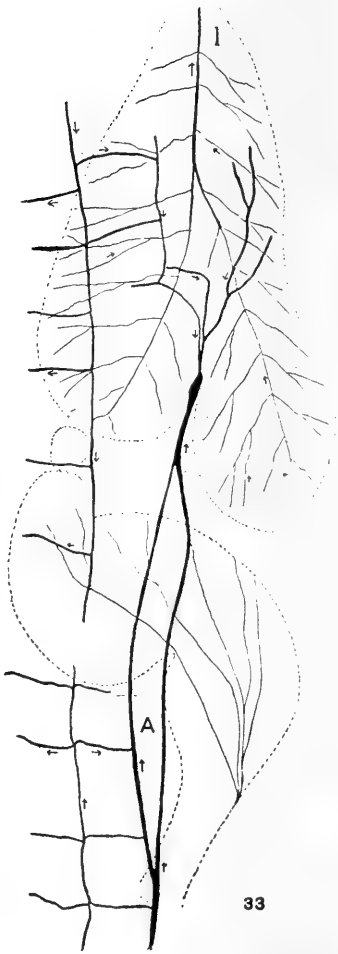

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Fig. 32 Diagram of the ventral vessels of a 14 mm. larval Desmognathus, shown from below.

Fig. 33 Diagram of the vessels of a 20 mm. Desmognathus larva, showing ventral abdominal (*A*) flowing into the liver (*l*) and the lateral cutaneous vessels flowing into it.

A large vessel from the ventral body-wall, double except for a little of its cephalic and caudal ends, opens into the capillary network of the liver. This is an early stage of the ventral abdominal vein. Laterally a series of somatic vessels are easily seen to correspond with the earlier parallel systems already traced; perpendicular branches from these run into the two lateral parts of the ventral abdominal which may be considered to be the last of the series of parallels, and in this stage, is not so completely fused into one as it is later. The lateral parallel and perpendicular vessels have a complicated course similar to that already traced in earlier stages. The cephalic portion of this system anterior to the ventral abdominal vein has broad but rather indirect connections with the liver at about the same point as that at which the abdominal enters it. I am inclined to think that these liver connections are part of an earlier one.

The study of serial sections brings out some points not shown in surface views of the living embryos. Probably partly because of the larger yolk mass, the mesoderm does not develop in quite the same way as in Amblystoma. At the close of segmentation the smaller cells at the animal pole migrate about the yolk; some of them form a thin, single-layered ectodermal covering, others form mesoderm. With the closure of the blastopore many of these small cells are carried to the dorsal side of the embryo. By this time some of the mesoderm has been formed lateral to the notocord, but this does not penetrate far ventrally. A dense mass of cells at the caudal end of the embryo, mostly in front of the blastopore, but also behind and lateral to it for some little distance, represents a region of undifferentiated germ layers. It is from this mass that mesoderm is formed in all directions. This fuses with the lateral and cephalic mesoderm formed earlier. Because of the size of this area and the position of the blastopore in it, it is impossible to be sure how much mesoderm is ventral and corresponds to that part which Mollier recognizes as giving origin to the blood.

At an early period, when these two chief areas of mesoderm can be recognized—that lateral to the notocord, and the caudal mass—there is still a large area of yolk not yet covered by a middle germ

layer, especially on the ventral side towards the head end. After the mesoderm has penetrated over a large area, between the ectodermic covering and the yolk, it becomes very thin except near the body of the embryo; so thin that it is difficult to make out more than a very slender line of cells. In the later development of mesoderm there seems to be little evidence that the yolk or entoderm contributes to its formation, for the yolk cells are large and have large granules, but it is possible that some small cells left behind by the migration from the animal pole may earlier or later join with the mesodermal ones.

In sections of embryos of about 13 somites (such as figure 34) the first indication of blood is shown by little lateral thickened masses of nuclei; these, I believe, are derived from mesoderm which has penetrated under the ectoderm at an earlier stage, but is now for the most part so thin as to be hardly noticeable (fig. 38). The first mesodermic indications of blood correspond in position to the first signs of blood island seen in surface views. These are compact groups of nuclei in which I was at first unable to see anything of a vessel wall (fig. 39). There is usually at least one longer thickened mass on each side but it was not considered to be a particular part of the early mesodermal sheet. Later, smaller masses become more numerous and may be somewhat isolated at first, but soon join one another, as the surface views indicate. In later stages other masses develop over the yolk, while some of the earlier ones become larger and may begin to show signs of a cavity as certain of their cells develop walls of the vessels (fig. 35). By this time the body of the embryo has developed blood vessels but they are without blood. Spaces occur on the yolk and for a time they are without endothelial linings (figs. 40, 41, 42); the walls afterwards gradually form in them from special vascular cells which probably bud out from the blood masses. The formation of spaces on the surface of the yolk goes along with the development of additional blood masses not inclosed in an endothelium, but when the blood is circulating, such spaces are probably penetrated by corpuscles almost as soon as formed, although it may be some time before the spaces are lined by endothelium. Figure 40 shows a space without endothelium but with blood cells.

In specimens a little later than those figured for the earliest blood masses, the heart is well formed, the mesoderm encircles most of the yolk, blood vessels within the embryo are without blood and the lateral channels are to some degree lined with endothelium (figs. 43 and 44). The early blood vessels are largely dorso-lateral in position as regards the rest of the yolk; right and left vitelline veins connect with the heart, the left being larger. In later stages the left vitelline remains, the vessels on the yolk are prominent, the corpuscles are well formed and there are communications of the aorta with the yolk vessels by a number of small vitelline arteries.

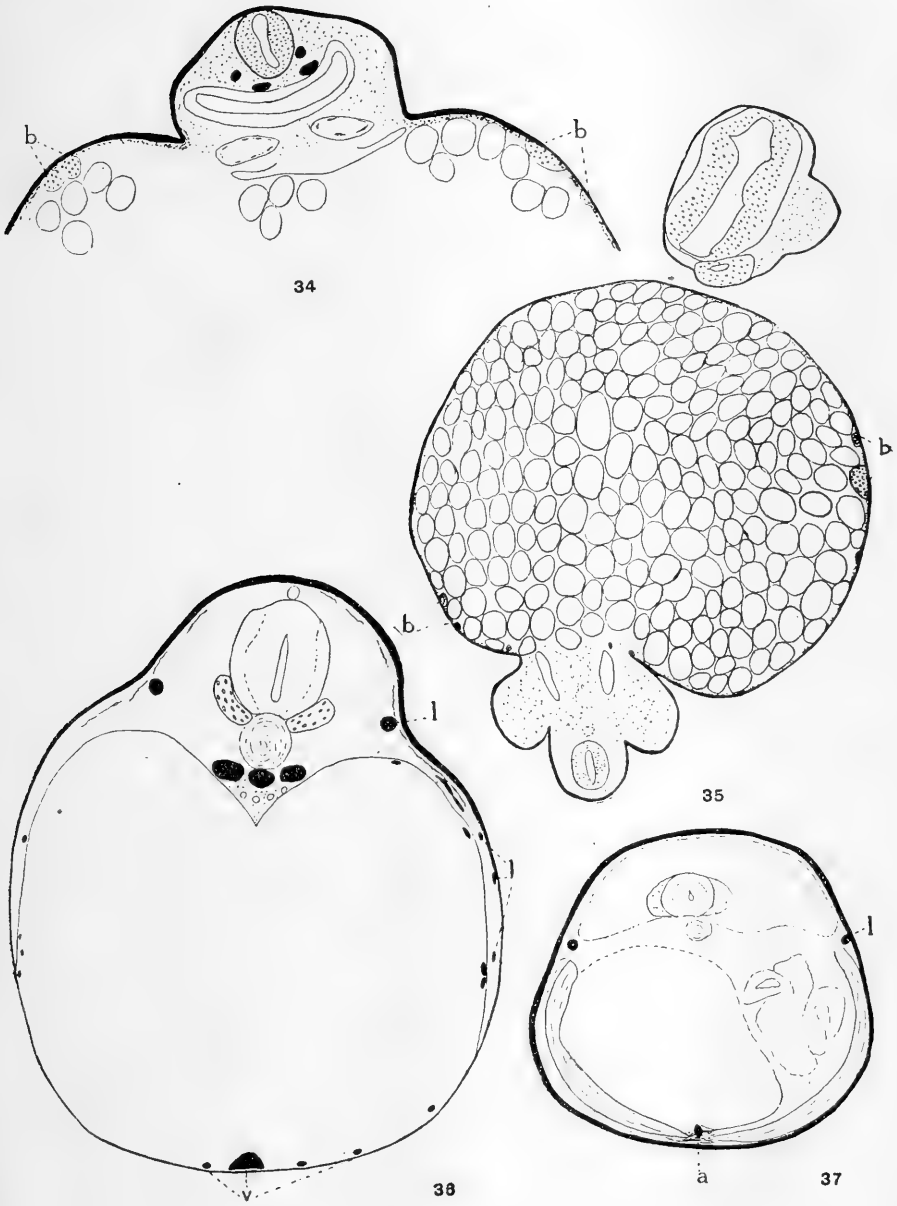
Some little time before hatching and before the embryo has uncoiled from the yolk and shortly after pigment is evident along the sides of the body, the lateral cutaneous vein is seen as already described. Sections of embryos of 10 mm. or less and before hatching show the lateral cutaneous on either side, a little ventral to the nervous system and near the surfaces of the body. It is not seen in later stages from the surface because of the development of pigment over it. In successively older embryos, as the extra cutaneous vessels develop as seen in surface views, sections show them extending down into the body-wall as it comes to encircle the yolk (fig. 36). These blood vessels, as could be seen to some extent from living specimens, were in the somatopleure. In sections of very much later larvae (those 18 to 20 mm. in length) the yolk is very much reduced, the vitelline system has in part become the hepatic-portal, the lateral cutaneous vessels have extended down ventrally into all parts of the body-wall. Connected with these cutaneous vessels, near the middle line ventrally, are two vessels partly fused into one. These evidently represent

Fig. 34 Section of the head end of an embryo of Desmognathus, showing the two vitelline vessels within the body and the beginnings of blood islands (*b*) on the yolk. × 25.

Fig. 35 Later embryo of Desmognathus, showing blood islands (*b*) on the surface of the yolk. × 25.

Fig. 36 Larval Desmognathus, about 14 mm. long showing the position of blood vessels in the body wall (*l*) and on the surface of the yolk (*v*). × 25.

Fig. 37 Section of a Desmognathus larva of about 20 mm., showing the position of the lateral cutaneous vessels (*l*) and the ventral abdominal vein, (*a*). × 25.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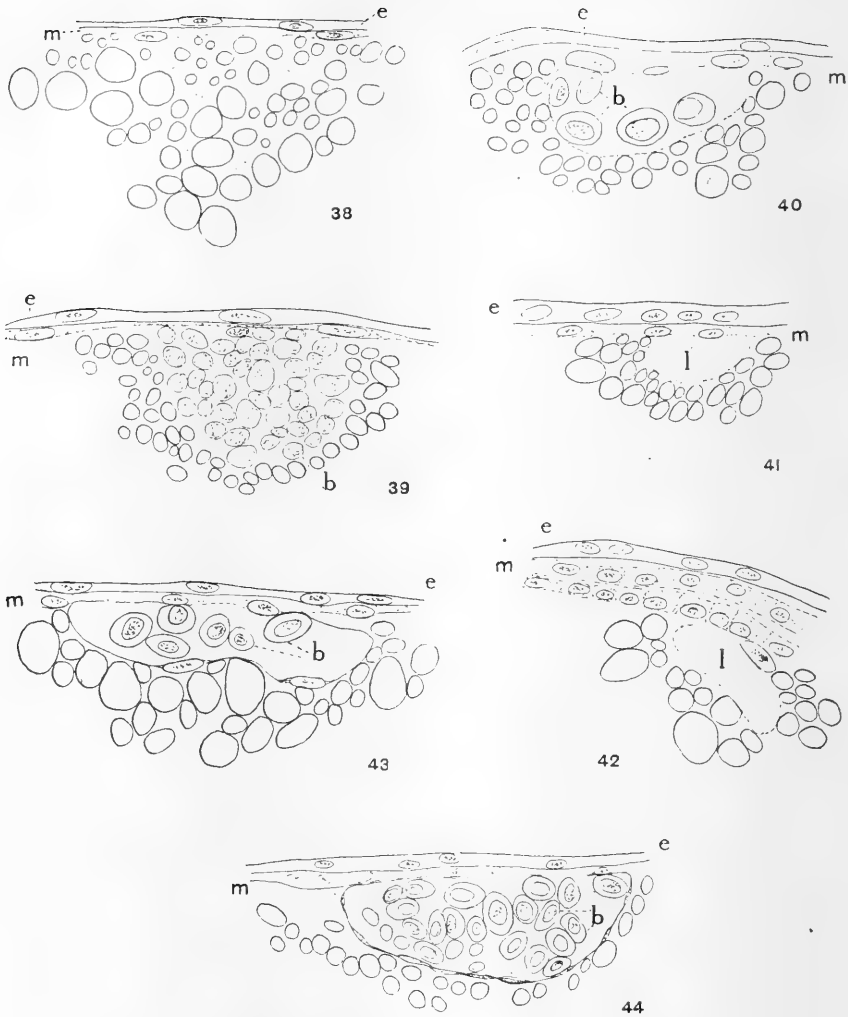

Fig. 38 Section through the ectoderm (*e*) and mesoderm (*m*) on the surface of the yolk of a Desmognathus embryo, such as is shown in figure 21. The section does not pass through a blood island; yolk granules in outline. × 300.

Fig. 39 Section through a blood island (*b*) of a Desmognathus embryo, such as is shown in figure 21. × 300.

Fig. 40 Section through a blood channel of Desmognathus before the formation of an endothelium; (*b*), blood cells. × 300.

Fig. 41 Section through a lacuna (*l*) on the surface of the yolk of a Desmognathus embryo. × 300.

Fig. 42 Section through a blood channel without blood (*l*) in the dorsal region of an embryo of Desmognathus. × 300.

Fig. 43 Section through a yolk vessel of an embryo about 12 mm. long, showing capillary endothelium. × 300.

Fig. 44 Section through yolk capillary of a 14 mm. embryo of Desmognathus, showing endothelium and blood corpuscles (*b*). × 300.

an early stage of the ventral abdominal vein which seem to develop in connection with the cutaneous system (fig. 37). This ventral abdominal gives evidence of its paired origin even up to the adult. In early larval stages it lies in the ventral body-wall, but in later larvae it becomes somewhat separated from the somatopleure. In sections, the cephalic portion of the vein is found to be very close to the vessels of the liver during an early period; later, this cephalic part of the ventral vein is found connected to the liver capillaries. Just when it first becomes united to liver vessels I was unable to determine, nor can I say anything now of the possibility of its cephalic portion being developed from part of the vitelline system, but it develops in the somatopleure, while the vitelline is formed in the splanchnopleure.

GENERAL CONCLUSIONS

The blood develops from the mesoderm on the surface of the yolk. In Amblystoma it may be more clearly followed from *ventral* mesoderm and it develops more ventrally on the yolk. In Desmognathus its development does not begin from continuous thickenings of mesoderm but from isolated areas.

The exact limits of axial and peristomial mesoderm are hard to determine, as Greil ('08) seems to recognize, so the conclusions of Mollier as to the early origin of the blood and the even earlier history of the blood- and vascular-forming cells given by Greil ('08) must be considered to be somewhat theoretical. It is not difficult to duplicate in Amblystoma, the stages which these authors figure and describe, but it is much more difficult to do the same for Desmognathus. From the position of the blood islands in this species I cannot feel sure that some of the more dorsally placed blood masses are not from axial mesoderm.

The reason why Desmognathus fusca forms blood islands as it does is probably due to the fact that the larger amount of yolk causes a more meroblastic type of development as is shown in its earlier stages.

The development of blood from the surface of the yolk in the case of Bufo, as described by Mollier ('06), and its development in

other Anura may simply mean that the cells are of similar origin in all cases, but there may be difficulty in recognizing them, due to slight differences in position and character of the cells in the two groups of Amphibia.

I examined a number of series of Rana sylvatica and found in them the first blood apparently being formed from ventral yolk cells. This appearance I think was due to the fact that the mesodermal cells and yolk cells in this species are so nearly of the same size and stain so much alike in early stages, that it was impossible to tell them apart in certain regions.

The first vascular endothelium in Amblystoma may be formed from some of the cells recognized in a position similar to those described by Mollier, but for a long time the early blood spaces on the yolk are without endothelium and much of the more ventral vessels(and probably some of the more dorsal ones as well) may receive their endothelium from the general ventral thickened mass. In Desmognathus, the first blood masses were the first indications of blood vessels and evidently, here at least, the cells which go to form the endothelium come from these first groups. Later developed vessels in this species seem to get their endothelium from the first areas. I was not able to determine vascular cells coming from the somites such as Greil ('08) described.

The heart was only studied in Amblystoma, where there was a close agreement with the work of Muthmann ('04) and Mollier ('06), as to the position of the cells which form the endothelium.

To what extent blood circulates in the early blood spaces which have no endothelium is a question, but it is evident in both species that there is some circulation in these before they receive their lining.

In Amblystoma at least, the vitelline veins develop first from the ventral thickened mesoderm. This is fused in one mass behind, but towards the heart it forks to correspond with the place where the mesodermal sheet is divided ventrally. Greil ('08) considers the heart and vitelline veins as continuous from the beginning, but in Amblystoma in some early stages there is a separation, or at least spaces are developed on the yolk which are for a time separate from the heart.

In Amblystoma and Desmognathus both a right and a left vitelline vessel develop; in later stages the left becomes the larger and persists as the vitelline vein. As the liver develops, branches from the vitelline vein are formed in connection with it and these become the hepatic veins. As the yolk sac becomes somewhat reduced and as the intestine begins to differentiate, the anterior vitelline vessel becomes changed and the posterior portion, which remains about the same, may now be called the subintestinal. The anterior part of the left vitelline of early stages comes to be a more dorsal vessel which develops more and more with the development of the intestine and this, with the subintestinal vein or posterior portion of the early vitelline, forms the hepatic-portal. This posterior part of the hepatic-portal is not a new structure in the strict sense.

The development of the first blood vessels in the body-wall of the embryo which form the lateral cutaneous, may be due to the penetration of yolk vessels at an early stage, but later the two systems develop practically independently of each other. The lateral cutaneous vessels in Amblystoma are formed a little differently from those of Desmognathus, probably largely because of the differences in yolk, but from the later developed vessels of these, the ventral abdominal is formed.

In both species there seems to be some indication that the first vessels formed after the lateral cutaneous, have some communication with the liver or the sinus. It may be that the ventral abdominal retains its connection with the liver through the modification of some of these early vessels. However this may come in much later, for as set after set of lateral vessels is formed, each one makes different connections forward and the third set has no relation at first to the liver. The position of the anterior ends of the last, just under the liver, would bring it into the proper position to join the hepatic capillaries as somatic and visceral vessels would be in contact.

The development of the ventral abdominal vein is, as Hochstetter ('94) pointed out, from paired somatic vessels. These are not early vessels or transformed parts of such, but correspond to

the last somatic set which develops as the body-wall grows about the yolk.

In Amblystoma the ventral abdominal cannot be easily traced because of so much pigment on the outside; in sections the body-wall has, even in an early stage, grown down quite a distance and contains large blood spaces, some of which remain as cutaneous vessels, others become transformed into the ventral abdominal.

Hochstetter compares the formation of the ventral abdominal to the allantoic vessels of higher vetebrates. These capillaries which migrate in this way about the yolk mass may give some indication of how such vessels originated or how they might have been formed, even though the vessels themselves may not exactly correspond.

The reason for the development in stages of perpendicular and parallel systems is evidently due to the gradual overgrowth of the body-wall upon the yolk. This may explain why such a progress is not so striking in Amblystoma. The development of systems of parallels is probably due to different periods of overgrowth when the advancing edge becomes thick enough for a vessel.

As the somatic system is increased the vitelline becomes more and more diminished; some of the functions of the vitelline system are apparently taken over by the somatic vessels, for at such a period the yolk mass is still large.

In general, I see no serious conflict between the investigations of Evans ('09) and the results from these observations on Amphibia. In certain regions of various sectioned specimens there is some indication of the formation of blood vessels from early capillary networks, such, for instance, as in the body-wall of Amblystoma near the heart. Also on the surface of the yolk and in the body-wall of living embryos the development of later vessels is through the selection of certain channels of the capillary networks, but the very first vessels or those developed on the yolk surface at an early period are more or less isolated and gradually anastomose with each other to form the yolk network. Many of these early capillaries, in both Desmognathus and Amblystoma, are without endothelial walls. At first many of them have no blood, but later there comes to be a circulation in some of the

spaces, while other channels are being formed, so that in the truest sense many of these early vessels are not capillaries because they do not yet have endothelium. Whether or not the spaces within the embryo which develop into capillaries and vessels, are, like some of the early yolk channels, without endothelium, and are merely spaces in the connective tissue which later receive a lining from vascular endothelium, I cannot tell, but I am inclined to believe that many of these early vessels are merely spaces between the mesodermal cells. That the lacunae themselves in the body of the embryo develop endothelium from the surrounding tissue I am inclined to doubt, and whether the spaces come to be lined by the growth from sprouts of earlier vascular areas as described by Clark ('09) for lymphatics, or whether vascular cells migrate in and gradually form an endothelium, as seems to be the case on the yolk at least, will have to be left for other investigations to decide.

The history of the development of the vessels in Amphibia may be summarized as follows:

The cells which form the endothelium of blood vessels seem to be from mesoderm. Some, at least, are from the rather solid thickened masses found in connection with the early development of the blood. Other cells may be formed from rather isolated areas such as described by Mollier and Greil and shown in some of the figures in this paper. However formed, these cells probably soon penetrate into spaces on the yolk and possibly also into the body of the embryo. But on the yolk at least, there seems to be a circulation for a time before the endothelium lines all these spaces. Perhaps not all of these channels are selected for the circulating blood after the establishment of an endothelial lining of the vascular system. New blood vessels are apparently formed by budding from those formed earlier, in a manner possibly similar to the growth of lymphatic vessels in frog larvae. Such vessels were seen in the process of formation in the growing edge of the body-wall as it comes to enclose the yolk in Desmognathus. Some of these fine vessels are kept for blood channels, others, in the constant reorganization which is going on in the advancing edge, may be much modified or lost.

May not these two conditions and periods of development of the blood vascular system in Desmognathus be applied theoretically to explain and reconcile some of the conflicting opinions of the day regarding the development of lymphatic vessels?

Some of the early vessels in which there may be a circulation for a time are mere spaces. Soon an endothelium is formed in these spaces, extending in or being formed from rather isolated cells; later after the establishment of endothelium there is a development of sprouts from the functional vessels, such growths as Clark ('09), describes in the frog tadpole.

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GERM CELLS OF COELENTERATES

I. CAMPANULARIA FLEXUOSA

GEORGE T. HARGITT

Zoological Laboratory, Northwestern University

TWENTY-ONE FIGURES

CONTENTS

INTRODUCTION

In an earlier paper (G. T. Hargitt '09) an account was given of the maturation, fertilization and segmentation of two of the tubularian hydroids, namely, Tubularia crocea and Pennaria tiarella. In the present consideration of one of the campanularian hydroids the complete oogenesis will be referred to, though not with equal attention to all phases. It may seem superfluous to add more to what has already appeared on the cytology of the coelenterates, and to call attention once more to the old view of Weismann concerning the origin and behavior of the germ cells of the Hydromedusae. But some features, such as continuity of the chromosomes, peculiarities and uniqueness of the germ cells, have come to have such a large place, on account to their relation

to heredity, that an examination of some of the assumptions on which the theories of heredity rest is not only of interest but more or less necessary. Weismann, who has been the great exponent of the uniqueness (one might almost say the sacredness) of the germ cells, based his hypothesis principally and primarily upon his work on the Hydromedusae. It has been found by Goette and others that many of his views on the place of origin of germ cells in hydroids, their place and manner of ripening, what he called the 'germinal track,' etc., are quite erroneous, but they are still referred to as bases for conclusions on other problems and theories.

Since it was found that Campanularia flexuosa offered such an unequivocal answer to some of these very questions it has seemed to be worth while to make a careful study of it and the results are here set forth.

MATERIALS AND METHODS

The material was collected, some in 1908 in Great Harbor at Woods Hole, some in 1909 in Casco Bay, Maine, at South Harpswell. I wish to thank the directors of the biological stations at these places for the courtesies extended to me. The material so collected was killed in Zenker's fluid, in Bouin's picro-aceto-formol, in Mann's picro-corrosive-formol, and in several of the other common killing mixtures. As noted in the earlier paper, it was found that material allowed to remain in alcohol for any length of time deteriorated somewhat, particularly as regards the finer details of the nucleus. When this was demonstrated the material not imbedded at once and preserved in paraffin was used sparingly or for comparison on grosser points of structure; the details here shown have all been worked out from material which was imbedded as soon as possible after being killed, in each case within two weeks after the capture of the material. Since then I have made a practice of imbedding the material within a few hours or a day after killing, with excellent results. As has been suggested by others (Smallwood '09, C. W. Hargitt '11) this is a very necessary precaution, since it does make a noticeable difference in the results.

Material killed in the mixture of Bouin seems to be especially favorable for study, showing in fine detail even very delicate structures; on the whole the fixation from this mixture seems to behave better for coelenterates than any other I have used. One can depend on the results given by it, since a close comparison of results obtained from this and from other fluids, as mercuric chloride, osmic acid, picric acid, potassium bichromate, platinic chloride, and so forth, show no essential differences, it being mainly a difference as regards staining. The very granular appearance found after the use of mercuric chloride mixtures, however, is in large part artificial, due to the vigorous precipitation of the colloid substances by the mercury. The material killed in Bouin's fluid has been used more than any other, though the results have always been checked by reference to other methods of fixation.

I should like to call especial attention to the fact that during the study of this form there has been a study of living material as well. Of course there are many features that could not be seen in the living eggs, but a great many structures of the nucleus and of the cytoplasm are almost as clear in the living material as in the sectioned and stained preparations. To check the work on Campanularia, living eggs of Obelia were also observed and the conditions found are the same as the ones figured for Campanularia. The sections show that all these features observed in life were faithfully preserved and clearly shown, and one may conclude, therefore, that the things not seen in life have probably been well preserved also.

In the paper on the tubularian hydroids I discussed the matter of stains; some or all of these have been used here. Heidenhain's iron-hematoxylin, as might be expected, has given the best results in the delineation of the form of the nuclear and cellular constituents. Combinations of various sorts have been used to determine, as far as possible, the similarity or dissimilarity of various constituents and their possible significance, origin and fate. The sections were usually cut 5 to 7 μ thick in order to have the nucleus in few sections, with the result that where chromosomes were present they usually appeared in a single section.

OBSERVATIONS AND DISCUSSION

1. The gonophore

The general position of the germ cells and their relation to each other is shown in the longitudinal section of a single gonophore in figure 1. In this particular instance the eggs are all in different stages of growth, though there is great variety in this. Especially characteristic is the definite arrangement of the eggs, so far as the stage of development is concerned, the older eggs at the distal end while in the proximal region the eggs are so young as to have hardly started their growth. It is this arrangement of the eggs which, in part, makes Campanularia so favorable for study. There need be no uncertainty of the stage of development of a certain egg, for if this egg does not itself show clear evidences of its stage of development there are those distalwards which are certainly older and the ones lying proximally are younger; both of these, therefore, serve to indicate the probable age of the egg under consideration.

Another advantage comes in the ease of comparison of stages. It often happens that the eggs at the proximal end of the gonophore are just beginning to grow, or are half-grown, and the distal eggs are in stages of cleavage or perhaps are planulae ready for liberation. In a single gonophore therefore, it is possible to get a number of different ages and to be certain of their relative stage of development, a condition that is not easily attained in eggs that develop freely and separately in the water, particularly in those cases, as the hydroids, where there are not many eggs ready for fertilization at the same time. This makes possible, not only a more certain but also a closer gradation of stages than is easily obtained under the ordinary conditions of development. These things make very clear the order in which certain things happen and therefore make a logical interpretation more precise. It seems very certain that, under the circumstances, no mistake has been made in the arrangement of the series, and no important stages have been omitted.

2. Origin of the egg

In figures 2, 3 and 4 the earliest recognizable egg cells are shown, and from this to the mature egg ready for fertilization an unbroken line has been traced, so there is not the slightest question that the

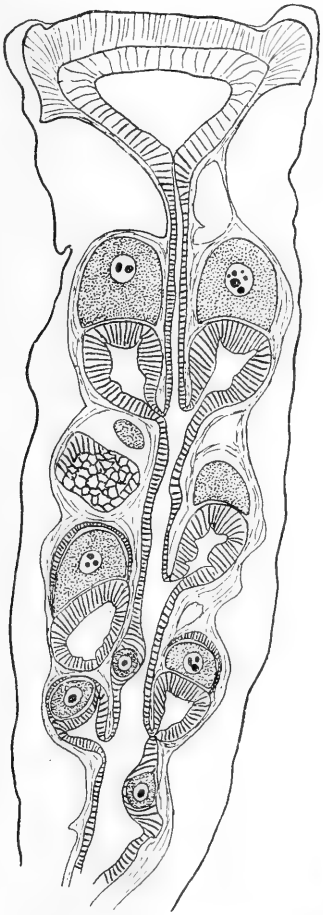

Fig. 1 Longitudinal section of an entire gonophore, showing eggs in various stages of growth. × 130.

cells shown are indeed egg cells. These cells are developed in the pedicel of the gonophore only and in no case were they found in the stem adjoining; and in the pedicel they came only from the

entoderm. In the ectoderm of this region no cells were found which looked at all like those figured, or any others that had any of the characteristics of germ cells, so it is certain that we have an entodermal origin of the egg cells. Goette ('07) found also that the eggs of Campanularia flexuosa which he examined, as well as those from most other campanularian hydroids, came from the entoderm and from the entoderm alone. He was not able to determine whether in this case the cells came from a single transformed epithelial cell as he had found in some hydroids or from the basal half of a divided epithelial (entoderm) cell as he found in other cases. Figures 3 and 4, especially the former, leave no doubt that an entodermal cell divides, the basal half forming the egg cell. Figure 2, however, seems to suggest the transformation of a single entodermal cell into an egg cell. There is no reason why both methods may not be active; certainly after it has been found, as Goette did, that some hydroids produce germ cells from ectoderm or entoderm indifferently, it might be expected that some would produce germ cells from entire or from half-cells indifferently, and this I believe to be the case in Campanularia flexuosa.

The place of origin of the egg cells of the hydromedusae, once of great interest, has ceased to have very much importance. Weismann ('83), in his actual observations, found evidences of some germ cells arising from the ectoderm and others coming from the entoderm. (His statement on p. 145 concerning Campularia flexuosa, that in the ectoderm there are cells which appear similar to the youngest egg cells, is not correct for the material which I have studied.) But later, especially in his volumes on Evolution ('04) he refers the origin always to the ectoderm, and if they are not actually to be distinguished there, says they must at any rate originate there and later migrate into the entoderm where they become demonstrable as egg cells. Entirely aside from the theoretical importance which he claims for the place of origin and the subsequent migrations, views long since shown to be without any firm foundation or real significance, it is interesting to see the many cases where an undoubted origin from the entoderm is demonstrated. C. W. Hargitt ('06) showed in Clava

leptostyla that the entoderm was the place where the egg cells originated; Goette ('07), in his very extensive paper on the germ cells of hydromedusae, showed conclusively that in many forms the germ cells came from the entoderm, others from the ectoderm and still others indifferently from one or the other. He says, for example, referring to Podocoryne carnea, that egg cells were found in the entoderm of the bud, in the ectoderm, and from all developmental stuffs of the medusa bud (p. 81). Again: "For me, therefore, no doubt exists that the germ cells of Clava multicornis proceed only from transformed half-entoderm cells" And later (p. 414) he says: "However, after it has once been established that the germ cells of Hydropolyps originate sometimes in the entoderm, sometimes in the ectoderm . . . . it is naturally of little fundamental concern which mode is current for the separate species." Smallwood ('09) in Hydractinia finds the egg cells arising in the entoderm. C. W. Hargitt ('11) in a general paper on coelenterate ontogeny calls attention to the work of Goette and of others and of the stand which Weismann has taken in regard to these facts, and reference should be made to these papers for a fuller discussion.

It may not be amiss, however, again to call attention to the fact that the work of Goette, the present paper and others furnish exactly the evidence demanded by Weismann himself as a proof of the origin of germ cells from the entoderm. Weismann says (p. 237) "If the egg cells were of entodermal origin, they must proceed by cross division of ordinary entodermal cells, with the result that the distal half bordering the enteric cavity remain epithelial cells, the basal half becoming only germ cells." The work of Goette shows this, and the present paper in figure 3 represents that just this thing has occurred, the basal half of the cell becoming a germ cell, the remainder continuing as an ordinary epithelial cell. Figure 4 is another drawing of a similar stage; in this the nucleus of the distal half of the original epithelial cell is present in another section. As already stated, not all egg cells of C. flexuosa are so formed, some coming from a transformed entire entoderm cell (fig. 2) but in all cases from the entoderm.

The entodermal cells from which the egg cells have arisen appear not to differ in any way from any of the other entoderm cells of the region; they are all cells which line the coelenteric cavity of the pedicel of the gonophore. Before the multiplication of the cells which led to the formation of the gonophore took place these cells were regular entodermal cells lining the coelenteric cavity of the stem of the hydroid; there is nothing that would set one cell apart from any other in position, size or appearance. In other words, previous to and in the early stages of gonophore formation *all* the entodermal cells of this region would be considered as ordinary differentiated epithelial cells. And any entoderm cell has the power of becoming a germ cell, provided only that it is in the position where the gonophore is to form. If no question of assumed theoretical importance came up, as of the origin of the germ cells, probably no one would even think that one of these cells had any different history, or any different potentiality than every other cell. Much less would he say that even though no difference could be discovered by the most delicate technic and with the latest and most refined optical apparatus, yet one particular cell must have had a different history, must have different capabilities, must be of a sort fore-ordained for a particular purpose since, forsooth, it came to a different end from its neighbor. And yet is not this just the argument of those who insist that the germ cells must of necessity be different, and have a different history from the somatic cells?

In Campanularia flexuosa there seems not to be the slightest doubt that the egg cells have arisen from differentiated epithelial (entoderm) cells lining the coelenteric cavity, which are not different from any others in the same place. The first indication of any difference comes when the nucleus increases very much in size; forms a spireme of chromatin, more or less regular in its arrangement (figs. 2, 3, 4); and a little later the cytoplasm is a little more compact and stains more deeply (figs. 3, 4, 5). As already mentioned, this does not occur in the prophase of dividing cells not forming germ cells. And in the egg cells the apparent prophase is not followed by any division, but from this time on, as shown in figure 2 to 6, and so forth, there is an unbroken series

of stages through the entire history of the egg. Furthermore, there is no division of the cell to form generations of oogonia and oocytes, but from the earliest stage found, up to the mature egg, it is the growth and development of a single cell. In other words, an individual cell (or half of a single cell) of the entoderm transforms directly into a single mature egg without any divisions in the process, until the polar bodies are formed.

Smallwood ('09) found in Hydractinia that egg cells are transformed directly without any immediately preceding cell divisions and C. W. Hargitt ('11) refers (p. 525) to similar occurrences in Pachycordyle and Eudendrium, and others have found similar cases. The point here made is that the form under consideration shows, in a manner so clear and striking as to leave no doubt, that it is not possible to speak of a 'continuity of the germ-plasm' in the sense of cells early set aside and more or less carefully guarded from contact with, and participation in, the activities of the so-called somatic cells. For it is clearly shown that the egg cells arise from, and are a further development of, an individual (already differentiated) entoderm cell, which up to the time of the transformation was performing the same functions as its neighbors lining the coelenteric cavity. Here, somatic and germ cells, that is somato-plasm and germ-plasm, are one and the same thing. It is proper to speak of a continuity of germ-plasm in the sense that all cells in the body, germ cells and somatic cells, are descendents of the original fertilized egg cell; but no cell in the body appears to retain any initial peculiarity that does not also apply to every other cell. If it be objected that this removes from the concept 'germ plasm' the very thing that has characterized it, I can only reply that such is precisely the case; but from the evidence as presented in C. flexuosa no other conclusion can be drawn. The work of Goette ('07) and of others give the same results when they say germ cells may be formed from any of the developmental stuffs of the body. Jorgensen ('10) finds the same thing to be true in sponges and comes to the same conclusion. It is not necessary to insist that these conclusions be applied to all other groups of animals, for exactly the opposite conclusion has been drawn from the evidence shown in forms such as Ascaris,

where there appears to be little doubt that there is an early separation of the primordial germ cells. Just as the weight of evidence in this form is admitted, so it must be in Campanularia.

3. Later development of the egg

a. The cytoplasm. Reference has been made to the fact that in the entoderm cells which are just starting their development as egg cells, there is little or no difference in the appearance of the cytoplasm from that of other cells. Figure 2, for example, shows at *a* and *b* two developing egg cells and one other entoderm cell; also some ectoderm cells, in all of which the cytoplasm is the same in appearance. But very soon there occurs the change which has been familiar to all workers for a long time, namely, that the cytoplasm which is granular becomes more compact and the stains take hold with greater intensity (figs. 3 to 5). Whether this is due to physical or to chemical changes within the cytoplasm is not certain; chemical changes are assuredly taking place and the physical configuration is also altering. No further changes occur while the egg is in its place of origin, but when it is migrating the short distance up the pedicel of the gonophore into the latter, it is increasing in size, due to the absorption of food from the enteric cavity. In a stage like that shown in figure 6, in which the egg is in the base of the gonophore, and figure 7, which represents an egg in its final position within the gonophore, the cytoplasm shows well marked changes which appear as irregular spaces within the cytoplasm. This is not purely an artefact (though it may be in part) since the spaces show some definiteness, as though caused by currents or streams within the cytoplasm. At the same time there is certainly an exchange of substances between the nucleus and the cytoplasm, as shown in figures 6 to 8.

All this time the cytoplasm has remained finely granular, with few or no protoplasmic bodies, even at the time when the egg has grown to be a quarter of its final size. This growth occurs very rapidly up to this point, and I interpret the lack of deutoplasmic bodies as meaning that food taken into the body of the cell is used up immediately, with nothing left over for a reserve.

In the stage represented in figure 8 (the egg being a quarter of the final size) we have the first indication of these yolk bodies, present in greater numbers near the periphery of the egg; when these bodies become more abundant (figs. 9 to 13) they are present near the nucleus as well. Figures 9 and 10 show lines in the cytoplasm radiating from the nucleus, which is evidence of currents which are proceeding outward from the nucleus, I conceive, therefore, that the yolk is built up in the cytoplasm out of material which has come from the nucleus, or from the material in the cytoplasm through the aid of material which has come from the nucleus. The simplest explanation of this appears to me to be that perhaps an enzyme from the nucleus gets into the cytoplasm and there elaborates and synthesizes the food brought into the egg. The presence of radiating lines—that is, of currents going out from the nucleus—is present for a considerable time; indeed up to the time when the polar bodies are about to be formed. This activity contines, then, throughout the entire history of the egg cell, and when it ceases the egg is loaded down with yolk granules which are so closely packed as to leave little space between them. The granules of the cytoplasm, at first the only material present, are now arranged around these yolk bodies and between them in a sort of an alveolar arrangement, but apparently not much greater in amount than in the young eggs. The difference in the appearance of the yolk bodies shown in figures 9, 10, and so forth, is apparently without significance, being due to the variation in the tenacity with which the bodies hold the stain.

b. The nucleolus. It is in the peculiarity of the nucleolar structure and history that one of the most interesting phases of the development of the egg of Campanularia flexuosa lies. And let it be remembered that many of the conditions and appearances referred to and figured have been seen in the living egg. The living egg of Obelia has also shown similar things so that there is no question of the effect of killing agents and so forth, for these appearances are faithfully shown in the sections; that is, the nuclear constituents have been normally preserved in the killed eggs, even to the delicate features.

In figures 2 to 4 the youngest egg cells show a single spherical nucleolus which stains deeply with iron-hematoxylin, though, with

double stains of acid and basic reactions, this body assumes some of the protoplasmic tint. In these same figures the nucleoli of the entoderm and ectoderm cells are much larger than those in the egg cells, though with the nucleus the opposite is true. Until the eggs assume their place in the gonophore, the nucleolus remains single; sometimes it is present as a single body after the eggs have come to rest and have increased in size in their permanent place in the gonophore (fig. 1). In the short migration which the egg cells go through from the pedicel of the gonophore into the body of the latter certain changes occur, principally in the cytoplasm and nucleus. The chromatin thread of the youngest eggs disappears and the nucleus contains only a delicate reticulum in which, as figures 5 and 6 show, are several deeply staining strands. In figure 6 there is also shown a darker and denser mass of cytoplasm just outside the nucleus, and scattered throughout this, but principally close against the nuclear membrane, several very deeply staining small granules. These are of interest because of what happens in later stages and are explained as being due to some substance passing from the nucleus into the cytoplasm.

After the eggs have reached their place in the gonophore, the nucleolus soon undergoes great changes, consisting of the breaking up of the nucleolus into pieces of various sizes and shapes, and never again in the history of the egg is the nucleolar matter in one body. It is the history of the changes of these nucleolar bodies and their possible significance that this section hopes to describe and make clear. Concerning the staining reactions of these fragments the following will show in general, and may suggest something of the nature of the changes involved. Double stains such as hematoxylin and eosin, hematoxylin and picric acid, picrocarmine and Lyons blue, hemalum and eosin and so forth, show some selective action. The result is that some of the nucleolar fragments show the colors assumed by the protoplasmic portion of the cell, some those assumed by the chromatic constituents of the nucleus, and some a tint intermediate between, or rather compounded from, the two tints. That is, the nucleoli behave as chromatic material, as non-chromatic material, or as a mixture

of the two substances. In some egg cells all the nucleolar fragments appear as non-chromatic, in others all appear to be mixtures, and in still other eggs some of the fragments seem to be chromatic and some non-chromatic or mixtures; in no case were the fragments in one egg all chromatic. In some instances these differences have been indicated in the drawings by differences in shading. The term nucleolus is simply a general term, as used here, for it has some of the characteristics of a plasmosome and behaves in part as a karyosome.

In the stage represented by figure 7 (about one-quarter the size of the mature egg) the fragmentation of the nucleolus begins and thereafter is characteristic, the variety in size and shape of these bodies being well shown in figures 6 to 16. The shape of the nucleolus is apparently of no significance, but the small spheres or spirals or the irregular masses as shown in figure 11 and 12 would present a greater surface than a spherical body. This may be a matter of some importance.

Of much greater interest is the condition shown in figures 7 to 9, in which there is seen a sort of ring of finely granular matter in the nucleus near and inside the nuclear membrane. Let it be noted that the groundwork of the nucleus is reticular and the ring of granular matter is not reticular nor arranged with any reference whatever to the reticulum and it will then be evident that the nuclear reticulum and the ring of granular matter are not the same thing and are probably not directly related. Rather it is just the condition that we might find if there were a wave of matter spreading outward from the nucleolus toward the edge of the nucleus; this material, being somewhat different chemically from the nuclear sap and reticulum, it would present a different staining reaction. Figure 9, indeed, is proof that some such thing is happening, for, in addition to this ring of granular material in the nucleus, there is in the cytoplasm a radial arrangement of the small granules as though a similar current were passing outward from the nucleus into the cytoplasm. This same radial arrangement of cytoplasmic granules just outside the nucleus of the growing egg is very characteristic and continues up to a very late stage, indeed it is present just as long as there is nucleolar

matter left in the nucleus (figs. 10, 12, 13, 15, 17, etc.). This last condition is strong evidence that the suggested interpretation is correct; certainly the fact that breaking up of the nucleolus and streaming currents from the nucleus into the cytoplasm co-incide; the fact that when the nucleolus has disappeared there is no longer such an arrangement of the cytoplasmic granules near the nucleus (i.e., no strong currents going from the nucleus into the cytoplasm) can not indicate other than a causal relation.

The nucleolus entirely disappears in the process just described. First of all, there is a very great increase in size of the nucleolus during the early growth of the egg, as a comparison of figures 3, 4 ($\times$ 1900) with figures 5, 8, 9, 10 ($\times$ 1228) shows, an increase which is much greater in amount than the increase in volume of the nucleus itself. Very soon, however, the nucleolus begins to undergo the modifications figured. It is certainly significant that a rapid increase in the body of the egg should be evident at the time when the nucleolus becomes vacuolated and breaks up, and streams of matter are going from the nucleus into the cytoplasm (figs. 8 to 10, 16). These are evidences that changes are occurring within the nucleolus, apparently quite rapid and considerable. In figures 6 to 11 there appears to be a change in shape of the nucleolus only, with little breaking into fragments, but a great vacuolization (these fragments, vacuoles, etc., are visible in the living egg). In later stages (figs. 12 to 17) the entire nucleolus breaks into many pieces, usually quite small, which, by the time the stage shown in figure 17 is reached, have almost entirely disappeared and a little later nothing of the nucleolus is left.

Another phase of these same activities is indicated in the character and appearance of the material which is leaving the nucleus and getting into the cytoplasm. In figure 6, as already noted, there are a few small deeply staining granules in the cytoplasm, close against the nuclear membrane; in figure 7 this is more evident. In the stages which follow this the same thing is seen, sometimes very plainly, at other times not so clearly (figs. 7 to 15). What becomes of the material when it gets into the cytoplasm? Let it be noted, as figures 3 to 7 show, that the cytoplasm is at first finely granular, in very early stages very closely packed

(figs 3 to 5), but a little later (figs. 6 and 7) becoming vacuolated and alveolar. In the periods represented by figure 8 (which may be variable as far as the size of the egg cell is concerned) for the first time there appear deutoplasmic bodies in the cytoplasm, and, since this coincides with the great activity of the nucleolus, it seems that there must be some connection between the two conditions.

If it be objected that figure 8 shows the deutoplasm closer to the periphery of the egg than to the nucleus and therefore there can be no connexion between nuclear emissions and yolk formation, let the following also be noted. First it is not assumed nor believed that all the *material* which forms yolk bodies comes from the nucleus; on the contrary, I believe a greater amount of it comes into the cytoplasm from the food stream in the enteric cavity of the gonophore and never enters the nucleus. This would probably be more abundant near the periphery of the egg than elsewhere. In the next place, the first emissions from the nucleus occur quite a while before yolk bodies form (figs. 6, 7). It is believed that at least the first emissions are of a ferment nature and not until they get into the cytoplasm is it possible for the material there to be synthesized into reserve food. It is even conceivable that the first nuclear contributions to the cytoplasm and the later ones are the same. And it is conceivable and possible that the same substances will at first hydrolize the food material coming into the cytoplasm, hence there will appear no yolk bodies, and later the same substance synthesizes the dissolved food and the result of this synthesis is yolk bodies. The reversible action of enzymes is too well known to call for any particular explanation in regard to the relation of enzymes and yolk formation. If this possibility be granted, there is no difficulty in accounting for deutoplasmic formation near the periphery rather than near the nucleus; indeed it would take place where the concentration of the hydrolized substances was greatest and this would be near the place the original material entered the egg, namely, near the periphery. Furthermore, the presence of yolk near the periphery is only at the first; the yolk is formed so quickly when it once starts that it fills the whole of the cytoplasm and is especially

prevalent near the nucleus (figs. 9, 10; fig. 9 is of the same stage as fig. 8).

With regard to the direct connection between the nucleolar matter and the yolk bodies, it is true that the nucleolus dissolves within the nucleus and there is certainly some commingling of nucleolar matter and nuclear sap, probably also a mixture of some of this matter with the chromatic network. But the growth of the nuclear reticulum and the dissolution of the nucleolus do not go on together, the reticulum showing almost no change until the nucleolus has disappeared. Again it is found, as figures 13 to 17 show, that the nucleolar fragments are always surrounded by a space; that is, they lie in a vacuole. Further, the smaller particles, when abundant, are often arranged in a rather definite row close to the nuclear membrane (figs. 14 and 15) as though it were here that dissolution was most rapid and the current outward had carried them here. These facts show that the dissolving nucleolar material is not being added directly to the nuclear reticulum, but, since the nucleus is increasing in size during this period, it is probable that the nuclear sap is increased very much in amount by the dissolved substance. Figure 16 shows well that the nucleolus is dissolving little by little; in this case the different particles showing different staining reactions, since the lightly stained nucleolar fragments show small drops of the material passing into the vacuole surrounding the fragment. The most satisfactory and crucial evidence of the connection between the nucleolus and yolk lies in this: during all the time that yolk bodies are being formed there is evidently a considerable exchange of material between the nucleus and the cytoplasm as shown by the currents already mentioned. During this same time (figs. 7 to 12) there is no change in the nuclear reticulum, which remains very fine-meshed, exceedingly finely granular and staining very faintly; that is, the reticulum is apparently unchanged and unmodified by any of the striking and active modifications that are going on. Since the nucleolus is the only portion of the nucleus showing signs of activity during this period and since there is clearly great activity going on in the cytoplasm in the synthesis of food matter, there appears to be no other possibility than to

conclude that the nucleolus actually stands in some causal relation to these changes.

Now plainly the whole egg during the period of growth is in a most active state of metabolism. In addition to the ordinary functions which it has to conduct in order to remain alive there is the further task of preparation for the cleavage period which is to come, in which it will not receive food from the outside. This rests principally in the storing of food as reserve for that period of activity. Even if we assume that the food which enters the egg from the enteric cavity has already been digested, there is the necessity for a great amount of it to be synthesized into the stored products needed later, and also a lot to be assimilated and used for the present needs of growth. Perhaps this is the most strenuous period of activity of any single cell in the life story of the cells of the body. Apparently, then, the nucleolus stands in some rather close relation to this activity of the egg cell during the active growth period. If not actually taking part in the transformation of the food itself it is closely related in some other way. The odd shapes assumed by the nucleolus may, therefore, be for the purpose of securing as much exposure of surface as possible. The nucleolus certainly aids in transforming some of the material, since this body alone is not sufficient to account for the increase in substances within and without the nucleus.

The origin of the nucleolus in the egg cell was not definitely determined, for in the earliest recognizable egg the nucleolus was already present. But the behavior is sufficiently clear. In the young eggs (figs. 2, 3, 4) the nucleolus is very small, smaller even than the nucleolus of the neighboring ectoderm and entoderm cells, though the nucleus is larger in the egg cell. But coincident with the disappearance of the spireme in the young egg cell, which takes place very quickly, the nucleolus enlarges considerably, even before the body of the egg increases. The nucleolus arises then, within the nucleus and evidently from the chromatic spireme (at least in part), but all of the chromatin does not enter the nucleolus, for in addition and at the same time a chromatic reticulum is formed in the nucleus; also the staining reactions show that the nucleolus contains a lot of non-chromatic matter.

The fragmentation of the nucleolus and the transference of its substance into the cytoplasm is, therefore, in correspondence with the staining reactions, for the material which is emitted from the nucleus has the same staining reaction as chromatin and the origin of the nucleolus from the nuclear spireme would explain the appearance. It has been shown that the chromidia, so-called, in the cytoplasm have come from the nucleolus and their chromatic relation is thereby explained, since the chromatin earlier entering the nucleolus leaves it later and goes into the cytoplasm to serve a particular purpose.

It may be added that Gonothyraea lovenii from Naples was sectioned and showed almost the same relations of nucleolus as Campanularia flexuosa. There was perhaps a little less variation in the form of the particles (none of them showed the arborescent-like forms shown in figs. 11 and 12) but there was always a fragmentation about the same period, and it continued about as long, so the agreement is very close. Bergh ('79) described the breaking up of the nucleolus into many pieces of various sizes and shapes and observed it in the living eggs of Gonothyraea.

The nucleolus, then, as its activities and functions have been conceived and outlined in the foregoing, would appear to be a 'trophonucleus' in the sense of Goldschmidt ('04). As is well known, this author conceived the nuclei of all animal cells to be double, a somatic (or better, vegetative) and a reproductive nucleus, which were usually united within a single nucleus which he called the 'amphinucleus.' He found from his own investigations that in the egg cells a separation came when a portion of the nuclear matter passed into the cytoplasm in the form of grains, to which were given the name of 'chromidia,' and this emission from the nucleus came only during the time of yolk formation. His point of interest was, not whether this extruded chromatin went to form yolk or whether it was a sort of regulative process for the reproductive chromatin, but that this process did establish a close relation of chromidia formation with a specially active somatic function. For the chromidia were determined to be isolated particles of the chromatin of the nucleus and they were in the place of highest somatic functioning, that is, in the cyto-

plasm of the cell. The behavior of Campanularia flexuosa seems to agree very closely with this theory, the actual facts being almost the same as those that Goldschmidt observed. But there in an incidental difference in that the chromidia proceed directly and immediately from the nucleolus, though this nucleolus, as shown, originated in part from the chromatic spireme and in its dissolution still showed reactions similar to those of the rest of the chromatin in the nucleus.

As early as 1895, Van Bambeke found in one of the fishes (Scorpaena scrofa L.) that chromatic substance passed through the wall of the germinal vesicle, but in this case the nucleolus had nothing to do with the process. Lubosch ('02) found what he called 'by-products' of the nucleus to pass into the cytoplasm and there take part in yolk formation. He further states that the phenomena of growth of the cell suggest that material taken in from the cytoplasm is synthesized in the nucleolus and transformed into chromatin. Henschen ('04), in Helix pomatia, finds a migration of chromatin from the nucleus into the cytoplasm and thinks it may have some relation to yolk formation. Brooks and Rittenhouse ('06), in the coelenterate Turritopsis, found the yolk to form close to the germinal vesicle as a result of nuclear activity. Popoff ('07) says chromidia come from the nuclear chromatin and in egg cells chromidia formation is least active in the first phase of growth and most active when the yolk is forming; the richest chromidia formation agrees with the strongest cell activity. The wide distribution of chromidia in strongly functioning tissue cells is suggestive of a physiological condition of the cell, and Popoff says: "I consider the chromidia as morphological consequences of cell growth and cell activity" (p. 104). In 1910 this same author makes the general statement that chromidia, mitochondria, and so forth, are different stages in the same genetic series, originating in nuclear chromatin, and the various appearances are due to differences in diffusion currents, peculiarities of the cytoplasm and the like. Yolk may form from these but "chromidial formation is only an expression of purely physiological cell conditions and these can in no way be specific for the germ cells alone" (p. 41). Others have found chromidia related

to yolk, as Jorgensen ('10) in sponges, Schaxel ('10, '11) in various Hydrozoa and in Ascidia. Nowikoff ('09), in Haliotis tuberculata tissue cells, found the nuclear chromatin assembled into nucleoli, worked over there and extruded into the cytoplasm in the form of chromidia.

The described behavior of the nucleolus is also characteristic of other forms of the Coelenterata since Trinci ('06) found, in members of the family Eucopidae, the nucleolus dividing into many and variously formed bodies in constant transformation. Merejkowsky ('83) saw the same thing in Obelia, as I have also: Wulfert ('02) noted similar conditions in Gonothyraea, as did Bergh earlier. On the other hand, Harm ('03), in Clava squamata, and C. W. Hargitt ('06) in Clava leptostyla, say the nucleolus sometimes migrates bodily into the cytoplasm. The latter believes the nucleolus has nothing to do with yolk formation.

c. History of the nucleus in the germ cells. When the egg cells are first distinguishable the nucleus is characterized by the presence of a chromatin skein. This skein appears to be a series of chromatin loops, more or less centralized at one pole of the nucleus (figs. 2 to 4), a condition similar to that found by Bigelow ('07) in eggs of Gonionemus and by the author ('09) in Tubularia crocea. In both these cases the arrangement occurred in oocytes after the last oogonial division and at the stage just before growth started; the author interpreting this as the synaptic stage or period of the conjugation of the chromosomes, which, as Montgomery ('04) says, takes place in metazoa in the early portion of the growth period of both oocytes and spermatocytes. Certainly in the cases just cited, this condition was not a prophase of division, and in Campanularia flexuosa, although no divisions have occurred previously, this condition of the chromatin does not lead to division, and at the proper time the reduced number of chromosomes appears in the first maturation spindle. It seems safe to interpret this condition, therefore, as the stage of the 'reduction,' so-called.

The next stage in the egg is that of the migration of the egg into the gonophore, a stage marked by certain peculiarities of the nucleus, as well as of the cytoplasm. The loops of chromatin very

soon disappear and the nucleus contains only a very fine meshed and slightly staining reticulum (figs. 5, 6). Since the reticulum is present only after the loops have disappeared it is evidently formed from the loops, though the nucleolus secures some of the material. After the egg reaches its place in the gonophore a rapid and marked growth takes place (fig. 1). It is during this period that the peculiar nucleolar changes occur which lead to yolk formation. Let it again be noted that the dark bodies within and just without the nuclear membrane in figure 7 to 9 are bodies which have left the nucleolus and are passing through the nuclear wall into the cytoplasm. These bodies are chromatic in character, since the nucleolus has been formed from the dissolving chromatin loops of the earlier spireme, and therefore they should present essentially the same staining reactions as the rest of the chromatin, and such we find to be the case. The nucleolus, therefore, appears to be a synthetic or transforming center of the nucleus where the chromatin is to be changed somewhat for the function it is to perform in the cytoplasm. The fact that these bodies do not belong in the reticulum, as an integral part of it, is shown by figures 7 to 9; they do not lie in the reticulum itself and the latter shows plainly as a delicate and finely granular affair.

In the late growth period of the egg (figs. 11 to 17) the nucleolus breaks into parts of a greater or less size and, as these are surrounded by vacuoles, it is evident that they are not a part of the nuclear reticulum. And while it may be that the dissolving nucleolus adds some material to the reticulum, this does alter the general appearance or behavior to stains which the reticulum has shown during the earlier part of its growth. Figures 11 and 12, for example, show the same sort of a reticulum as is shown in figures 6 to 8, though a considerable portion of the nucleolus has disappeared. The point made here is simply this; the chromatin in the nucleus is of two sorts or if not actually different in composition, at least it serves two different functions in the cycle of the cell. A certain portion of the chromatin (that which has gone, or goes, into the nucleolus), after some probable transformation within the nucleolus, passes out of the nucleolus into the cytoplasm, there to serve a particular purpose, and this portion does

not take part in the reproductive activity of the cell. The second portion of the chromatin plays little part in the described activities of the growing egg, but at a certain period is essential for the reproductive phase. It is not considered that the chromatin intended for differing functions is fundamentally different, for, as already noted, both the reticulum and a portion (if not all) of the nucleolus has come from the same original source, namely, the chromatin skein in the young egg cells. Nor is it conceived that the chromatin, thus having one function, does not take any part in the other function or receive additions from the other portion of the chromatin. There is certainly an interaction between the nucleus and the cytoplasm, as well as between the nucleolus and the rest of the nucleus; the whole cell is a unit and it so works. But there appears to be some division of labor, and in analyzing conditions, functions, and substances, it is convenient to think of them separately.

To summarize the previous paragraphs: There has been no apparent modification in the nuclear reticulum by the dissolving nucleolus, nor is the preparation of the nucleus for division dependent upon a certain stage of nucleolar dissolution, for, as figure 13 shows, a large number of nucleolar fragments are still present and the chromatin of the reticulum is beginning to condense into strands at certain places. On the other hand, some nuclei, not included in the figures, show the nucleolus practically gone and there is only a faint reticulum. But there comes a time, at the end of growth, when the chromosomes begin to form. This may be by the formation of strands in the nuclear reticulum, as shown in figures 13 and 16, or it may be initiated by the condensation of the chromatin at the nodes of the reticulum (figs. 14, 17). In some cases the whole reticulum appears to become coarser, the grains composing it larger and staining more deeply (fig. 15). In many nuclei all these methods are active. But figures 13 to 17 show clearly that at the end of growth the nuclear reticulum, hitherto very delicate and lightly staining (figs. 7 to 12), shows the beginning of a segregation and a condensation of its substances which go to form the chromosomes, and the latter form only from the reticulum. This appears to involve the nuclear reticulum

alone, since the nucleolar substance remaining continues its dissolution and discharge into the cytoplasm. However, there is in no case any indication of the formation of a spireme previous to the formation of the chromosomes. Nor is there such a spireme in the eggs of Tubularia crocea or Pennaria tiarella, the chromosomes coming from the delicate reticulum of the nucleus.

Sections of eggs of Gonothyraea lovenii from Naples, show the same general relation of the reticulum, the nucleolus, and the nucleolar fragments, and the same position and behavior of the chromosomes as already described for Campanularia flexuosa. Whether this applies to all the details of the behavior of the eggs and their ingredients has not been determined, but there is a very close similarity in general.

4. Polar body formation

In whatever manner the chromatin of the nuclear reticulum condenses, there comes a time when the nucleus is without any trace of a nucleolus and the chromatin within is grouped into grains arranged in a very close reticulum—a stage between figures 17 and 18. When this condition is reached the nuclear membrane breaks, the chromosomes form and enter the spindle, and the divisions into the polar bodies and the egg occurs. Figure 18 shows the formation of the polar spindle outside the nucleus, the nuclear membrane broken and the chromatin granules escaping into the cytoplasm. In this figure, and in the egg from which the figure was made, the chromosomes were not yet formed. Whether it is usual for the chromosomes to delay their actual formation till after the rupture of the germinal vesicle I do not know, but such was the case in this particular egg. A point of significance should be noted in figure 18, namely that some of the chromatin granules, are escaping from the germinal vesicle through the broken wall and may be seen in the cytoplasm, while others are evidently attracted toward and are arranging themselves along the fibers of the developing spindle. This means that, of the chromatin which has as its function the division of the egg, there is only a portion needed for the new cells, the rest is superfluous and passes into the cytoplasm. In spite, therefore, of the large amount

of chromatin matter which has already passed from the nucleus into the cytoplasm during growth, there is still a super-abundance at the end of the cycle and only a portion is handed on to the next generation of cells. In figure 19, in which the chromosomes of the spindle are dividing, this extra chromatin is seen in the cytoplasm of the region as dark granules. These granules are the same as the granules which are found when the membrane first breaks as shown in figure 18. The very fact that there is a superfluity of chromatin after the considerable emission of chromatin during the growth period, is an indication that there has been new chromatin formed. For all the chromatin the egg had to start with came from the entoderm cell which was its progenitor, and this was approximately the same amount as is needed for the formation of the chromosomes. To have the amount necessary to go through two divisions (in the formation of the polar bodies) with a superfluity of apparently double this quantity, and, in addition, the extrusion of a large amount during the whole of the growth period, there must have been the formation of an enormous quantity of new chromatin during the growth period. This synthesis of chromatin I judge to be one of the functions of the nucleolus.

The objection has been raised and will doubtless again be offered that the chromosomes present in the first maturation spindle (fig. 19), which correspond closely in amount to the chromatin received by the primordial germ cell from its entodermal progenitor, do precisely represent these chromosomes. The aim of the objection is to force the conclusion that there has been a direct continuity of the chromosomes of the cell giving rise to the germ cell, and the chromosomes of the mature egg cell. This claim would thereby ascribe to the chromatin emissions no significance as far as relation to the chromosomes is concerned, and the chromatin left over after the chromosomes had formed would be more or less foreign or extraneous matter, or chromatin-like substance, of a different origin and fate but predestined to have no part in chromosome formation.

This does not appear to be a fair position to take, for with the foregoing insisted on as a premise, the significance of the loss of

chromatin from the germinal vesicle could not be properly considered. It is assumed, to start with, that the chromosomes of one generation are the same chromosomes as of the previous generation. Therefore, the chromatin emitted from the nucleus during growth and that remaining unused when the chromosomes are formed—in short all that does not enter into the chromosomes —is of a different sort, had a different origin and cannot be considered as related to the chromatin of the chromosomes. In essence the argument then proceeds, that since the chromatin which leaves the nucleus during growth has a different fate from that entering the chromosomes, it belongs in a different category, and this is evidence of the genetic similarity of the chromosomes of the two generations. We thus arrive at the same point from which we started, the whole argument being based on the a priori assumption that the chromosomes of one generation continue essentially unchanged to the next generation. Even if it should be granted that there is evidence of the continuity of chromosomes in molluscs, echinoderms, insects and so forth, it should not be forgotten that certain cellular activities, as cleavage in some of the coelenterates, do not follow the plan of cleavage of the molluscs and others. Since this difference has been established, it might be expected that differences would exist in other processes. We must, then, *examine the evidence* in Campanularia and not *reason* insect conclusions into our data.

The facts are these: The earliest recognizable egg cell has all its chromatin arranged in a spireme. This is relatively small in amount, for the primordial germ cell and its nucleus is little different in size from any other body cell. This spireme of chromatin entirely disappears and there is present in the nucleus a reticulum and a (partly chromatic) nucleolus. During the growth of the egg there is a very considerable loss of chromatin from the germinal vesicle into the cytoplasm; all of the chromatin of the nucleolus goes into the cytoplasm; whether much, little, or any of the chromatin of the reticulum is lost now is not possible of demonstration; it is assumed that little if any is lost. Here is perhaps, the first place for disagreement, the claim being made that the chromatin in the nucleolus is of a different sort from that

in the reticulum, and the latter is retained while the other goes into the body of the egg. But the chromatin of the assumed two sorts came from the same original source, the chromatin loops of the conjugation phase (synapsis) of the primitive egg cell. Is it sufficient to say that, since there was a different ending, there must have been a different source? To claim this would be assumption, not observation, for there is no way of demonstrating a difference in the chromatin of the reticulum and of the nucleolus. But let us grant for the sake of argument that this reticulum retained in the germinal vesicle is the essential chromatin. We have the chromatin scattered over a very fine-meshed, extensive reticulum in the minutest grains. There is no difference in any part of the network so far as can be discovered by differential staining or by the use of apochromatic lenses. Later there appear larger masses of chromatin at the nodes of the network, or the whole reticulum appears to condense into larger masses of chromatin. These appear the same everywhere; again no difference can be discovered by staining methods or by careful use of apochromatic lenses. But some of these chromatin masses enter into the chromosomes of the maturation spindle and some escape into the cytoplasm in the form of grains when the membrane breaks, not having been used to help form the chromosomes. Here the claim will again be made that the chromatin which escapes is of a different sort from that which goes into the chromosomes. But once more, the claim has no basis at all in *observation;* it is simply a position assumed by the necessity of making the facts agree with the theory which is held to apply in this case.

My position is that we can depend on facts more than on interpretation of those facts, particularly in our attempted generalizations of interpretations. So, in the absence of any evidence whatever that the chromatin in the nucleolus is different from that in the reticulum of the germinal vesicle, that the chromatin of the reticulum which forms chromosomes is different from that which remains unused, it must be granted that we are forced to the conclusion that all the chromatin is of the same sort, and not that a portion is fore-doomed to be cast into the cytoplasm and another part destined to form the chromosomes. This would not, of

course, be in agreement with those who hold that the chromosomes are continuous entities from one generation to the other. But the facts do not seem to warrant any such conclusion.

It can not be claimed that, because some chromatin comes to an end different from other chromatin, there is in this very fact an indication of its essential unlikeness. Some of the young egg cells of Campanularia flexuosa, in moving from the pedicel into the body of the gonophore, pass by the only place where there is room for them to develop, so they continue their migration (as shown in fig. 1) into the distal end of the gonophore, where they come to naught but degeneration. Can we say that since this egg came to a different end from the one which entered the gonophore where there was more room, it was from the first predetermined for failure? In other forms—as Tubularia, Pennaria, Clava and so forth—it has been clearly shown that two eggs have behaved alike for a considerable time, and it is only the chance of better position as regards food and room and the like which shall determine which has the opportunity to develop into a mature egg and which shall degenerate.

The chromosomes formed in the manner described in a previous paragraph, arrange themselves in a spindle in the usual way. They are ten in number, the reduced or haploid number, and appear, as figure 19 shows in the metaphase, as single bodies. No centrosomes and little indication of polar asters are present in the spindle. Two polar bodies are formed (fig. 20 shows one of them somewhat degenerate). The spermatozoon appears to enter by an attraction cone but leaves no path to indicate its movement into the egg. Figure 20 shows the fusion of the two pronuclei and the formation of the cleavage spindle. Figure 21 is a section through a second cleavage spindle, the right end of which contains all the chromosomes, which are seen to be twenty in number; the spindle having been cut somewhat obliquely, only a part of the chromosomes of the left end of the spindle were present in this section.

Briefly summarizing the nuclear history of the egg cells, we find that after the division of one of the epithelial cells of the entoderm of the stem of the gonophore (or by the transformation of a single entire cell) the basal half has its chromatin arranged in a

spireme or into loops more or less regularly and definitely grouped together. All the chromatin in this new cell came from the old cell. These chromatin loops are transformed into a nuclear reticulum and a nucleolus. There is a great increase in the size and volume of the nucleolus at an early period; later it is believed there must be a synthesis of new chromatin, since the original chromatin of the nucleus is not sufficient to account for all that is used during the growth period by being passed into the cytoplasm, and that which still remains over when the chromosomes of the maturation spindle are formed. When the nucleolus has disappeared the reticulum undergoes a condensation of its chromatin into large grains and eventually these form chromosomes. Only a small portion of the chromatin which remains in the nucleus at the time of polar body formation is actually used in the formation of the chromosomes and the rest is scattered in the cytoplasm and there dissolved. This sort of thing, which from published accounts is not limited to the hydroids or to the coelenterates but is more or less common, leads us to consider whether the matter of the continuity of the germ-plasm, the individuality and continuity of the chromosomes, the alleged supremacy or uniqueness of the chromosomes in heredity and so forth, are not after all mere names or phrases. There should be more careful thought as to whether the things connoted by these names are not also without real meaning or significance. Once they were undoubtedly useful and served a valuable purpose, but are we not allowing ourselves to be unduly handicapped and hemmed in by these older conceptions? Must we not come to look more to the ultimate chemical composition and constitution and not to morphological entities really to harmonize and explain the various and varying functions and activities of all cells, somatic as well as germ cells?

CONCLUSIONS

The egg cells of Campanularia flexuosa arise in the entoderm of the pedicel of the gonophore, by the transformation of a single epithelial cell, or from the basal half of a divided cell, the distal half of which remains an epithelial cell and retains its epithelial functions. Therefore the egg cells have come from differentiated body-cells (so-called) and there is no differentiation of the germplasm in the sense that germ cells are early differentiated and set aside and do not participate in the body functions. Any cell of the entoderm of Campanularia flexuosa may become an egg cell if it is in the position of the developing gonophore. There is no division of the primitive egg cell but each transforms directly into a single mature egg cell.

The chromatin of the primitive egg cell, at first arranged in definitely arranged loops, disappears, forms a fine-meshed delicate reticulum and a nucleolus (the latter also contains non-chromatic matter).

The nucleolus becomes greatly vacuolated, breaks up into fragments of various sizes and shapes, and the chromatin contained in these passes through the membrane of the germinal vesicle to form the chromidia in the cytoplasm. Co-incident with this chromatin emission, the rapid growth period of the egg begins. So long as the dissolution of the nucleolus continues there is a considerable outflow of material from the nucleus, shown by currents in the cytoplasm. The chromatin particles in the cytoplasm become, or have something to do with the formation of the yolk bodies. Yolk formation, chromatin emission, strong currents from the nucleus, and growth of the egg cease when the nucleolus has disappeared. The nucleolus is, then, a dynamic center, concerned primarily with the nutritive activities of the egg cell. It also aids in the formation of new chromatin.

The nuclear reticulum is apparently unchanged by the dissolution of the nucleolus, but when the nucleolus has disappeared, or nearly so, the chromatin of the reticulum forms the chromosomes. There is not the formation of a spireme and not always the formation of strands in the reticulum, but the chromosomes may form by the segregation of the chromatin granules of the

reticulum. The first maturation spindle is formed outside the membrane of the germinal vesicle, the membrane breaks and the chromosomes enter the spindle.

Not all the chromatin of the germinal vesicle enters into the formation of the chromosomes, but the apparently larger amount escapes into the cytoplasm when the membrane of the germinal vesicle breaks. The chromatin which escapes is of the same sort and has the same history as the chromatin granules which form the chromosomes. This is evidence against a continuity of chromatic material from generation to generation.

There are ten chromosomes in the maturation spindle, the reduction apparently having taken place at the time of the polar arrangement of the chromatin loops in the primitive egg cell. Two polar bodies are formed.

On account of the evidence against the continuity of chromatic matter from one generation to another, and because there is shown to be no difference between the germ plasm and the body plasm until after the egg has begun to grow, it is suggested that we must come to look to the ultimate chemical composition and constitution for explaining cellular activities and relations.

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PLATE 1

EXPLANATION OF FIGURES

All figures have been drawn with the aid of the camera lucida. The magnification indicated is the original magnification, the figures as they appear in the plate have been reduced to three-fourths the original size.

2 Primitive egg cells in the pedicel of the gonophore, arising from entire entoderm cells. × 1900.

3 and 4 Egg cells in the pedicel of the gonophore. These have been formed from the basal half of a divided epithelial (entoderm) cell. The chromatin of the nuclei arranged in loops. × 1900.

5 Egg cell passing along the pedicel into the gonophore. The chromatin has lost its polar arrangement and has formed a reticulum in the nucleus. × 1228.

6 Egg cell in position in the gonophore. Beginning of chromatin emission. × 715.

7 Egg grown to about one-fifth its mature size. Nucleolar fragmentation shown, chromatin emission taking place. × 1228.

8 Egg about one quarter grown. Shows nucleolar fragmentation and vacuolization; chromatin emission; beginning of yolk formation. × 1228.

9 In addition to the features shown in figure 8, this egg shows the granules of the cytoplasm arranged in radial lines, an indication of outgoing currents from the nucleus. × 1228.

10 Growing egg. Vacuolization of the nucleolus very marked. Chromatin emission. × 1228.

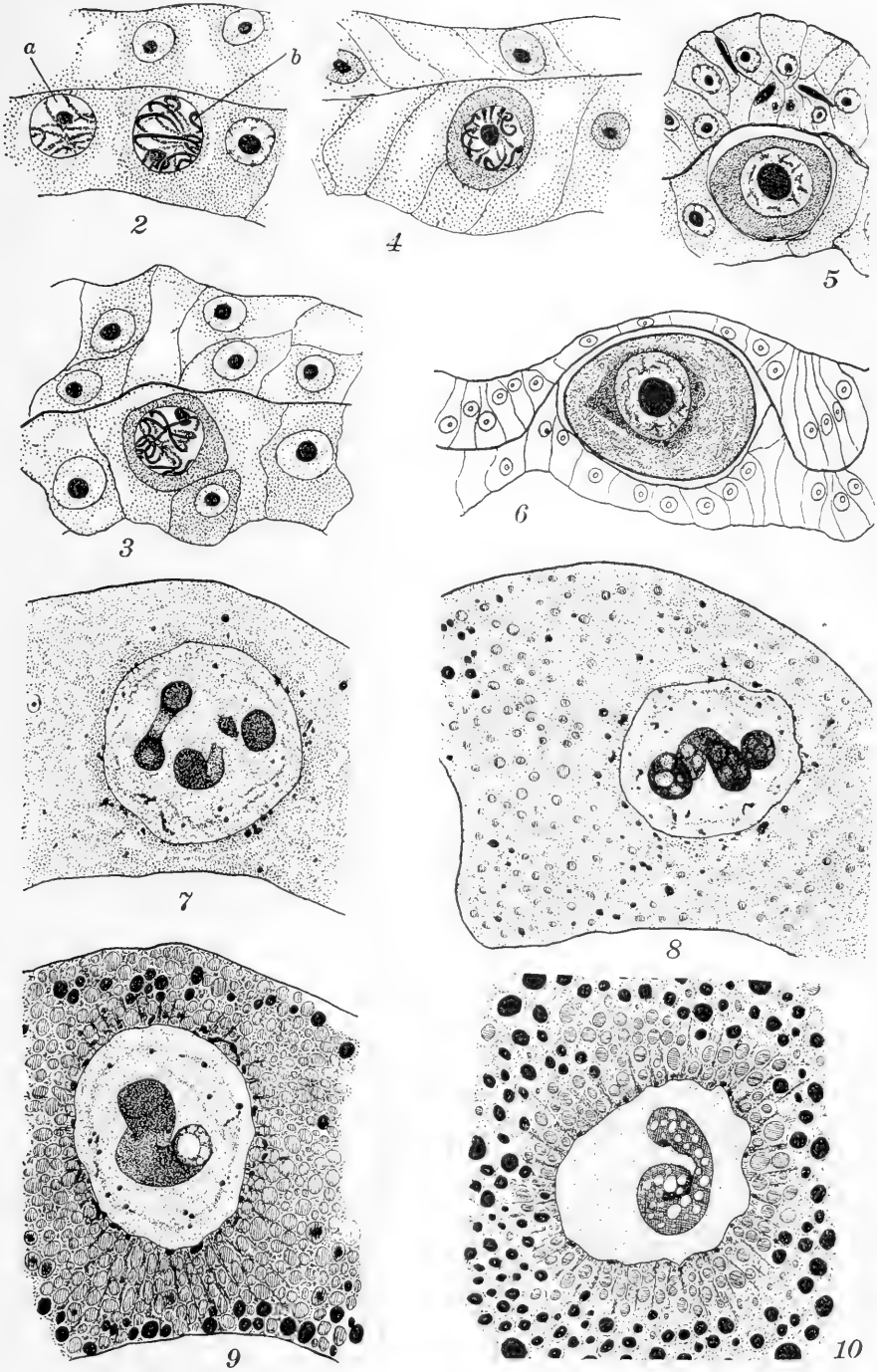
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PLATE 2

EXPLANATION OF FIGURES

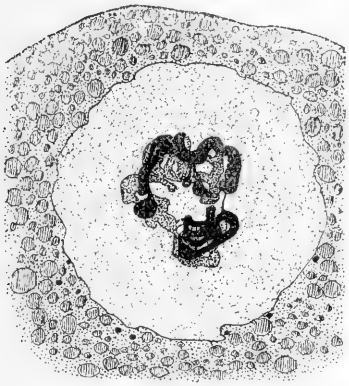
11 and 12 Growing eggs showing extreme nucleolar fragmentation. Note the delicate, fine grained reticulum in the nucleus. The two eggs were treated by different killing fluids and stained in different ways. × 1228.

13 Large growing egg. Nucleolus represented only by small fragments lying in vacuoles in the nucleus. In certain parts of the nuclear reticulum the chromatin is forming strands. × 1228.

14 Egg near the end of the growth period. Nucleolus in fragments, the nuclear reticulum growing denser at the nodes. × 1228.

15 Egg nearly mature, the nucleolar fragments mostly in a ring near the periphery of the nucleus. The whole nuclear reticulum is becoming denser and more deeply staining. × 1228.

16 Egg at the end of the growth period. The nucleolar fragments, within vacuoles, are of different composition, as shown by the different reactions to stains. Chromatin in the reticulum forming strands. × 1228.



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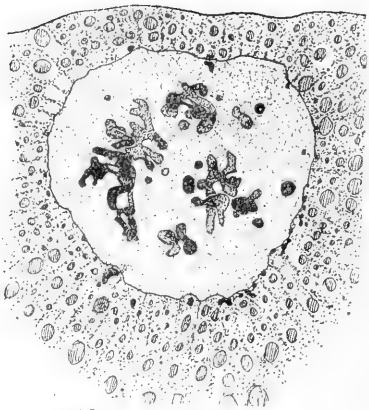

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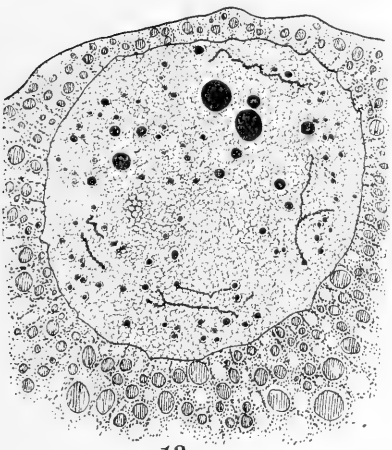

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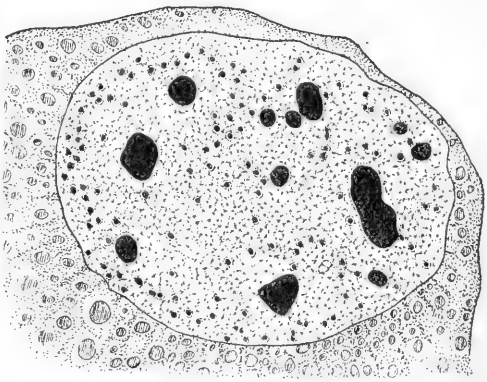

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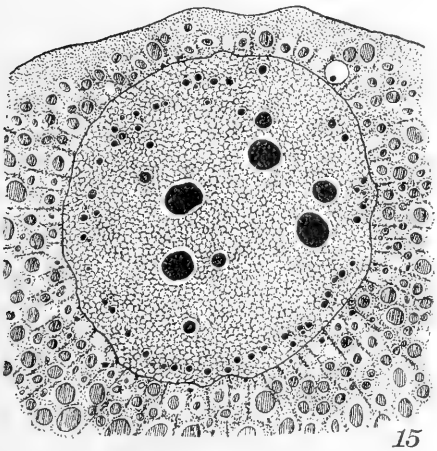

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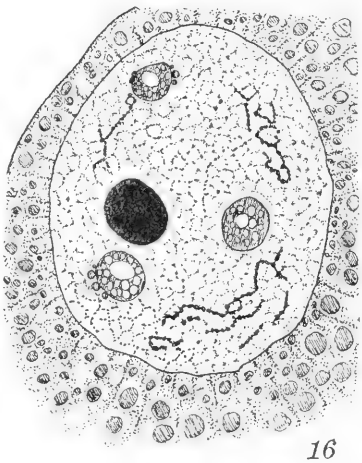

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PLATE 3

EXPLANATION OF FIGURES

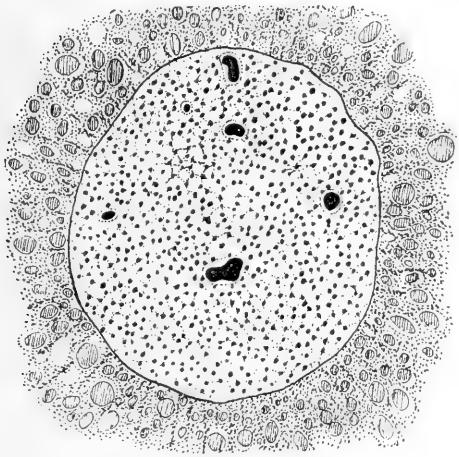
17 Germinal vesicle of egg preparing for maturation. The nucleolus is represented by only a few small fragments. Chromatin of the nuclear reticulum segregating into granules at the nodes of the net. × 1228.

18 First maturation spindle forming outside the germinal vesicle. The chromatin granules are arranging themselves along the fibers of the spindle or are escaping into the cytoplasm through the broken membrane. × 1228.

19 Metaphase of the first maturation spindle, with the chromosomes splitting. Note the granules of chromatin in the cytoplasm in the region of the spindle. × 1900.

20 Copulation nucleus, first cleavage spindle forming. Remains of one polar body present. This drawing is compiled from two sections. × 1900.

21 Telophase of the second cleavage spindle. The section is cut somewhat obliquely. At the right end of the spindle all the chromosomes present in the spindle are shown (20 in number). Not all of the chromosomes are present in the left end of the spindle in this section, but are found in adjacent sections. × 1900



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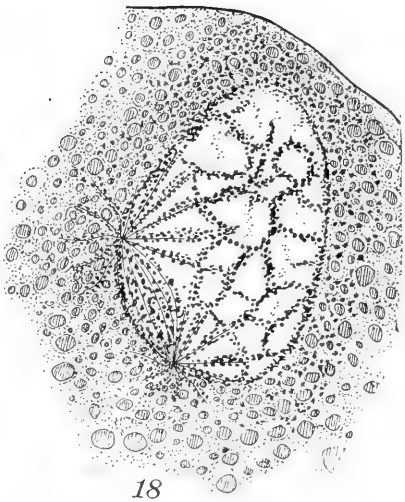

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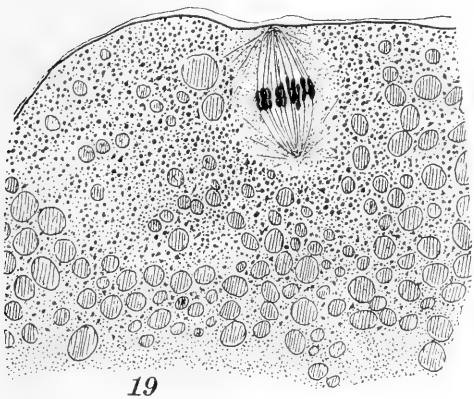

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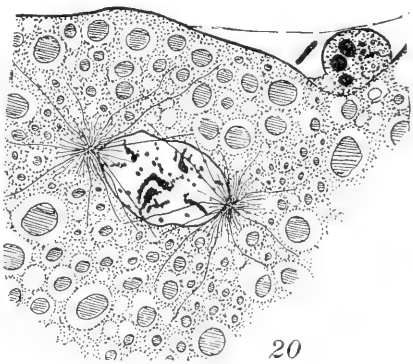

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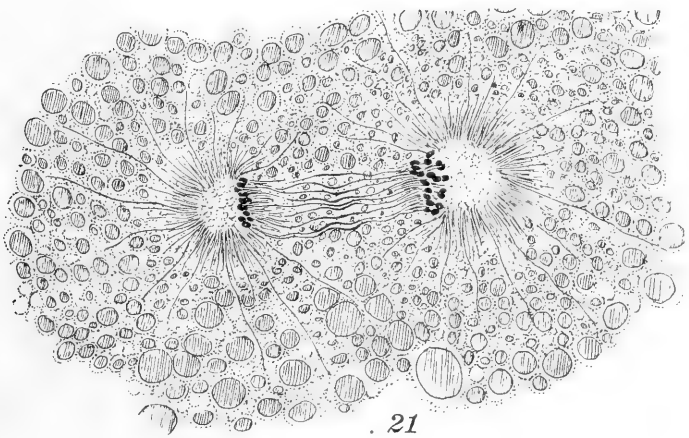

21

Thesis presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

THE SPERMATOGENESIS OF ASCARIS MEGALOCEPHALA WITH SPECIAL REFERENCE TO THE TWO CYTOPLASMIC INCLUSIONS, THE REFRACTIVE BODY AND THE 'MITOCHONDRIA': THEIR ORIGIN, NATURE AND RÔLE IN FERTILIZATION

EDWARD E. WILDMAN

Zoological Laboratory of the University of Pennsylvania

FORTY-EIGHT FIGURES

In 1906 Marcus made two notable contributions to our knowledge of nematode spermatogenesis. The first was his discovery that the refractive body is formed within the vas deferens in Ascaris canis, thus completing the spermatogenesis within the male. Previously this most unique and characteristic structure of the nematode spermatozoon had been seen only in those cells which had already entered the uterus of the female. This discovery was confirmed by Mayer ('08) and Romieu ('11), both working with A. megalocephala.

Marcus also suggested that the 'protoplasmic corpuscles' or 'microsomes,' observed by Van Beneden, Hertwig and others surrounding the chromatic mass in the spermatid, were mitochondria. This interpretation has been supported by Mayer ('08), Romieu ('11), Meves ('11) and Faure-Fremiet ('11), all working with A. megalocephala.[1]

But none of these workers made any attempt to learn the origin or to trace the development of either the refractive body or the mitochondria up to their appearance in the complete spermatozoon. It seemed very desirable to do this, now that we know that the entire development of the paternal germ cell takes place

[1] Since this paper was written, Romeis has published a study of the degeneration of the 'condriosomes' in Ascaris spermatozoa, in which he also calls these bodies mitochondria.

within the male. At the suggestion of Prof. T. H. Montgomery, Jr., I undertook this study. I wish here to express my deep appreciation of Professor Montgomery's kindly interest in my work, and of his helpful advice and criticism, during the course of the observations. I very much regret that this report was not finished in time to be reviewed by him, for this is a field of research in which Professor Montgomery stood preëminent.

THE REFRACTIVE BODY

Place and manner of its formation

Notwithstanding the fact that the spermatogenesis of the nematodes has been studied since 1858, the history of the refractive body had remained unknown. During this time the study of no animal has contributed more to our knowledge of the cytology of the germ cells than that of the common nematode parasite of the horse, A. megalocephala. The brilliant researches of Van Beneden, Hertwig, Brauer, Tretjakoff and others leave little to be desired concerning the nuclear phenomena during spermatogenesis. The laws of nuclear structure and behavior here discovered have been found to be of very general application. But the observations of these authors on cytoplasmic structures, accurate and extensive as they are, lack the completeness of a final interpretation, because none of them saw the last stage in the process of sperm development.

Throughout the incomparable researches of Van Beneden on the development of the Ascaris spermatozoon there is no indication that he even suspected the formation of the refractive body within the vas deferens. His excellent figures represent the various stages of its development as entirely within the uterus. He believed that the paternal element left the male in the form of the spermatid, spherical or slightly conical in form but with no trace of the refractive body.

At the proximal end of the uterus one always finds a great number of spermatozoa crowded among the eggs and uterine epithelial cells. This region Van Beneden named the 'receptaculum seminis.' Among this crowd of spermatozoa are to be

found some that are only slightly conical, with no trace of any inclusion; some having a thin axial rod in the cone which stains readily in iron hematoxylin; some in which this rod is thicker and longer, and more deeply staining, and others contain the fully formed refractive body. In these four forms or 'types' Van Beneden thought he saw the complete development of this body. His reasoning seemed so logical and his figures so in keeping with all observations that most of the students in this field have accepted his conclusions.

O. Hertwig ('90) and Brauer ('93) did not follow the development of the sperm beyond the formation of the spermatid. These authors gave special attention to the nuclear phenomena, although they describe and figure cytoplasmic inclusions as they appear throughout the growth period and maturation divisions.

Tretjakoff ('05) followed Van Beneden in a study of the complete spermatogenesis, and concluded with him that the spermatid enters the uterus, there to become the spermatozoon. He points out that in many animals the uterine epithelial cells act as nurse cells for the developing spermatozoa, and he interprets the crowding of the spaces between the epithelial cells of the uterus wall with sperm cells of the four types of Van Beneden as an effort to get at this necessary food supply.

But Marcus showed that, in A. canis, throughout the later growth period, maturation divisions and also in the spermatid, the cytoplasm contains numerous bodies, rodlike or spherical in form, which in the living cell are refringent, like the refractive body, and in fixed and stained material resemble that body in all staining reactions. In his study of the developing spermatid Marcus finds that these bodies, now uniform in size and spherical in outline, gradually fuse together and thus ultimately form the refractive body itself. Mayer showed that this body is formed in exactly the same way in A. megalocephala. His work is confirmed by Romieu,[2] and I have seen the same phenomenon.

[2] The authors just named explain the long delay in the discovery of the place and manner of formation of this body. They find that in only one male out of thirty killed is the development of the sperm cells in just the right stage to show this formation. This proportion is so small that we are not surprised that the

Thus the destiny of the refringent granules and vesicles, which have long been observed in the spermatocytes and spermatids of nematodes and of A. megalocephala in particular, is no longer a mystery. Every observer since the time of Munk ('58) has seen them, and many have been struck with the similarity between the appearance of them and the refractive body, both in life and in fixed and stained material. Indeed, Nussbaum ('84) and Schneider ('02) suggested their true relationship, but neither saw any proof of it. Now, however, we have this proof, and the search for the true origin of the refractive body leads us to a study of the complete history of the refringent vesicles.

THE REFRINGENT GRANULES AND VESICLES

Technique

These bodies are readily seen in unstained, living cells as spherical, shining granules or droplets scattered throughout the cytoplasm. No division has ever been observed in them, though if it occurred it could easily be seen. Such intra vitam stains as neutral red, methyl-green or methyl-blue are readily taken by them. But some of the nuclear structures also take these stains. They are fixed equally well by all the osmic fixatives and by most of the acetic mixtures. After fixation, as well as in life, the similarity of their staining reactions to those of certain nuclear structures is striking. Most of the stains in common use give results which would lead one to conclude that these refringent bodies are made of nuclear material escaped into the cytoplasm. Indeed Scheben ('05), Struckman, and Auerbach all agree with Munk ('58) in the belief that the refractive body exudes from the nucleus, reasoning only from the similar staining reactions of this body and the chromatin.

Although it is upon such evidence that we must rely for our knowledge of the true relationship of the various structures in the cell, both nuclear and cytoplasmic, the accuracy of our conclusions will depend entirely upon the selectiveness of the stain

earlier workers considered the absence of the mature spermatozoon in the male to be the normal condition; and even if the rare case of its presence was observed it was probably interpreted as abnormal or the result of poor technique.

we use. For example, osmic acid stains all cytoplasmic inclusions in the developing sperm cell black or gray-black, while iron hematoxylin stains them all dark blue or black. But it would be quite inaccurate to say on the evidence given by these stains that all these bodies are composed of fat, or that they are all made of chromatin. For if we use a counter stain with the iron hematoxylin, such as Bordeaux red, we find that certain of these bodies retain the blue-black color while others readily give it up and take the red. They must therefore be quite different in chemical nature.

The physiological cytologist insists that the true chemical nature of no protoplasmic structure can be told by its reaction to a stain. Many workers in this field have shown that the staining reactions of such material are almost wholly determined by the ionic sign of the fixative used, and so give little or no information concerning the chemical nature of parts. This is true; yet when two structures always stain differently after a given fixative it is fair to conclude that they are unlike chemically. But the converse of this statement is true only when we use a very selective stain. In the hope of finding a truly differential stain, one which would show in this broad way a difference of chemical nature, I used in the present study combinations of the following fixatives and stains: Flemming's strong solution, without stain; Benda's modification of this solution and his 'krystal violet' stain; Altmann's fixative and his acid fuchsin stain, washed in picric alcohol. Besides these reciprocal stains suggested by the men who designed the fixatives, I used the Ehrlich-Biondi-Heidenhain preparation and iron hematoxylin-Bordeaux red as double stains, not only after these fixatives but also after Carnoy and Lebrun's fluid, sublimate-acetic, zur Strassen's, Tellysnickski's, Zenker's, Müller's, and Bouin's fixatives. The unstained osmic preparations are of little value from the point of view of differentiation, no matter how carefully treated. Altmann's acid-fuchsin-picric-alcohol method is somewhat better, but it does not differentiate clearly the refractive body and the mitochondria or 'plastochondria' (Meves) from chromatic structures. But most of the acetic fixatives show good differentiation

of both nuclear and cytoplasmic structures when followed by either iron hematoxylin-Bordeaux red, or the Ehrlich-Biondi stain. These combinations and those suggested by Benda were indeed the only ones that proved to be of value in this study.

The most instructive reactions obtained were those caused by Benda's fixative and stain.[3] A late spermatocyte with this technique shows the numerous refringent bodies stained blue, the karyosome or chromatic nucleolus red-brown, and the plastosome and certain small granules throughout the cytoplasm (Meves' 'plastochondria') yellow. While these different colors tell us nothing concerning the chemical composition of these structures we must conclude that they are not alike chemically. The prime value of Benda's stain for our present purpose, therefore, is that it is more selective than any other used. The other combinations mentioned will distinguish the plastosome and small granules from the karyosome and the refringent vesicles, but the latter so closely resemble each other in color that they might easily be

[3] Owing to frequent injury to sections in following Benda's directions for staining, I modified his method slightly. As used by Benda, Meves, Duesberg and others, the treatment of material for this stain is as follows: after fixation in strong Flemming's or Hermann's fluid, the tissue is washed in water, then left in a mixture of equal parts of pyroligneous acid and 1 per cent chromic acid to mordant. Then for a like period of time, say twenty-four hours, in a 2 per cent solution of bichromate of potash for the 'second chromization.' The material is then washed, dehydrated and imbedded as usual. After sectioning, iron alum in a 2 or 4 per cent solution is used as a mordant, and also an aqueous solution of sodium sulfalizarine. After washing in water, a solution of 'krystal violet' is poured over the sections and the slide is held over a flame until steam arises from the solution. The stain is then poured off and the sections are washed in water. Next they are destained in 30 per cent acetic acid, and after being thoroughly rinsed in water they are dried between sheets of blotting paper. The sections are then put directly into absolute alcohol for a moment, and are cleared and mounted as usual. I found, however, that heating the sections, destaining them in 30 per cent acetic and finally drying them between blotting or filter paper ruined most of the sections. I got just as clear-cut and permanent stain differentiation by using the following method: after washing the excess alizarine solution from the sections by carefully dipping the slide into water, they were stained by putting the slide into a 3 per cent solution of 'krystal violet' (3 cc. aniline stain in 100 cc. distilled water) for ten minutes. They were then rinsed in water and put into 80 per cent alcohol for about five seconds; then into 95 per cent and finally into absolute alcohol, where the destaining was watched carefully. Then the sections were cleared and mounted as usual.

considered to be alike chemically. But with Benda's stain such a misinterpretation could not be made; for the red-brown of the karyosome and the karyochromatin is quite distinct from the deep blue of the refringent vesicle.

What then does the use of Benda's stain teach us concerning the material which makes up these vesicles before they appear in the late spermatocyte?

Origin

There is no trace of this blue-staining material in the cytoplasm of the spermatogonium, neither during division stages nor in the 'rest stage' nor yet in the synizesis period. But within the nuclear membrane throughout all of these stages a blue-staining material is unmistakably present. It is most abundant in the prophase of the spermatogonial divisions, where it lines the nuclear membrane in thin sheets and irregular masses, apparently covering the karyochromatin (figs. 2 and 3). Often however the karyosome itself is cut, showing a red-brown color inside, though it is covered by the blue staining material. During division this material is seen covering the chromosomes. Figure 1 represents a fortunate section of a cell in metaphase in which two chromosomes were cut nearly lengthwise. The karyochromatin at the center of each is stained red-brown while the surfaces are blue.

Throughout the synizesis period the chromatic skein is in contact with the nuclear membrane at many points. As this period closes, the greater part of the blue-staining material passes toward the nuclear wall, while the karyochromatin and the plastochromatin aggregate to form the karyosome and the plastosome respectively. As a rule these bodies take positions near the center of the nucleus.

The young spermatocyte thus shows a fairly definite localization of three nuclear materials, which stain quite differently from each other (fig. 4). The migration of the blue staining material does not cease when it reaches the nuclear membrane, though as a rule practically all of it has come to line the nuclear wall in the form of cords and plates before any of it pushes through the membrane into the cytoplasm (fig. 4). In figures 4 and 5

we see the actual penetration of the nuclear membrane by this material.[4] Once in the cytoplasm it takes spherical shape as tiny granules, or as droplets easily visible under the low power lens, according as the quantity of material transuding at that point is larger or smaller. These droplets have long been known as the 'refringent granules.' Their refringent appearance, however, is acquired after they have reached the cytoplasm. It is due to the formation of yolk within them, as will be shown later. The material of which they consist is therefore a yolk-forming substance.

Such cytoplasmic inclusions in gland and sex cells, controlling secretion there and staining blue after Benda's stain, are called by many cytologists 'mitochondria.' But these are thought to be wholly cytoplasmic in origin, having nothing to do with the nucleus or its contents. We cannot therefore use this term to represent these yolk-forming granules in Ascaris spermatocytes, for they arise in the basichromatin or karyochromatin of the nucleus and pass out into the cytoplasm.

Meves uses the term 'plastochondria' to represent cytoplasmic granules arising in the oxychromatin or plastochromatin, and so indicates their origin and nature. I would suggest the term 'karyochondria' for these yolk-forming granules, because it likewise indicates their origin and nature.[5] The observation of the migration of the karyochondria from the nucleus leads to an

[4] I am indebted to Professor McClung for confirming this observation.

[5] The following table will define the terms 'karyochondria' and 'plastochondria' as they are used in this paper:

	Origin	*Function*	*Equivalents*
Karyochondria	Arise in the basichromatin: i.e., chromatin network, chromatin nucleolus or karyosome, and chromosomes	Yolk-forming granules, which very soon become yolk vesicles. These fuse to form the 'refractive body' of the spermatozoon	Cytoplasmic nucleoles Pseudochromosomes Accessory nuclei Vitelline body Trophochromatin Ergastoplasm of Prennant and Bouin Mitochondria of Meves and Korff

interesting interpretation of the condition of the cytoplasm of the spermatocyte as shown in figure 10. Here we find granules and yolk vesicles of varying sizes scattered throughout the cytoplasm. Small granules may be close to the cell wall, while near the nucleus we may find fully formed yolk vesicles. Since the karyochondria do not arise de novo in the cytoplasm there must be a constant movement of the cytoplasm, or these inclusions could not become thus scattered. Such protoplasmic movement is well known in resting plant cells, and it occurs, of course, in all dividing cells; but it must occur also in the resting spermatocyte of Ascaris (figs. 6 to 10).

Development

The karyochondria, almost as soon as they first appear in the cytoplasm, increase in size and become less deeply staining. They remain spherical in outline as they grow; no irregular or dumb-bell shapes appear amongst them. Though their growth seems to be quite definitely limited, ceasing when the diameter is about ten times that of the original granule, I have not been able to find undoubted cases of division or even fragmentation. For some time before the maturation divisions, the cytoplasm is about half filled with vesicles evenly distributed through it and uniform in size and staining reaction. A count of the vesicles in the median plane of such a cell corresponds so closely with one made in a cell in the midst of the growth period, when only a few of the granules have become fully formed vesicles, that it

Plastochondria	Arise in the oxychromatin: i.e., the linin network, true nucleolus or plasmosome or plastosome, the centrosome and centrosphere	Minute granules which make up the interalveolar reticulum, the fibrillae, the rays and fibers of the division figure. After reaching the egg cytoplasm they dissolve and help to form the fertilization membrane	Mitochondria of Marcus, Mayer, Romieu, etc. Microsomes Plastidules Archoplasm Protoplasmic corpuscles. Paraplasm or metaplasm (Wilson)

is evident that no division occurs among the vesicles. Their number, which is remarkably uniform in all the cells of a tube, seems to be determined, therefore, by the amount of transuding karyochondria.

Destiny

Just before the first maturation division the centrosomes emerge from the nucleus. The refringent vesicles at once begin to arrange themselves in concentric arcs about them. This symmetrical arrangement is kept throughout the divisions. The close of the anaphase of the second division is marked by two interesting events; the fusion of the centrosome with the two chromosomes in each spermatid, and the withdrawal of small, dense granules (plastochondria) from the refringent vesicles (fig. 34).

When the new spermatids separate they are spherical, and in them the vesicles are arranged radially and concentrically in four or five layers. Each spermatid has at its center the fused centrosome and chromosomes, surrounded by a clear mass of cytoplasm, or the 'perinuclear zone' of Van Beneden. In this zone lie the plastochondria, or 'microsomes' of Van Beneden. In the great majority of males killed one finds only spermatids of this form filling the vas deferens (figs. 12 and 35). Among them, however, are many which show 'cytoplasmic reduction,' a phenomenon now recognized in the development of the spermatids of a good many animals. In these, probably the older ones, a mass of cytoplasm containing plastochondria but without refringent vesicles, gradually flows out from the spherical spermatid and is cut off from it (figs. 13 and 14). Romieu ('11) was the first to describe this phenomenon in Ascaris. As a result of this loss, the volume of the spermatid is often reduced one-half, and the vesicles are thus brought close together.

In extremely rare cases a male will be found in which the spermatids are transforming into spermatozoa. This transformation probably occupies only a few minutes, and takes place just before copulation occurs.

In these the vesicles gradually fuse together until they form three or four large globules which surround the clear zone (figs. 25 and 36). Indeed, this is usually encroached upon, and some-

times the central mass is temporarily pushed aside (fig. 37). Fusion continues, however, until a single hemispherical body is formed which comes to lie in the cytoplasmic cone of the spermatid, stretching the latter until it forms a mere sheath around it (figs. 15 and 38). The refractive mass now elongates, taking the form of a truncated cone. Its base is slightly concave, and adjoins the clear zone, which has regained its former position (fig. 48).

The refractive body is now completely formed. Before the fusion of the refringent vesicles begins sections of them show a different reaction to stains on the surface and in the interior. This difference is very marked in sections of the globules and of the refractive body itself. While the surfaces of all these inclusions remain a deep blue after Benda's stain the interiors of them become more and more yellowish as fusion progresses, and finally the entire inner portion of the refractive body stains yellow. In the older spermatozoa even the surface of this body loses the blue color (figs. 12, 15, 16 and 25).[6]

I believe this is the final step in the history of the blue-staining material, or karyochondria, in Ascaris; that it is entirely consumed in the formation of the refractive body of the spermatozoon by changing chemically into the material of which that body is made, namely, yolk.

For we know that all secretions whether glandular or yolk, stain yellow after Benda's stain. Indeed, Bouin ('05) considers the growing spermatocyte homologous with the gland cell so far as the secretion of yolk in it is concerned. We know that in many gland cells a substance appears in the cytoplasm just before secretion begins, and gradually disappears as the secreted material accumulates (Faure-Fremiet, '10, for literature). This substance has been called a 'prezymogen'—a factor which directs secretion, and may later transform into the secretion itself. In other forms this factor is known as 'mitochondria,' and its behavior seems to be followed precisely, as we have seen, by the karyochondria in Ascaris. Mitochondria, however, are considered to be wholly cytoplasmic in origin.

[6] Romeis ('12) confirms this observation.

RÔLE OF THE REFRACTIVE BODY

In our search for the nature and function of this unique structure, a review of the normal behavior of the Ascaris spermatozoon will be of interest. So far as we know at present, these cells are injected into the distal end of the uterus at the time of copulation, and have to make their way to the proximal region of this organ by their own powers of locomotion, for the unfertilized eggs are to be found only at the proximal end. The uterus is usually not less than 12 and may be more than 15 cm. in length, and it is always full of eggs and developing larvae. The spermatozoa have, therefore, a relatively long and difficult journey to accomplish; and since their only known method of locomotion is amoeboid creeping, their rate of travel must be very slow. Indeed, the total distance to be traversed by them is far greater than the length of the uterus, because the inner walls of this organ are thickly studded with epithelial papillae, so that their course cannot be a direct one. It is evident, therefore, that a considerable amount of energy must be expended by the spermatozoa in making this journey, and hence a definite food supply is necessary for their use.

A very brief examination of the sperm which have reached the proximal or 'entrance region' of the uterus shows a great variation in the form and size of the refractive body, as we have seen. While Van Beneden interpreted these various shapes as indicating different stages in the growth of this structure, basing his opinion upon the hypothesis of the uterine origin of it, Mayer, Romieu, Romeis and others interpret them as stages in the phagocytosis or degeneration of superfluous spermatozoa. I believe, however, that they merely mark the normal consumption of a proper food supply by the spermatozoon itself. While figures 40 and 48 represent sperm in which very little change in the form of this body has taken place, such are rare. In figures 40, 46 and 47 are shown those in which only a remnant is left in the form of a corroded axial rod.[7] In figures 39, and 41 to 45

[7] Since iron hematoxylon used after any acetic fixative stains both the inner and outer parts of the refractive body blue-black, it is an excellent stain to use to reveal even slight traces of this structure.

spermatozoa are represented which not only have lost all trace of the refractive body, but are entering eggs in this condition. In sections of one tube I found that nearly half the eggs had been entered by spermatozoa of this kind. To be sure that these were not merely lying upon the sections, I examined many whole eggs which had been fixed and stained just as the sectioned eggs were, and found many eggs which were being entered by such spermatozoa. I have examined a great many egg tubes and find them all alike with respect to this phenomenon (figs. 42 to 45).

Probably no point in the study of Ascaris spermatogenesis has called forth greater difference of opinion than the nature and function of the refractive body. Van Beneden first observed spermatozoa entering eggs without it, and concluded from this fact that it plays no part whatever in fertilization. His further conclusion that its presence in the spermatozoon is purely accidental must be judged in the light of the fact that he had seen this structure only in the proximal region of the uterus, and thought it was formed there.

Boveri agrees with Van Beneden that it is not necessary for fertilization, since this is accomplished with or without it. Scheben, on the other hand, believes it to be most important in fertilization, for he thinks it gives rise to the male pronucleus. Tretjakoff believes that its only function is to serve as a mechanical support for the sperm, corresponding in this respect to the capsule of the decapod spermatozoon as interpreted by Koltzoff.

Romieu agrees to this suggestion, but adds that it may feed the egg as well as to help in effecting penetration. Romieu thinks that its presence is the sign of maturity of the sperm, and that it is present in every functional male cell.

Marcus suggests that in Ascaris canis it may be a food supply. On page 460 we read, "Ich muss mich also in Gegensatz zu Scheben der Ansicht v. Benedens und Boveris ausschliessen die dem Glanskörper keine unmittelbare Bedeutung bei der Befruchtung zuschreiben. Nach meiner Auffassung ist er ein Nahrungskörper. Ob die Dotterkugeln bei den Spermatocyten ursprünglich aus dem ins Plasma hinausgetreten Trophochromatin entstanden sind, kann ich nicht entscheiden." He adds

that this may occur, but he thinks it is not probable because the yolk forms first at the periphery of the cell.

Marcus thus approaches very closely the true origin and nature of the refractive body. He lacked only the actual observation of the escape of the 'trophochromatin' or karyochondria from the nucleus into the cytoplasm, and the formation from this of the refractive vesicles, to complete his history of the refractive body in Ascaris canis. As we have seen, this gap is filled by this study of A. megalocephala. If it be true that the yolk vesicles first appear near the cell wall in A. canis, it must be due to chance aggregation of them there as a result of protoplasmic movement. The smaller granules from which others would be derived must be scattered throughout the cell, though these might have been overlooked.

At any rate, the suggestion of Marcus is the correct one—borne out fully, I believe, by the observations recorded in this paper—that the refractive body plays no essential part in fertilization, but that it is merely a food supply for the spermatozoon alone, derived from the cytoplasm through the activity of a substance which escapes from the nucleus, the karyochondria.

THE 'MITOCHONDRIA'

When spermatocytes are stained in iron hematoxylon alone, after fixation in an acetic mixture, and destained almost completely, one finds small dense granules in the refringent vesicles which stain black. In those cells which are approaching division the vesicles are slightly oval, and the granules arrange themselves in chains or rods which lie in the long axes of the vesicles. They are most conspicuous during the metaphase of the second maturation division, because at this time the refringent vesicles are arranged concentrically around the centrosomes, and the chains of granules are therefore perfectly radial with respect to these (fig. 34).

But during the anaphase of this division the chains of granules are drawn out of the vesicles and break up, and the separate granules crowd close up to the chromatic mass, but in radial and concentric lines. The refringent vesicles, also acted upon by the

attractive force of the centrosome, arrange themselves in concentric circles. The size and number of those vesicles in the nearest circle prevent them from coming up to the attracting mass at the center, so that a clear zone,—the 'perinuclear zone' of Van Beneden, is left around it. In this zone the granules just described stand out clearly.

The vesicles still stain blue in Benda's stain, though of a lighter shade than before the divisions, *but the granules always stain yellow;* and, since they surround the closely massed centrosome and chromosomes, the red-brown of the latter is completely masked by the yellow, unless seen in section (figs. 12). After iron-hematoxylin-Bordeaux red the granules stain red, while the vesicles stain blue; and in Ehrlich-Biondi the granules take the red dye and the vesicles are purple (fig. 25). Thus these granules must be quite unlike the vesicles chemically. Their origin and history will be of interest.

The plastosome and the plastin grains or plastochondria

The plastosome appears in the nucleus of the spermatogonium very soon after division ceases. It is a small granule throughout the 'rest period' and stains yellow after Benda's stain (figs. 3 to 11), bright red after Ehrlich-Biondi (figs. 19 to 24) and black after iron hematoxylin (figs. 29, 30 and 31); while the karyochromatin stains red-brown, green and blue-black respectively, after these stains, as shown in the figures just mentioned.

During the synizesis stage the plastosome increases in size, and often small granules staining just like it are to be seen scattered throughout the nucleus (figs. 19, 20 and 21). Throughout the long growth period these remain evident, and their number increases.

Early in the growth period small granules like those just described in the nucleus appear in the cytoplasm. They are very small, 0.1 to 0.5 μ in diameter (figs. 5, 6 and 7). At the close of the growth period, when the centrosome leaves the nucleus it takes these granules with it into the cytoplasm. They are often clustered around it in the form of a hollow sphere, as shown in section in figure 24. As the cleavage figure is formed, many of

the granules scatter throughout the cytoplasm, and these cannot be distinguished by size, form or staining reaction from those already there.

At about this time one finds similar granules inside the refringent vesicles, as stated above. The plastosome itself sometimes survives the growth period as a unit, though often it does not. It is never found in the spermatid nor the spermatozoon, but in both of these the plastochondria are very conspicuous. Usually it exists only as scattered fragments by the time the maturation divisions occur, and these form the rays and fibers of the cleavage figure. Whether these plastin grains or plastochondria reach the cytoplasm by passing through the nuclear membrane at many points, or by going out with the centrosome they are undoubtedly of plastosomal origin and nature.

But some of these granules develop within the refringent vesicles, escaping from them just after the second maturation division. These cannot have come from the plastosome. Yet their staining reactions indicate that they are exactly like all the other plastochondria. We have, apparently, in this observation, a proof of the true origin of all of the plastochondria, and so of the plastosome itself. Their staining reactions, whether within or outside of the nucleus, show them to be secretion or excretion products. Here we see what substance it is which first secretes and then excretes them. In the cytoplasm the only secreting agent is the karyochondrial material in the form of the refringent granules and vesicles. In the nucleus the plastosome does not appear until this material is present in considerable quantity. I believe we must conclude that the plastosome and its derived plastochondria, therefore—indeed, the plastochromatin in all its various forms—arises in the karyochromatin as a product of its metabolism. Montgomery ('11) reaches this same conclusion, so far as the origin of the plastosome is concerned, from his study of the history of the nucleus of the spermatocyte of Euchistus. That the plastochondria cannot be used as food by the spermatozoon is shown by the fact that they are excreted by the refringent vesicles just before these fuse to form the refractive body, which is the food supply of the spermatozoon. Earlier authors

have called them 'plastidules,' 'plastin granules' or 'microsomes.' Van Beneden first observed and figured them clustered around the chromatic mass at the center of the spermatid. But he did not trace their origin. Mayer first saw them in the refringent vesicles, and leaving these to take the position just mentioned. He did not trace their origin, however, and seems to have overlooked the fact that they always stain with acid dyes, for he calls them mitochondria, although all cytologists agree that mitochondria take basic dyes.

When the plastochondria reach the perinuclear zone fusion occurs to a limited extent, resulting in fewer and larger granules, while many are still scattered throughout the cytoplasm of the spermatid. Many of these are lost when cytoplasmic reduction occurs. Though the diameter of the spermatid is reduced about one-half at this time, the granules lost seem to be only those lying by chance toward the periphery of the cell. There seems to be no selection or segregation here.

Those that remain keep a fairly definitely symmetrical position around the center of the spermatid during its transformation into the spermatozoon, regaining it if it is temporarily lost. The smaller ones lie in the thin sheath of protoplasm which surrounds the refractive body, while the larger ones lie in the sponge-like protoplasm of the 'crown' or head (figs. 25, 27, 28 and 48).

In these positions they are carried into the egg; but, as soon as they enter the egg cytoplasm, the symmetrical arrangement is lost, as a consequence of the absorption of the sperm cytoplasm by that of the egg. The larger ones in the perinuclear zone remain in a fairly close group long after the outlines of the sperm have been lost, and can be distinguished easily by their larger size, as Meves has recently pointed out ('11), from similar granules which are scattered throughout the egg cytoplasm. After the fusion of the pronuclei, however, they also become dispersed, fusing with those of the egg, or with those of there own kind, or remaining as small granules which take part in the formation of the rays and fibers of the cleavage figure. Romeis ('12) confirms the observations of other authors who found that they help to form the fertilization membrane of the egg after they have dissolved in the egg cytoplasm.

This is the complete history I believe, of the 'mitochondria' of Mayer, Romieu, Faure-Fremiet, and Romeis, or the "plastochrondria' of Meves. Their behavior is such as any inert excretion grains would and do show in other cells. They undergo no division, show no inherent power of growth, and do not transform into any other substances. They are shifted about in the cell by any forces that are set up in it. Their staining reactions are always those of secretion products, yellow after Benda's stain, and red after Ehrlich-Biondi. They do not take intra vitam stains.

DISCUSSION

Faure-Fremiet's definition of true mitochondria

It remains only to consider briefly the observations recorded in this paper in the light of the study of cytoplasmic inclusions in the germ and body cell of other animals, now recognized as 'microsomes,' protoplasmic corpuscle,' 'archoplasm,' 'mitochondria,' 'ergastoplasm,' 'plastochondria,' and so forth, by various authors. The essential point in the study of such inclusions is to determine which of them are living, formative elements, and which are inert, formed products.

Ever since the panstaking work of Dujardin, this problem has claimed the attention of the most eminent cytologists. Faure-Fremiet well says, (page 461, '10):

> When we find in a cell a fat globule, a vitelline corpuscle or an albuminoid granule which increases in size until it is absorbed or expelled, we do not consider this an integral part of the cell, since it results from the work of the cell, or it is utilized in this work and does not help to direct it. When we see, on the other hand, the nucleus of the cell or a leucoplast we consider these as part of the organization of the cell, controlling its work. We see them grow and divide like the cell itself, and we know that they are active factors in the life of the cell. We conclude therefore that the nucleus is a *living* factor, while the fat globule is *not*. This illustration is clear cut, but it is often far more difficult to apply the words *formative substance*, and *formed substance*.

His closing sentence on this subject is of interest in connection with this review: "For my part, when I see that these elements divide, that they contain fatty acids, or that yolk material ap-

pears around or within them, I merely say that similar facts have been observed in connection with the leucopasts of plants." These sentences are quoted from the close of the introduction to a very complete review of the literature on mitochondria.

Mitochondria as yolk-forming factors in spermatozoa and in eggs

In this comprehensive study, Faure-Fremiet shows that this material occurs in sex cells under four different types of formation and behavior. In the last of these he includes all those cases in which the mitochondria transform partially or wholly into yolk. The spermatocytes of batrachians and of myriapods, and the oocytes of many groups of animals are cited here.

Prennant ('87), in the spermatocytes of myriapods, observed certain granules and filaments which he named 'ergastoplasm,' because he considered them as active elements in the cell. In 1905 Bouin showed that these bodies transform into yolk. Meves and Korff ('01) call this material 'mitochondria,' as its behavior in the myriapods was like that of mitochondria in other forms.

Benda ('03) found the mitochondria in the spermatocyte of Rana in a compact mass in a cavity in the nuclear wall. He called this mass the 'corps condriogens.' Champy ('09) found, here and there, in the spermatocytes of Bombinator 'yolk vesicles' of various sizes among the mitochondrial granules and filaments. They resembled the nucleolus inside the nucleus in staining reaction but they are often made of two spheres, the inner one always staining lighter or more acidophilic than the outer. This author believed that yolk is formed in these 'cytoplasmic nucleoles' or mitochondria, just as in the oocytes of Bombinator or other forms.

Very clear cases of the transformation of mitochondria into yolk have been found in the developing oocytes of a number of animals. The simplest case recorded by Faure-Fremiet is found in the chilopods, where there is no yolk nucleus or vitelline body of any kind. The mitochondria themselves become yolk granules, then vesicles or globules, just as they do in the spermatocytes of these forms.

Bouin ('98) observed the 'paranuclear bodies' in the oocytes of Echinoderms breaking up into 'corpuscles;' these 'disappeared' at the moment of yolk-formation through loss of their stainability, but the cell seemed to be full of yolk granules and vesicles.

Loyez ('09) found in the young oocytes of certain tunicates the 'nucleoles' grouped around the nucleus. Later they form angular filaments, which then break up into granules. These increase in size, and show a clear, inner portion which does not stain blue in Benda's stain, while the surface does. These vesicles increase in size, the unstained central portion growing at the expense of the blue-staining surface material, until none of it is left. Van der Stricht ('05) finds the 'pseudochromosomes' in the oocyte of the white rat and the bat breaking up into mitochondria, which then transform into yolk. Lams and Doorme ('07) confirm these observations on the oocytes of the rat and the guinea-pig.

Mitochondria in the spermatogenesis of Ascaris megalocephala

Many other cases of this kind might be cited, but these are clear. In view of them my observations of the origin and development of the refractive body in Ascaris lead clearly to the conclusion that this form and probably all of the nematodes belong to this fourth 'type' of Faure-Fremiet, for the blue-staining material in Ascaris spermatogenesis is certainly a yolk-forming material, and eventually completely transforms into yolk. Its place of origin is also clear. This 'ergastoplasm' of Prennant and Bouin, or 'mitochondria' of Meves and Korff undoubtedly has its counterpart in the blue-staining material in the spermatocyte of Ascaris. The authors just named thought that 'mitochondria' were expelled from the nucleus of the myriapod spermatocyte into the cytoplasm. As we have seen, this is the origin of the blue staining material in the Ascaris spermatocyte. Here it forms the refringent granule, then the surface of the refringent vesicle and later of the refractive body. It is evident that the term 'mitochondria' is ambiguous, for as we have just seen, it is used to stand for cytoplasmic inclusions which form yolk and

also for those which do not, and which therefore must be quite different in origin and nature. While Meves and Korff use it to represent this yolk-forming material of nuclear origin, Benda, Duesberg and others use it to represent structures which they believe are wholly cytoplasmic in origin, aud give rise to the various connective tissus of the embryo. The authors just mentioned state that the mitochondria always take basic stains, though they do not arise in the nucleus. But Mayer, Romieu and other students of Ascaris spermatogenesis call the small, dense granules found in the 'perinuclear zone' of the spermatid, and in the 'crown' of the spermatozoon, mitochondria. But these granules not only take acid stains always, but they unquestionably arise in the nucleus. Besides, no one has yet found that they give rise to any yolk or embryonic tissue. They seem to be merely inert residua.

Manifestly, therefore, the term 'mitochondria' is useless. Meves recognizes the inappropriateness of this name for the granules in Ascaris just mentioned, and calls them 'plastochondria' because of their resemblance to the plastosome in all staining reactions, and their possible origin in it.

I would suggest the name 'karyochondria' for the yolk-forming granules because of their close relation to the karyochromatin. It remains to be seen, I think, whether all inclusions in the cytoplasm of sex cells of other forms referred to as 'mitochondria' cannot be identified with one or the other of these, the plastochondria or the karyochondria, for there is abundant evidence that both yolk-forming and organ-forming factors first arise in the nucleus and pass out into the cytoplasm, as will be shown later.

Among the earlier students of Ascaris spermatogenesis, Van Beneden spoke of Meves' plastochondria merely as 'protoplasmic corpuscles,' attributing no function whatever to them. Hertwig figures them in the refringent vesicles, just before the maturation divisions, but he does not discuss them. Boveri found them in the egg, surrounding the male and female pronuclei. He called them 'archoplasmic grains,' but he attributed no significance to them. Tretjakoff figures them scattered among the

refringent vesicles early in the growth period, but he does not refer to their origin or behavior.

Meves, however, finds that they come from the plastosome, as we have seen, and he considers them of great importance in fertilizaton. He interprets the fact that they fuse with similar but smaller granules in the egg as a phenomenon of great significance. Meves was not the first to observe this fusion; as he points out (p. 686), L. and R. Zoja ('91) first described it. But Meves thinks that these observers missed the significance of it. He believes that through the fusion of the plastochondria from the sperm with those of the egg, the male transmits to the offspring all the paternal structural characters that are to be inherited, and that these can be inherited in no other way. Thus, Meves' plastochondria agree in function with the 'mitochondria' of Benda, Duesberg and others; but the former are nuclear in origin, while the latter are thought to arise entirely within the cytoplasm. Even if the male pronucleus could be utterly obliterated before it fused with the female pronucleus, the offspring, according to Meves, would show structural characters inherited from the father.

But we may well ask what proof is there for considering the plastochondria to be of such great biological importance? Meves gives us none whatever except that these granules persist throughout the early cleavages. As we have seen these granules play the part of inert products in the cytoplasm forming the fibers or rays of the cleavage figure, or through their solution helping to form the fertilization membrane (Romeis). We are entirely without evidence of the great importance of these granules in heredity. On the other hand, there seem to be serious reasons for doubting the interpretation of them which Meves has given.

a. Inheritance in enucleated eggs. If Meves' interpretation be correct, Boveri's classic experiment of fertilizing the enucleated eggs of Sphaerechinus with sperm of Echinus should yield plutei which are not of the pure Echinus type, since the egg plastochondria should contribute Sphaerechinus structures to the hybrid.

Boveri, however, believes that the plutei so produced are of the pure Echinus type. This conclusion shows clearly that the maternal plastochondria are not bearers of structural characteristics, and it cannot be supposed that these bodies in the spermatozoon possess hereditary qualities not to be found in those in the egg.

b. Heredity in hybrids. The experiments of Loeb, Herbst, Baltzer, Tennent and others in crossing echinoderms, fishes, and so forth, show that heterogeneous hybrids are almost constantly maternal in structure. This should never be the case if the plastochondria function in inheritance of structure. But the larvae produced in these experiments show clearly, when studied cytologically, what it is that determines their maternal type of structure. Baltzer, Tennent and others have seen cross-fertilized eggs of echinoderms actually eliminate from themselves certain bodies brought into them by the spermatozoon. But these bodies are not plastochondria; they are chromosomes. Loeb finds that fish hybrids of this kind are always maternal, because synapsis is never successfully accomplished. Here again the chromatin alone is concerned.

As is well known, it is entirely possible, on the other hand, to produce 'intermediate hybrids.' But the study of cross-fertilized echinoderm eggs has shown that it is not the fusion of paternal and maternal plastochondria that produces these, but the retention of paternal chromatin by the fertilized egg. This always occurs, apparently, in forms sufficiently closely related.

c. Normal fertilization in Nereis. No clearer proof of the secondary importance of cytoplasmic structures in fertilization and inheritance could be desired, however, than that furnished by the observations of the normal fertilization of the egg of Nereis, made recently by F. R. Lillie. One of the enigmas in the study of the cytology of the sex cells has been for many years the nature and function of the middle-piece. The fact that it alone accompanies the 'head' or nucleus of the spermatozoon into the egg in fertilization has been generally accepted as proof that it plays some essential part in that process. Indeed, it was the discovery that the middle-piece and its accessory structures are com-

posed of 'mitochondria' that led Benda, Duesberg and others to claim for these bodies a continuity and significance in heredity quite equal to those of the chromosomes.

But Lillie finds that the middle-piece never enters the egg in Nereis. This discovery, therefore, renders this interpretation of the importance of 'mitochondria' exceedingly doubtful. On page 427 this author discusses this question as follows:

> The only characteristic thing about the cytoplasmic elements introduced by the spermatozoon is their great variability as to quantity and character in different animals. In Ascaris a very large quantity of cytoplasm containing characteristic plastosomes is introduced, as Meves has shown. In many, probably most, forms with flagellated spermatozoa, the entire spermatozoon enters; in some echinids the tail is left without, and in Nereis both tail and middle-piece fail to enter; and turning to plants, in phanerogams, apparently nothing but the nucleus is eventually concerned. There is nothing on the cytoplasmic side to correspond with the regularity of the nuclear phenomena in both animals and plants. In such precise phenomena as those of inheritance a mechanism of equal precision is to be expected, and it must be admitted that on the cytoplasmic side no such mechanism has been discovered. Moreover, as the laws of inheritance are the same for both animals and plants, a similar mechanism must exist for both, and such has been discovered only in the nuclei of the gametes. There is bad logic in the assumption that whatever parts of the spermatozoon enter the egg are necessarily concerned in the mechanism of transmission in inheritance, and the view that the cytoplasmic elements of the male gamete are concerned primarily in accessory functions of fertilization, such as locomotion and penetration, is still well founded.

The nature and function of 'mitochondria' or plastochondria

I believe the cases just cited argue strongly against Meves' interpretation of the importance of the plastochondria in Ascaris, and of the 'mitochondria' in other forms. If these plastin granules are of such great importance in heredity as Meves, Benda, Duesberg and others believe, it is difficult to understand why such a considerable proportion of them should be lost by the sperm during the course of its development. In Nereis we see the loss of this material just before fertilization occurs; but in many forms it takes place earlier. It has already been stated that a reduction in the amount of cytoplasm occurs in the young

spermatids of Ascaris by which the size of these cells is greatly lessened. Many plastin granules are lost when this cytoplasmic lobe is thrown off (fig. 13). Such a reduction in the spermatid with the consequent loss of these inclusions has been described by Struckmann in Strongylus, by Meves in the guinea-pig, by van Korff in Phalangista, by Broman in Myxine, by von Ebner in Rana and in certain mammals where he refers to these granules, probably, when he speaks of 'tingierbäre Körner.' Duesberg observed it in the rat and Jordan in the oppossum, while Rosenberg reports it occurrence in the Arachnida and Vejdovsky in Turbellaria. Faure-Fremiet reports the loss of all of these granules in the cytoplasmic lobe thrown off by the spermatid of Arion. Professor Montgomery discovered a remarkable case of the loss of 'mitochondria' in Peripatus. Just before the transformation of the spermatid into the spermatozoon all the mitochondrial material aggregates into a fairly compact body, and this is always included in the cytoplasmic lobe which is thrown off, so that the mature sperm never contains any of it. If, then, this material is of such great significance in heredity, it must be explained why it is so generally partially or wholly lost by the developing spermatozoon, in forms ranging all the way from the Turbellaria to mammals.

The true origin of 'organ-forming substances'

I cannot close this brief discussion of the significance of cytoplasmic inclusions in germ and body cells without referring to the work of Conklin on ascidian eggs and larvae. No one has traced the heredity of specific larval tissues from definite cytoplasmic inclusions more accurately and continuously than he. In the fertilized but unsegmented egg of various ascidians, notably Cynthia, Conklin found a constant distribution of pigment in the cytoplasm. This marks a definite localization of masses of protoplasm. These masses differ potentially from one another, as Conklin proved by the fact that he could trace the formation or origin of the various germ layers, tissues and organs of the larva as cleavage progressed to one or another of these masses,

since the identity of each was made certain by its specific pigmentation. But the localization and pigmentation of these masses of cytoplasm—'organ-forming substances,' as Driesch calls them —is not complete until after the break-down of the nuclear membrane. Indeed, the material which forms the 'clear zone,' from which arise the ectoderm and its derivatives, actually comes out of the nucleus. All the other masses also are derived as certainly from the nucleus, though not so directly, according to Conklin's observations. After describing the formation of the clear zone in gasteropod and Ascidian eggs, he says (page 101):

> This truly remarkable condition in which considerable portions of the cytoplasm are traceable to the nucleus is of the utmost theoretical importance. From all sides the evidence has been accumulating that the chromosomes are the seat of inheritance material, until now this theory practically amounts to a demonstration. On the other hand, all students of the early history of the egg have observed that the earliest visible differentiations occur in the cytoplasm, and that the position, size and quality of the cleavage cells and of various organ bases are controlled by the cytoplasm. However, in the escape of large quantities of nuclear material into the cell body and the formation there of specific protoplasmic substances we have a possible mechanism for the nuclear control of the cytoplasm; and when, as in the case of the ascidians and fresh water gasteropods, these substances are definitely localized in the egg, and can be traced throughout the development until they enter into the formation of particular portions of the embryo, a specific mechanism for the nuclear control of development is at hand, and the manner of harmonizing the facts of cytoplasmic organization with the nuclear inheritance theory is clearly indicated.

SUMMARY

1. Cytoplasmic inclusions of whatever kind found throughout the course of spermatogenesis of Ascaris megalocephala are reducible to two materials, both of which are of nuclear origin: (a) the karyochondria, which are derived directly from the karyochromatin, and (b) the plastochondria, which are derived from the plastosome, in part, and in part from the karyochondria.

2. Both of these appear first in the nucleus of the spermatogonium. The karyochondria form the surface layer of the karyochromatin, whether this is in the form of chromosomes or not. The plastochondria form the plastosome.

3. In the young spermatocyte the karyochondria pass through the nuclear membrane into the cytoplasm, where they form the 'refringent granules.' These at once begin the elaboration or secretion of yolk within themselves, and so become the 'refringent vesicles.' These then fuse to form the 'refractive body,' as the spermatid transforms into the spermatozoon. This transformation takes place entirely within the vas deferens.

4. The refractive body is purely a food supply for the use of the speratozoon, and frequently it is entirely consumed before the spermatozoon enters the egg. It therefore plays no part in fertilization; hence, the only function of the karyochondria in Ascaris is to form yolk.

5. The plastochondria are, like the plastosome, merely residua, and show negative behavior wherever they occur. While many are retained by the spermatozoon, many are lost by the spermatid.

6. Through the use of a single basic or nuclear stain, these two kinds of cytoplasmic inclusions have been confused. The simplest 'double stains' distinguish them clearly.

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All figures were drawn with the camera lucida, on the table level, with one-twelfth oil immersion objective and ocular 18, except figures 15, 16, 18 and 39 to 48 inclusive, which were drawn with ocular 4. Figures 41 to 48 inclusive have not been reduced. All others have been reduced one-third.

PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 18 inclusive represent cells fixed in Flemming's strong solution, and stained according to Benda's method, modified as stated on page 426. In all of these, the basi- or karyochromatin stains red-brown, the oxy- or plastochromatin and its derivatives, the plastochondria, yellow, and the karyochondria dark blue.

1, 2, 3 Spermatogonia of different ages.

4, 5, 6, 7, 8, 10, 11 Developing spermatocytes, which show the escape of the karyochondria from the nucleus into the cytoplasm, and the formation of yolk within them. Also the escape of the plastochondria from the nucleus into the cytoplasm, and the great increase in the relative amount of the cytoplasm.

12 A fully formed spermatid. The mitochondria of Mayer or plastochondria of Meves are distributed radially around the chromatic mass throughout the 'perinuclear zone.'

14 A spermatid undergoing cytoplasmic reduction.

15 A longitudinal section of a fully formed spermatozoon.

17 A spermatozoon in the 'entrance region' of the uterus, showing no trace of the refractive body.

18 An egg in the metaphase of first cleavage. The chromosomes are not cut, so that the karyochromatin is entirely hidden by the karyochondria. The astral rays, centrosome, etc. are distinctly yellow.

19 to 27 Cells fixed in the Carnoy-Lebrun acetic fluid, and stained in the Ehrlich-Biondi stain. In these the karyochromatin is green, the plastochromatin and plastochondria red, and the yolk vesicles purplish (not so dark as in the colored figures). The karyochondria are not to be distinguished from the karyochromatin in cells stained in Ehrlich-Biondi.

25 Fusion of the yolk vesicles preparatory to the formation of the refractive body in the vas deferens.

27 A spermatozoon in the 'entrance region' of the uterus which has almost entirely consumed its food supply (the refractive body) during its journey.

28 Here the food supply is entirely gone. This cell was fixed in acetic-alcohol, a modification of Zur Strassen's fluid, and stained in iron-hematoxylin and Bordeaux red. The plastochondria take the latter.

29 A spermatogonium fixed in acetic-alcohol and stained in iron hematoxylin alone. The plastochondria (plastosome) here stain jet black.

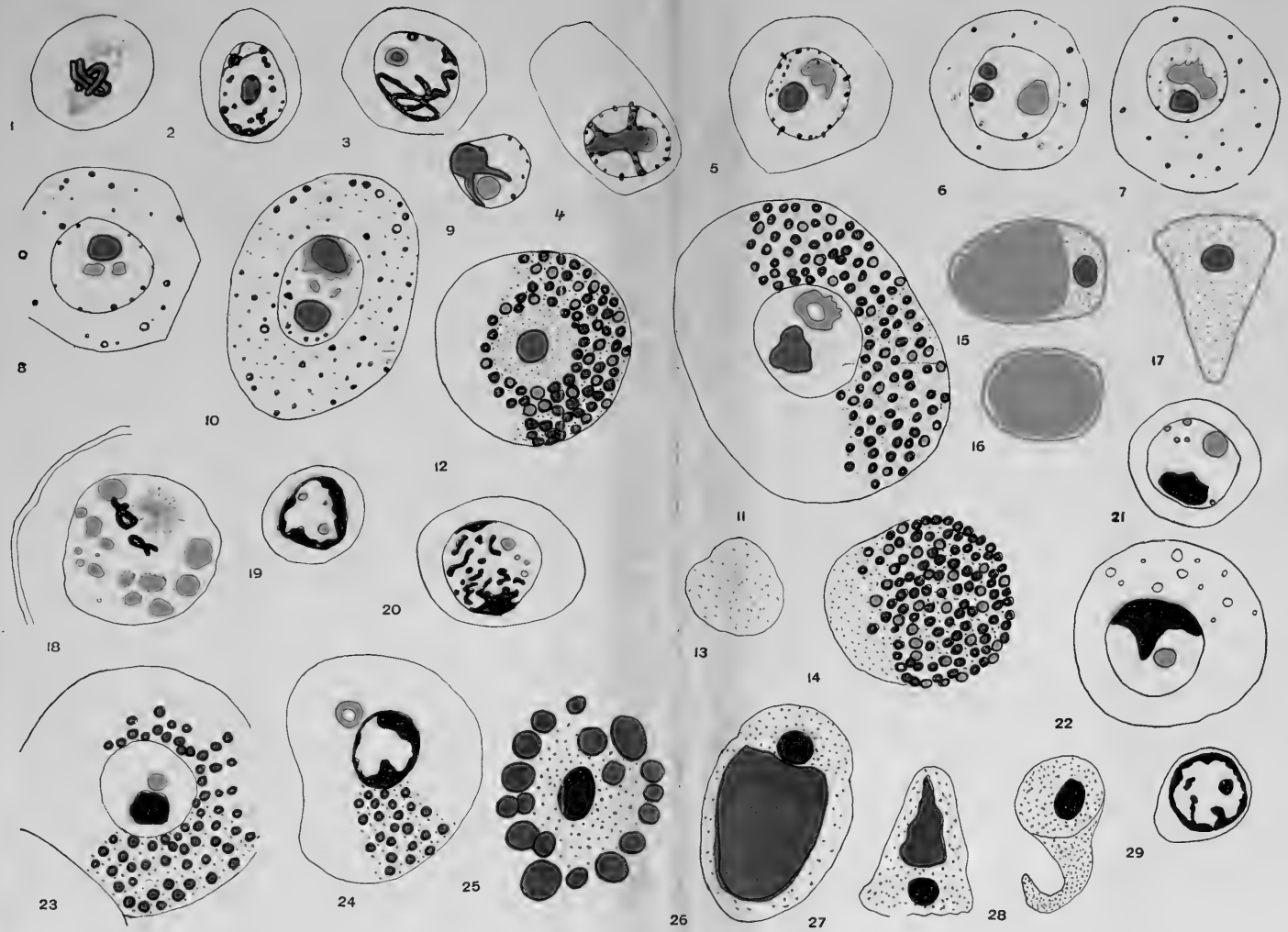
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PLATE 2

EXPLANATION OF FIGURES

30–40 All figures from cells fixed in acetic-alcohol.

30, 31 Older spermatogonia, stained in iron hematoxylin alone.

32 Young spermatocyte stained in iron hematoxylin and Bordeaux red. Karyochromatin blue-black, oxychromatin or plastochondria red and yolk vesicles blue to light blue.

33, 34 Maturation divisions of spermatocytes, stained in iron hematoxylin alone, and very heavily destained. The mitochondria of Mayer, Romieu, Faure-Fremiet, and others, form axial chains in the fully formed yolk vesicles.

35, 36, 37, 38 Various stages in the formation of the refractive body in the vas deferens.

39, 40 Eggs being entered by spermatozoa, which show various stages in the consumption of the refractive body.

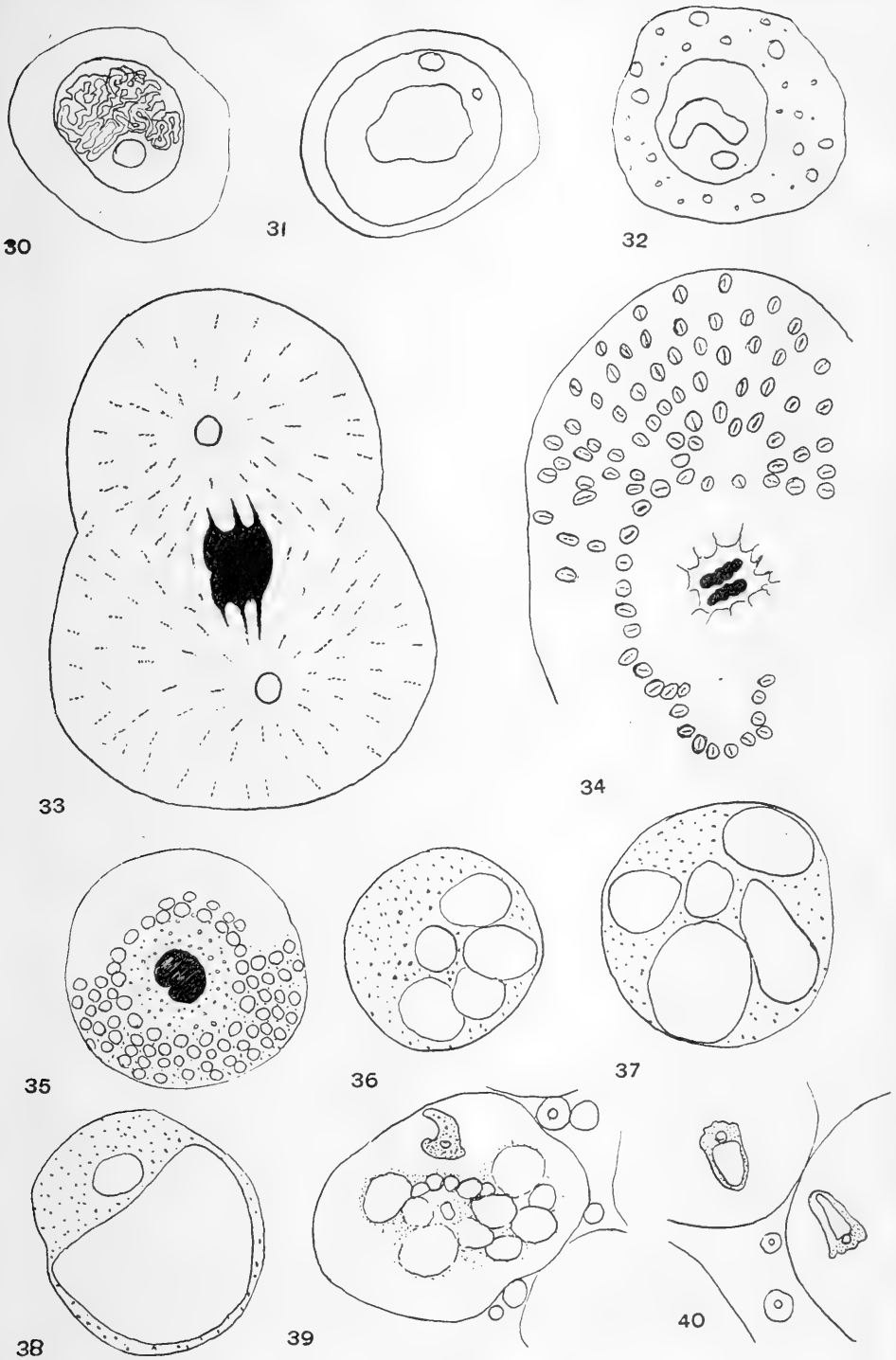
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PLATE 3

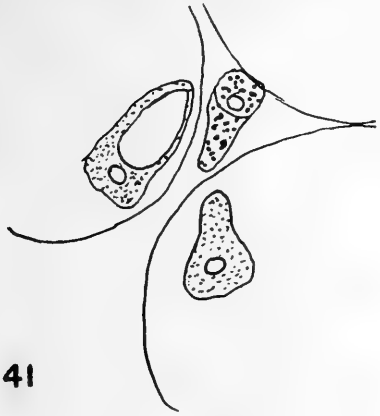
EXPLANATION OF FIGURES

41–48 All figures from cells fixed in acetic-alcohol and stained in iron hematoxylin and Bordeaux red.

41, 42, 47 Sectioned eggs.

43, 44, 45, 46 Whole eggs shown in optical section.

48 A mature spermatozoon with refractive body intact.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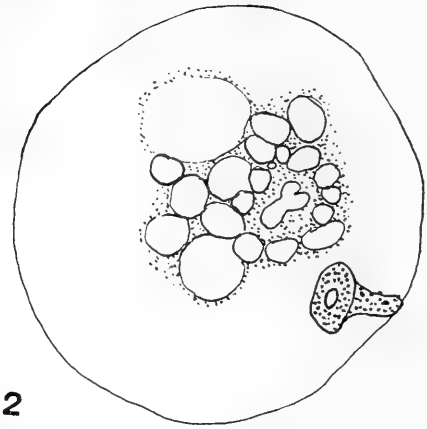
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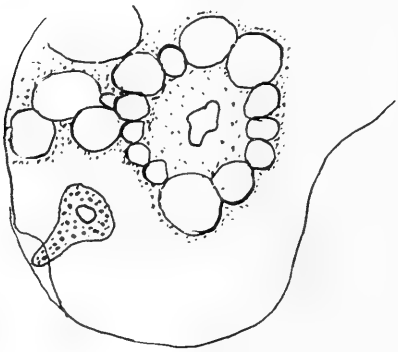
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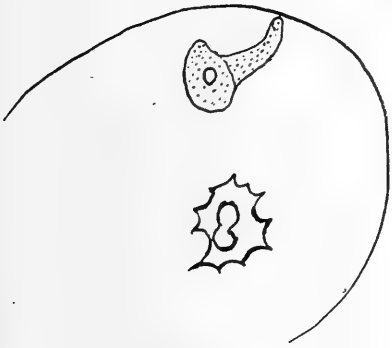
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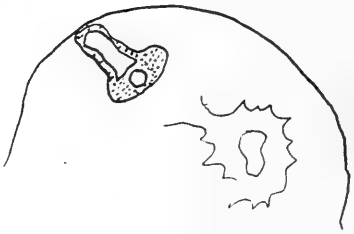
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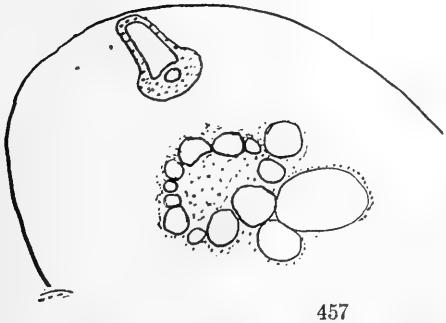
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48

THE AIR SPACES IN THE LUNG OF THE CAT

WILLIAM SNOW MILLER

Anatomical Laboratory, University of Wisconsin

FOUR PLATES

Since the time of Hippocrates the lung has been a subject of study and discussion. But little was known of its finer structure until the discovery of the circulation by Harvey and the introduction of the microscope into histological research made it possible for Malpighi to publish his letters to Borelli. Then, step by step, our knowledge of the finer structure of the lung increased: Rossignol, Kölliker, Waters, Schulze, Aeby and others contributing to the advancement.

Rossignol showed in 1847, that the essential part of the respiratory tract, that concerned in haematosis, is identical in all vertebrates; consisting in each instance of cells or alveoli. In the lower vertebrates the structure is simple, the lung consisting of a plain sac. According to Renaut the lung of Proteus represents an elongated uni-alveolar lung, showing the ideal morphological structure of this organ. As we ascend the scale of vertebrates the lung becomes more complex. In Siren we find what Renaut calls the lobular lung in which we have a number of alveoli instead of a single one. In Rana and Lacerta we have the single lobar lung. Continuing the ascent of the scale we find the organ becoming more and more complex until, in mammals, it reaches its highest development. Renaut describes the lungs of birds and mammals as being composed of many thousands of elementary lungs, such as that of Proteus, that is saccules having a respiratory surface.

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Oppel, discussing the above statements of Renaut, says that the simple and compound lungs of vertebrates are homologous structures inasmuch as they all arise originally from the foregut which possesses the property of producing the respiratory epithelium. The uni-alveolar lung of Proteus, as well as the compound lobular lung of the highest vertebrates, considered as a whole, corresponds to this original lung. Though a single alveolus of the highest vertebrates may correspond to the lung of Proteus in structure and function, qualitatively, both cannot easily be homologized; rather an alveolus of the lung of the higher vertebrates corresponds only to a part of the lung of Proteus. The profuse division to which the perfecting of the respiratory apparatus has led in higher vertebrates has necessitated the subdivision of the originally single organ into numerous small unit regions. As a result of this division, varying in manner in different species of animals, we have the origin and varying structure of the bronchial tree.

THE BRONCHIAL TREE

Aeby, in his work on the bronchial tree, based on comparative anatomical studies, denies that the older idea of a dichotomous division is the correct one. As the result of his studies he found the method of branching to be strictly monopodial: each bronchus retaining its individuality to its end and sending off lateral branches.

Ewart, in his work on the bronchi and blood vessels of the human lung, arrives at a very different conclusion from Aeby. He believes in dichotomy; not the even dichotomy of the older authors, but uneven dichotomy.

Although I do not agree with Ewart in the mode of bronchial division, I must call attention to what seems to me to be a general misunderstanding as to his position. He is usually quoted as asserting that dichotomy is "The Alpha and Omega of bronchial division." True, he did make this statement; but that is not all of it. What he did say is: "The further dissection is carried within the lung, the less rarely does *even dichotomy*

occur." Later on, under the heading "Even dichotomy unsuitable for the lung," he says in regard to dichotomy that

> It constitutes, so to say, the alpha and omega of bronchial division. But absolute evenness of dichtomy is not to be looked for. Due regard being paid to the shape of the thorax, unevenness is more likely than regularity. The products of a dichotomy which had been carried through with mathematical precision would have fitted ill within the pleural boundaries. Nay, even the more elastic principle of 'monopodial branching' requires, in its working, to be allowed some latitude. All so-called principles, or laws, are overruled by a higher law, the *law of adaptation.*

When this statement is taken into consideration it seems to me that we can no longer consider Ewart as believing in an even dichotomy, but must place him with those who believe in an uneven dichotomy. Moreover, when we reread the last statement in the above quotation, there does not seem any appreciable difference between it and the statement of Flint when he says: "The bronchi, apparently, show great adaptability both in the power and direction of their growth." In fact Ewart apparently was the first to advance this theorem.

In his summary Ewart says:

> (1) All bronchi are dichotomous; (2) that in any bronchial pair, the greater size of one bronchus is correlated with the greater mass of lung tissue which it must supply with air. Thus unevenness of size is not necessarily a negative evidence against dichotomy; and dichotomy does exist, at any rate in the limited sense that never more than two branches arise from any one division.

Huntington, through essentially different methods, arrived at the same conclusion as Ewart; that the type of division is dichotomous.

Müller studied the mode of division in the adult whale and came to the conclusion that "each main bronchus sends off branches in a strictly monopodial manner."

Schaffner bases his opinion on his study of the cardiac bronchus of man and agrees emphatically with Aeby. He says:

> The cardiac bronchus divides into two branches, a ventral, smaller and a dorsal, larger. The dorsal . . . . divides . . . . again into two branches, an inner and an outer . . . . These

two branches again divide, each into two branches, one of which shows a somewhat larger caliber than the other. Beyond this the branching becomes irregular and the monopodic type becomes obscured. Through this strictly monopodic method of branching of a bronchus . . . . Aeby's theory seems to me as much as proven.

Lühe, commenting on the above quotation, says he can find nothing in his description which would exclude the assumption of an unequal dichotomous division.

We have just seen that there is a wide difference in the conclusions reached by those who have studied the mode of division in adult lungs by means of corrosion preparations. If now we turn to developmental studies we find just as wide a difference, as a comparison of the work of d'Hardiviller and of Flint on the one hand with that of Justesen on the other will show.

His was the first, after the appearance of Aeby's brochure, to study the development of the bronchial tree. He describes in the lung of man a mixed type of division; the first divisions being monopodial, while in their growth the new bronchi originate by a dichotomous division of the end buds, not from the already cylindrical root tubes.

Robinson reached practically the same conclusion as His from studies made on the lungs of rats and mice; but in addition he describes single dorsal branches which arise, between bronchi already established, as buds from the walls of the main bronchus after the latter has attained its cylindrical form. These are the accessory branches of Aeby and of Narath.

It is somewhat difficult to place the type of division as described by Narath. If one follows the text of his various articles, he must be placed in the list of those who believe in the monopodial or, as Flint has classified him, a modified monopodial type of division. If, however, a careful study of his illustrations be made, it will be seen that they show a marked dichotomy.

Guieysse examined the musculature of the trachea and bronchi of the rabbit, guinea pig and other animals and compares his results with those of Aeby. The musculature of the trachea shows a variable position in different animals; it being attached in some to the outside of the cartilage, in others to the inner sur-

face while in still other instances it is attached to the free end of the cartilage. The bronchial muscle on the other hand is always situated between the cartilage and the mucosa. He furthermore states that the bronchus of the inferor lobe shows the same musculature as the trachea, while in the bronchi of the middle and upper lobes we have a different muscle. Guieysse applies this arrangement of muscle to Aeby's theory as follows. The fact that the musculature of the bronchus of the inferior lobe is the same as that of the trachea corresponds to Aeby's statement that the bronchus of the lower lobe is a continuation of the main bronchus. He also argues that this arrangement of muscle has an important bearing on the question of bronchial division. If the division were dichotomous the upper and lower bronchi ought to be alike in every respect; but this is not the case, for while the upper bronchi are soon enveloped in their muscle, the lower bronchus retains the tracheal muscle. This proves, according to Guieysse, that we are dealing with a continuation of the main bronchial trunk.

Oppel, commenting on the above statements of Guieysse, says that caution is necessary in applying his results as arguments for monopodial division, for, even if the branching were dichotomous, the peculiar arrangement of the muscle might be a secondary acquisition; on the other hand, it does not seem proven that in monopodial branching the upper bronchi should vary, in so far as their musculature is concerned, from the lower bronchi or the bronchial trunk.

Of all investigators, Justesen holds most decidedly that the method of division in the development of the bronchial tree is strictly dichotomous. His conclusions are based upon studies of the development of the bronchial tree of the ox. He followed carefully the branching of a single bronchiole throughout its entire course and found that "each of the side branches is homologous in all its branches to the continuation of the trunk."

d'Hardiviller emphatically declares that the mode of division is monopodial. In his work on the rabbit's lung he says that the end bud remains undivided and that the lateral branches originate by no means through true or false dichotomy, but from

hernia-like protuberances of the wall of the main bronchus which become gradually more marked and in the end develop into lateral bronchi.

Lühe, commenting on the results obtained by d'Hardiviller, says: "his work may be taken as a confirmation of the statement that new side branches always originate exclusively near the distal end of the growing bronchus, but no longer from the completed bronchial tube." d'Hardiviller takes this as a proof of the monopodial development of the bronchi. In a later study of the lung of the sheep d'Hardiviller states that the branching of the primary bronchi is exclusively monopodial, but their division into secondary bronchi is partly monopodial and partly dichotomous.

Nicholas and Dimitrowa also used the lung of the sheep in their study; they reached essentially the same conclusions as d'Hardiviller, but go further and claim that even the two main bronchi do not originate through a forking of the trachea but arise as buds from the dorsal portion of the lateral faces of the tracheal anlage.

Flint has made an extended study of the development of the bronchial tree in the pig. His results may be summed up as follows:

> The growth of the main series of bronchi is monopodial in character, that is to say, they are produced without a definite division of the end bud. New elements are not always produced from the end bud, but may be formed from the stem some distance from its terminus. The process is successive; that is to say, the elements are produced one after another from above downwards. . . . . Subsequent division of the branches may occur either by monopody or dichotomy. Often monopodial production of buds persists for one or two generations on the main bronchi, then the method becomes dichotomous, either equal or unequal in nature, depending somewhat on the space in which the bronchi have to divide.

My own studies of the bronchial tree of the cat lead me to the conclusion that, so far as the main series of bronchi are concerned, the mode of branching is monopodial. The mode of division in the terminal branches will be considered under a separate heading.

ULTIMATE ENDING OF THE BRONCHIAL TREE

For an extended review of the older literature I will refer the reader to my previous contributions and to the excellent résumé by Oppel.

In 1892 I published the first of a series of contributions on the finer structure of the lung which seem to have stimulated renewed interest in this complex organ. The introduction of Born's method of reconstruction placed in the hands of the investigator a method by which the relation of the air spaces to each other and to the bronchial arborization as well as the form of each could be definitely determined. By means of this method, using the lung of the dog for my study, I made a reconstruction of all the air spaces connected with a bronchiolus respiratorius.

By following a bronchus to its ultimate division we find that the smallest branches are no longer smooth but bear on their wall alveoli. The smallest division of the bronchial tree which has smooth walls I named in previous descriptions bronchus III (bronchiolus B. N. A.). The branches arising from the bronchiolus, the bronchioli respiratorii, bear alveoli which increase in size and number towards their distal end. Each bronchiolus respiratorius branches, giving rise to the smallest divisions of the bronchial tree, the ductuli alveolares. The air spaces connected with a given ductulus alveolaris, together with that ductulus alveolaris, form the lobule.

With the lobule thus defined it is necessary to describe what is situated peripheral to the ductulus alveolaris. During the past twenty years I have made numerous reconstructions of the air spaces in the lungs of various animals, and I have found no occasion to modify my original description.

Leading out of the distal extremity of the ductulus alveolaris are from three to six openings which are more or less circular in outline. These openings do not all take the same direction; usually one of them appears as though it were a continuation of the ductulus alveolaris, while the others open out at various angles or may take a course nearly recurrent to that of the ductulus alveolaris. These openings lead into the atria. Each at-

rium bears on its periphery numerous alveoli and opens into a variable number (two to five) of sacculi alveolares. The sacculi alveolares present a great diversity of form; they are very irregular and adapt themselves to the space they have to occupy. The irregular contour of one sacculus fits into corresponding irregularities of the adjoining sacculi. Each sacculus alveolaris bears on its periphery numerous alveoli, the true alveoli pulmonis.

While the original description applied to the lung of the dog, I have found by reconstruction, that it applies to the lung of the cat, ox, child and adult man. Flint found that it applied to the lung of the pig; Oppel that it could, however, be applied to any mammalian lung.

It has seemed to me, as I have read the papers of those investigators who have failed to recognize the presence of the atrium, that failure to recognize it is due to one of three causes: study of single sections; use of corrosion preparations; over distention. This last cause has made me trouble in times past. It was not until I learned to fix the lung in situ, that is, in the unopened thorax, that I obtained uniform results. Removal of the lungs from the thorax and filling them with the fixing fluid can so stretch and distort the atria that they may be mistaken for ductuli alveolares.

Atria do not possess the muscular walls of the bronchioli, nor the scattered muscle of the ductuli alveolares, but resemble the sacculi alveolares in structure, and, like them, are very distensible. May it not be possible that the atria take some important part in respiration, occupying as they do, an intermediate position between the ductuli alveolares, on the one hand, and the sacculi alveolares on the other?

NOMENCLATURE

In the following description of the air spaces I shall use the nomenclature which I have advocated ever since the B. N. A. made its appearance in 1895. In 1900 and again in 1902 I wrote as follows:

Recognizing that the B. N. A. is a decided advance in anatomical nomenclature I recommend the discarding of all previous nomenclature, the retention of all names given under the heading 'Pulmo' (B. N. A., p. 59) down to (and including) Ductuli alveolares, and the insertion then of:

Atria
Sacculi alveolares

The nomenclature would thus be made uniform and the objectionable term 'infundibulum' would be discarded. The finer divisions of the lung would then be:

Bronchioli
Bronchioli respiratorii
Ductuli alveolares
Atria
Sacculi alveolares
Alveoli pulmonis

In each instance I gave a list of the English, or English and German synonyms, not to recommend their general use, but that my English and German readers might fully understand the portion of the air space designated by the B. N. A. and myself. Notwithstanding my care I find some of the later authors consider the synonyms as a new nomenclature introduced by myself. I regret the misunderstanding and trust the above statement will make my position clear.

The distinction between bronchioli and bronchioli respiratorii should be easily recognized; but Laguesse and d'Hardiviller say there is no sharp boundary between them. I have had no difficulty in differentiating between the muscular wall of the bronchioli and the alveoli bearing bronchioli respiratorii; this is especially true of longitudinal sections. Laguesse and d'Hardiviller state that the 'alveolar canals' (and, in this instance, they evidently mean bronchioli respiratorii) "may be rather long and may branch once or twice before reaching the acinus" (lobule). I have found this true in the case of the cat, as the illstrations will show, and have designated the branches as 'a' and 'b'. The term ductulus alveolaris, as used in this study, corresponds to the acinous bronchiole of Laguesse and d'Hardiviller; it is situated between the bronchiolus respiratorius and the lobule and is the final division of the bronchial tree before it breaks up into the air spaces of the lobule.

In the preceding section I have discussed the presence and relationship of the atria, sacculi alveolares and alveoli pulmonis. It is not necessary, therefore, to enter into a detailed discussion of these portions of the lobule. I will only add that Oppel has demonstrated the presence of atria in the lungs of a long series of vertebrates. Justesen found them in the lung of the ox. Councilman has demonstrated them in the human lung and Flint in the lung of the pig. I have already given the list of reconstructions that I have made; each of which shows well marked atria.

THE LOBULE

The term 'lobule,' as applied to lung structure, is used at the present time to designate two different areas. By some it is applied to those large areas, faintly marked out on the surface of the human lung, but distinctly marked out by broad septa on the lung of the ox. By others it is applied to much smaller areas which consist of a ductulus alveolaris and the air spaces connected with it. Laguesse and d'Hardiviller call the larger areas the lobule, and give the name acinus to the structures connected with the ductulus alveolaris.

If one review the literature of the lung it will be found that almost universally the term lobule is applied to the structures connected with the last division of the bronchial tree. Various names are given to this last division and to the structures which lie beyond it; but, whatever the name, the ensemble forms the lobule. These lobules, primary lobules if you so choose to call them, are grouped together into secondary lobules and these secondary lobules collectively form the lobes.

The term acinus should be discarded as it is too indefinite. In its present usage, as applied to gland structure, it is not used consistently. If any part of the lung structure is to be compared to an acinus, it is an atrium and its sacculi alveolares; but until 'acinus' is used to describe a more definite portion of a gland than its present usage does, no intelligent comparison can be made.

Elaborating the definition of the lobule as given in connection with the air spaces, the complete lobule consists of a ductulus

alveolaris, the air spaces connected with it, their blood vessels, lymph vessels and nerves. Collectively this forms the anatomical unit of the lung.

The acinus of Laguesse and d'Hardiviller is not the unit of structure; their unit is the lobule as defined by them and consists of from fifty to one hundred acini.

Rindfleisch has given a description of the finer structure of the lung, which corresponds quite closely to that of Laguesse and d'Hardiviller, in which he says the acinus is the unit of the lung; that it is far more constant than the lobule. The lobule, composed of from twenty to thirty acini, is however, pathologically of much more importance. In other words he seems to make a distinction between an anatomical and a pathological unit.

MATERIAL

The present investigations are based on a study of the lung of the cat. In the preparation of the model I received material assistance from one of my students. Mr. G. H. Scheer.

The block from which the reconstruction was made was cut, perpendicular to the pleura, into sections 20 μ in thickness. The reconstruction ran through 115 sections of the series. The highest point of the reconstruction was situated three and a half millimeters below the pleura; it can be said, therefore, that the reconstruction represents the structure of the lung uninfluenced by the pleura.

The dimensions of the reconstruction are 23 x 24 x 20 cm.; as the amplification was 100, the portion of the lung entering into the reconstruction was approximately 2.3 x 2.4 x 2 mm. The amount of shrinkage was not estimated; as great care was exercised in the imbedding it probaby is a negligible quantity.

In studying the air spaces of the lung, the positive model is by far the most useful. Corrosions and corrosion models (negative models) of the lung do not exhibit its structure as clearly as do positive models. I have repeatedly called attention to this point and shown that the use of corrosions has led several investigators into error. Early in my work I abandoned their use except for demonstrating the gross arrangement of the bronchi.

DESCRIPTION OF RECONSTRUCTION

The portion of the lung entering into the reconstruction consists of a bronchiolus and the air spaces connected with it. The walls of the bronchiolus are practically smooth, showing only the rugae which are normally present. The bronchiolus divides into two branches whose walls bear scattered alveoli. These branches will be designated as bronchioli respiratorii a (plate 1). One of these branches is carried out. It measures 0.21 mm. in diameter and extends in nearly a straight line for about 0.5 mm. It then divides into two branches whose walls bear a greater number of alveoli; these branches will be designated as bronchioli respiratorii b (plate 1). Only one of these branches is carried out in the reconstruction. This branch measures 0.14 mm., in diameter and extends about 0.35 mm. It then divides into three branches, the ductuli alveolares. Each ductulus alveolaris is thickly covered with alveoli, some of which are of considerable size and resemble sacculi alveolares.

The ductuli alveolares vary in diameter from 0.11 to 0.12 mm. and are about 0.42 mm. in length, measuring to their most distal point; but, if we allow for the dilated extremity, they are about 0.22 mm. in length. Each ductulus alveolaris breaks up into a series of air spaces which collectively form a lobule. The three lobules thus formed enter into the reconstruction and for convenience of description will be designated by the numerals I, II and III (plate 1).

In the following description the terms of direction are relative, and describe the position of the parts in the reconstruction.

Lobule I. Ductulus alveolaris I (plate 1, I), extends to the left from the median line of the bronchiolus respiratorius from which it arises. It maintains practically a uniform diameter and shows only a slight dilatation at its distal end. From it arise three atria which will be designated 1a, 1b, 1c (plates 1, 2).

Atrium 1a lies at the distal end of the ductulus alveolaris and extends in the same direction as the latter. It measures 0.3 x 0.2 x 0.28 mm and communicates with the ductulus alveolaris

by an oval opening 0.17 x 0.12 mm. Two sacculi alveolares arise from this atrium.

Atrium 1b comes off from the ductulus alveolaris proximal to atrium 1a and communicates with the ductulus alveolaris through a nearly circular opening in its lower wall; its direction is almost directly downward. This atrium measures 0.30 x 0.23 x 0.31 mm. and the opening from the ductulus alveolaris 0.14 x 0.13 mm. Five sacculi alveolares arise from this atrium.

Atrium 1c arises from the roof of the ductulus alveolaris proximal to both of the preceding atria; it extends upward from and to the right of the ductulus alveolaris. The atrium measures 0.30 x 0.24 x 0.17 mm. and the opening by which it communicates with the ductulus alveolaris, 0.09 x 0.11 mm. Three sacculi alveolares are connected with this atrium.

Lobule II. Ductulus alveolaris II, and the lobule connected with it, lies to the right of lobule I. The distal end of the ductulus alveolaris shows the usual dilatation. Four atria are connected with this ductulus alveolaris and are designated 2a, 2b, 2c, 2d (plates 1, 2).

Atrium 2a arises from the left side of the distal end of the ductulus alveolaris and extends to the left and forward. The atrium measures 0.24 x 0.24 0.20 mm. and the oval opening by which it communicates with the ductulus alveolaris measures 0.19 x 0.12 mm. Two sacculi alveolares are connected with this atrium.

Atrium 2b comes from the right side of the distal end of the ductulus alveolaris and extends to the right and slightly upward. This atrium measures 0.33 x 0.34 x 0.23 mm. and the oval opening from the ductulus alveolaris measures 0.16 x 0.12 mm. Four sacculi alveolares are connected with this atrium.

Atrium 2c opens out from the floor of the ductulus alveolaris and extends downward. Its communication with the ductulus alveolaris is nearly round, measuring 0.08 x 0.09 mm. The atrium itself is 0.26 x 0.25 x 0.16 mm. Three sacculi alveolares are connected with this atrium.

Atrium 2d arises from the right side of the ductulus alveolaris, proximal to the other three atria, and extends to the right and upward. It measures 0.25 x 0.22 x 0.18 mm. and the rounded

opening into the ductulus alveolaris measures 0.1 x 0.09 mm. Two sacculi alveolares arise from this atrium.

Lobule III. This lobule lies below lobules I and II on a line about midway between them. Its ductulus alveolaris extends downward and forward and has a decided dilatation at its distal end, measuring at its widest point 0.21 mm. Three atria designated as 3a, 3b, 3c arise from this ductulus alveolaris (plates 1 and 2).

Atrium 3a arises from the distal end of the ductulus alveolaris and extends in the same direction. It measures 0.30 x 0.41 x 0.26 mm. and the opening from the ductulus alveolaris measures 0.20 x 0.19 mm. Four sacculi alveolares open out from this atrium.

Atrium 3b comes off on the left side near the distal end of the ductulus alveolaris and extends to the left and downward. It measures 0.33 x 0.25 x 0.20 mm and communicates with the ductulus alveolaris by a nearly circular opening 0.13 x 0.11 mm. Two sacculi alveolares are connected with this atrium.

Atrium 3c arises from the right side of the ductulus alveolaris proximal to the other atria and extends to the right and downward. It measures 0.30 x 0.23 x 0.22 mm. and the opening into the ductulus alveolaris measures 0.09 x 0.08 mm. Three sacculi alveolares arise from this atrium.

The above figures show, as we would naturally expect, that there is a gradual diminution in the diameter of the divisions of the bronchial tree as we approach the lobule. As soon as the lobule is entered the air spaces at once enlarge. The average size of the atrium is 0.29 x 0.26 x 0.22 mm. being, in its smallest dimension, twice the diameter of the ductulus alveolaris from which it arises. In previous communications I have shown that the atria are about half the size of the sacculi alveolares. These figures differentiate clearly the atria from the connecting air spaces. The communication between the ductulus alveolaris and the atria I have, from the first, described as being nearly circular in outline. The average of the communications in the lobules reconstructed gives practically a circular outline: 0.135 x 0.116 mm.

While in a complete reconstruction the differentiation of the atria is an easy matter, in sections it is sometimes difficult to distinguish them; this is especially true if only individual sections are available. In sections in which the air spaces and the ductulus alveolaris are cut longitudinally the recognition of atria is not difficult. The presence of smooth muscle and the character of the epithelium determine the distance to which the ductulus alveolaris extends and the immediate widening of the air spaces marks the position of the atrium (plate 3). In transverse or oblique sections through the lobule it is sometimes difficult to determine the position of the atria unless one has serial sections to study. Plate 4, 1b, illustrates the appearance of a section taken transversely through an atrium and the surrounding sacculi alveolares; it also shows an outline tracing of the principal air spaces in a section which includes the three lobules entering into the reconstruction.

In their general configuration the sacculi alveolares differ in no respect from those I have illustrated in previous contributions. Many of them are subdivided by deep clefts. In some instances this is undoubtedly associated with the course of the branches of the pulmonary artery which are distributed to the sacculi alveolares. No two sacculi alveolares are of the same size or shape; they apparently have grown along the line of least resistance.

Sacculi alveolares may arise from any of the smaller divisions of the bronchial tree. In the present reconstruction small sacculi alveolares were found connected with the ductuli alveolares and also with the bronchioli respiratorii. In my reconstruction of the lung of the dog I found an atrium, with two sacculi alveolares attached, arising from the same division of the bronchial tree.

When I first began my work, under the direction of Prof. F. P. Mall he taught me that when an artery gave rise to branches of various orders, any of the subsequent orders could arise from a preceding order. The same statement holds true for the bronchial tree and its ultimate endings.

TERMINAL BRANCHING OF THE BRONCHI

Although the main series of bronchi divide monopodially, the mode of division, as shown in the reconstruction, is quite different in the terminal divisions. Other investigators have also noted a difference.

Ewart says "strictly dichotomous branching predominates in the end branchings of the bronchial tree."

d'Hardiviller, in his description of the finer air passages, says that each bronchiolus respiratorius divides into two or more canals which show, on their part, several bifurcations.

His describes the primary bronchi as dividing in a monopodial manner but says that further growth takes place through the dichotomous fission of the end bud. Robinson reached similar conclusions.

Justesen studied the mode of branching of the bronchial tree both in the embryo and in the adult and he finds that in all the branches, from the largest to the smallest, the mode of division is exclusively dichotomous.

Flint believes in a monopodial division for the main series, but finds that subsequent division may be either by monopody or dichotomy. In some instances there may be alternation of the two processes.

With the possible exception of Flint, none of the investigators who have studied the smaller bronchi seem to have found anything but dichotomous branching. It is certain, however, that in the portion of the lung of the cat entering into the reconstruction dichotomy does not uniformly prevail. From the bronchiolus two branches arise and each of these divides into two; with this division dichotomy ends. From this last division three ductuli alveolares of practically the same size and length arise. Two of these ductuli alveolares have each three atria attached to them while the third has four; of the ten atria, four have two sacculi alveolares arising from each, three have each three sacculi alveolares attached to them, two have four sacculi alveolares each, while one has five sacculi alveolares arising from it.

Surely this is not dichotomy; neither is it trichotomy, although in one division it is the type and partially prevails in two others. It seems to me that, in a certain degree, it conforms to the statement of Flint that there may be alternation of the two processes, dichotomy and monopody.

ALVEOLAR PORES

A distinction must be made between the wide open communications of the older authors and the perforations which were first described by Adriani as occurring here and there in the walls of the alveoli, by means of which adjoining alveoli were in communication with each other; the so called alveolar pores. In 1892 I called attention to these openings, quoting Henle's statement that he did not consider them normal structures, but rather the result of atrophy and resorption of the lung tissue. In 1893 Kohn brought these structures prominently into notice by describing openings in the alveolar septa through which, in cases of pneumonia, fibrils of fibrin, could be traced from one sacculus alveolaris to another. He did not consider these openings to be normal, but rather the result of the pathological process.

The investigations of Ribbert, Hauser, Herbig and Bizzola on the side of pathology; of Aigner, von Ebner, Laguesse, Oppel and myself on the side of normal histology and of Flint on that of embryology lead to the same conclusion. On the other hand, Hansemann, Zimmermann, Merkel, Schulze and Marchand believe that they are normal structures.

It seems to me that history is repeating itself in this discussion as to the presence or absence of pores in the alveolar septa. In the present instance we have 'pores,' while in the earlier it was 'stomata.' The same factors which are capable of producing the well known artifacts, stomata, can produce these openings; that is, anything causing separation of the epithelial cells or rupture of the delicate frame work of the septa, as for example over distention, inflammation, desquamation of the epithelium, atrophy and age. The recent investigations of Walter show very

conclusively that stomata are, as I have maintained in the case of the pleura, artifacts. The statement of Flint, who has studied alveolar pores from the embryological side that they are not normal structures, should go a long way in settling this question of alveolar pores.

SUMMARY

1. The mode of division for the main series of bronchi is monopodial.

2. There are present in the cat's lung two series of bronchi to which the name 'bronchiolus respiratorius' is applicable.

3. The sacculi alveolares do not communicate directly with the ductuli alveolares; but, between the two, atria are interposed.

4. The lobule is the natural unit of structure. It consists of a ductulus alveolaris with its atria, sacculi alveolares, blood vessels, lymph vessels and nerves.

5. Communications between adjoining sacculi alveolares, the so called alveolar pores, do not exist in the normal lung.

6. The mode of division for the smaller bronchi is a mixed dichotomy and monopody.

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PLATE 1

EXPLANATION OF FIGURE

A purely diagramatic representation of the portion of the lung entering into the reconstruction. It shows schematically the method of branching of the bronchi, the number of atria arising from each ductulus alveolaris and the sacculi alveolares connected with each atrium. The sacculi alveolares of each lobule are differently colored so that they may be readily distinguished.

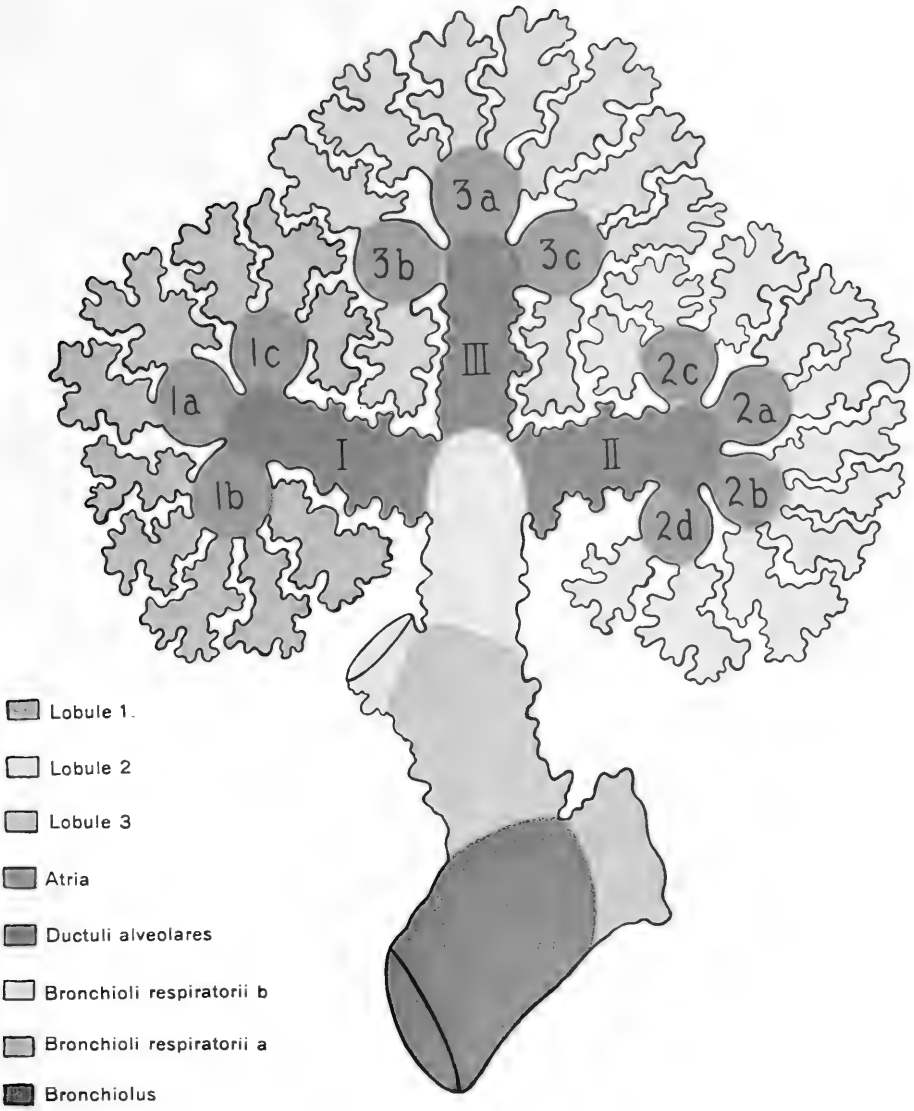

3a
3b
3c
1c
III
2c
2a
1a
I
II
1b
2b
2d
Lobule 1
Lobule 2
Lobule 3
Atria
Ductuli alveolares
Bronchioli respiratorii b
Bronchioli respiratorii a
Bronchiolus

PLATE 2

EXPLANATION OF FIGURES

Diagrams showing the relative position of the atria of each lobule to the ductulus alveolaris and to each other.

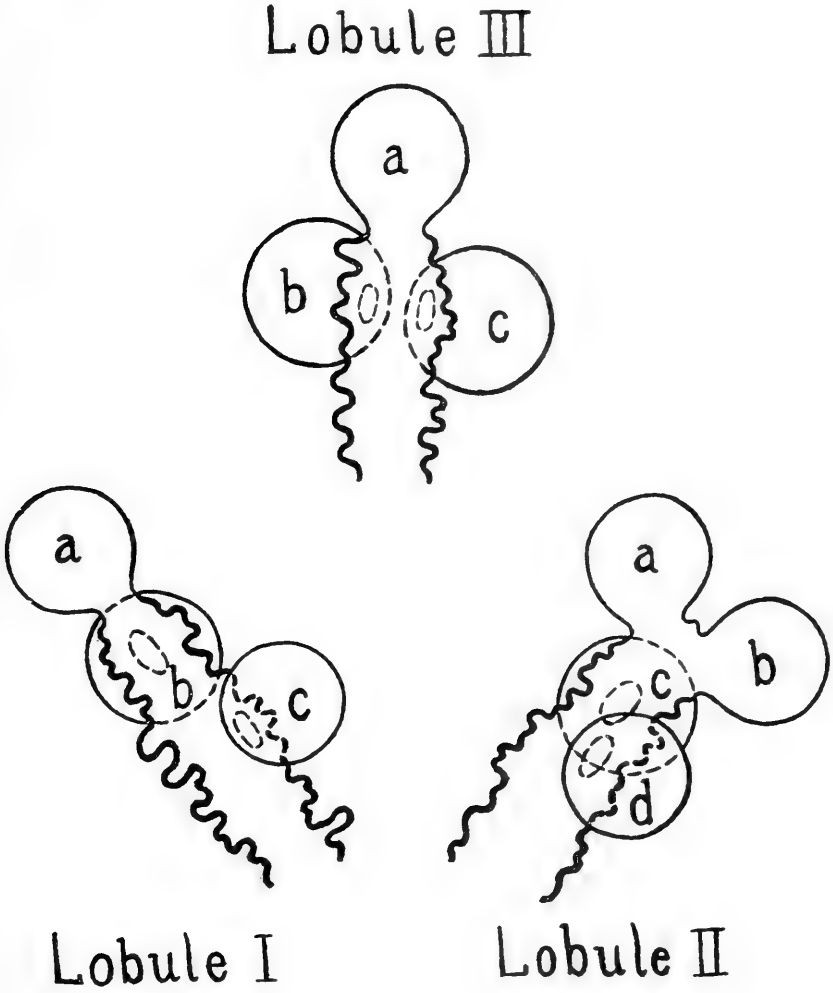

Lobule III
a
b
c
a
b
c
Lobule I
a
b
c
d
Lobule II

PLATE 3

EXPLANATION OF FIGURE

Camera tracing of the principal air spaces of lobule I. The section is cut in such a plane that all of the air spaces from one of the bronchioli respiratorii a., are opened longitudinally. Note the position of atrium 1a. The dotted line indicates the extreme dimensions of the reconstruction at this level. × 75.

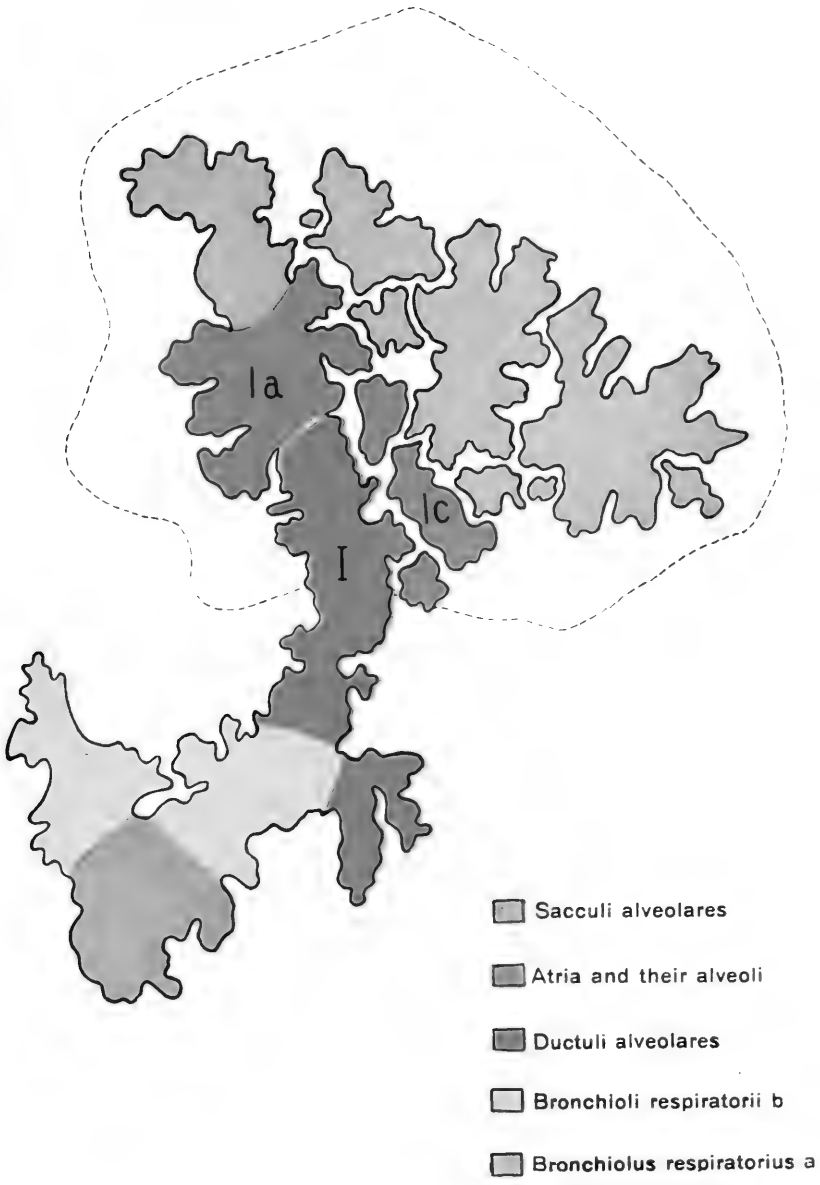
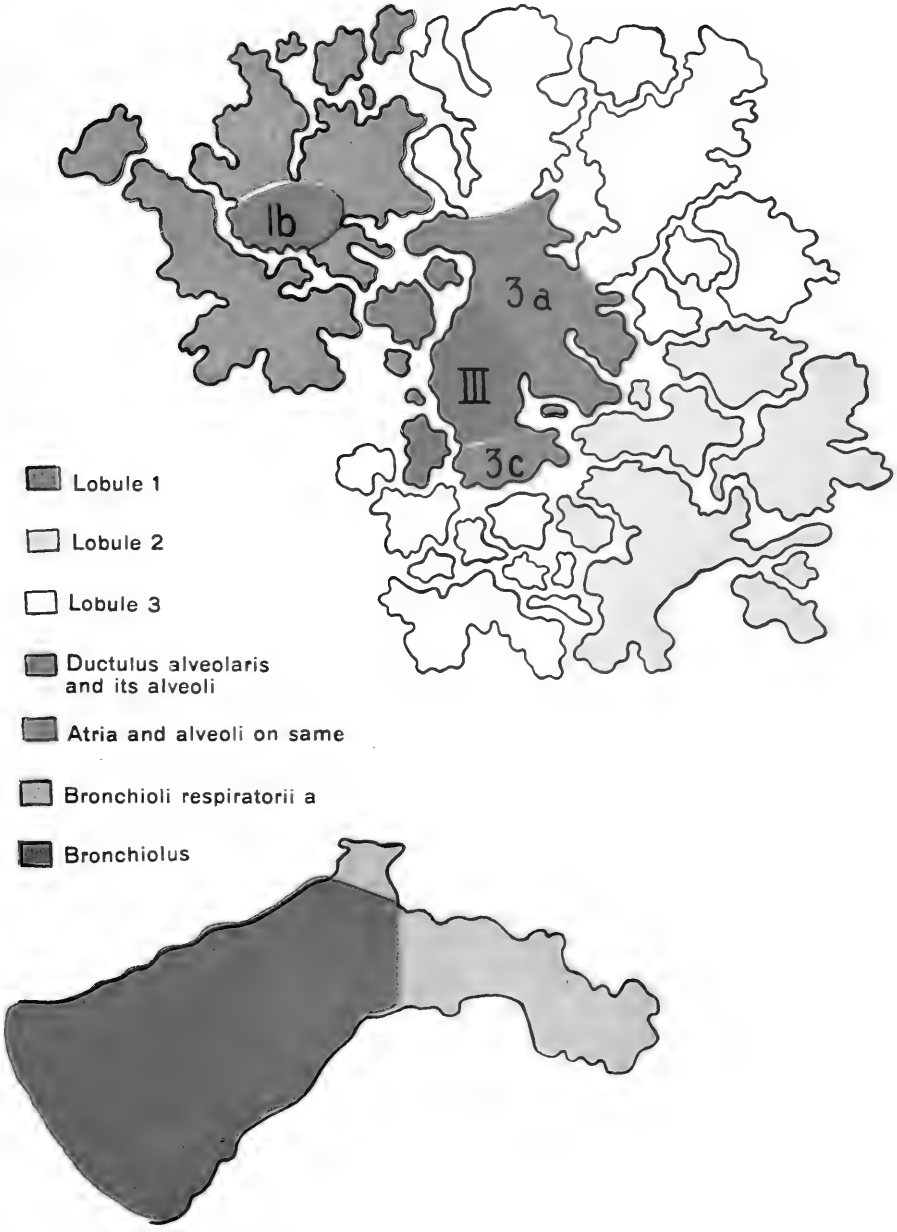

Ia
Ic
I
Sacculi alveolares
Atria and their alveoli
Ductuli alveolares
Bronchioli respiratorii b
Bronchiolus respiratorius a

PLATE 4

EXPLANATION OF FIGURE

Camera tracing of a section in which portions of all three lobules entering into the reconstruction are found. This section illustrates the difficulty encountered in attempting to study the structure of the lung from individual sections. The air spaces of the three lobules can be distinguished by the color scheme. × 75.


Ib
3a
III
3c
Lobule 1
Lobule 2
Lobule 3
Ductulus alveolaris and its alveoli
Atria and alveoli on same
Bronchioli respiratorii a
Bronchiolus

THE MENDELIAN RATIO IN RELATION TO CERTAIN ORTHOPTERAN CHROMOSOMES

E. ELEANOR CAROTHERS

SIXTY-NINE FIGURES

CONTENTS

INTRODUCTION

The aim of this paper is to describe the behavior of an unequal tetrad which occurs in the first spermatocytes of three members of the Oedipodinae: Brachystola magna, Arphia simplex and Dissosteira carolina. The distribution of the dyads of this tetrad, in relation to the accessory, follows the law of chance; and, therefore, affords direct cytological support of Mendel's laws. This distribution is easily traced on account of a very distinct difference in size of the dyads. Thus another link is added to the already long chain of evidence that the chromosomes are distinct morphological individuals continuous from generation to generation, and, as such, are the bearers of the hereditary qualities. McClung wrote ('05, p. 303), "In the absence, therefore, of definite knowledge of the chromosomes in the germ cells of organisms exhibiting Mendelian characters or mutations we are warranted in supposing them to be of the same general character as the ones

known till they are proved different." Since the above was written the increased knowledge of Mendelian phenomena has shown that they occur in practically every group of organisms. I hope that it may be possible to determine what the characters controlled by this particular pair of chromosomes are, through an experimental study of the living animals, correlated with a study of the developing soma of the embryo.

The credit for recognizing the importance of this tetrad belongs to Dr. C. E. McClung, as I was not sufficiently familiar with the ground to realize its general bearing when I found it. I am also indebted to him for consistent encouragement and guidance throughout the whole course of the work. The slides used were Dr. W. S. Sutton's, a few of Dr. McClung's and a number prepared by myself, from material in Dr. McClung's collection. In the work on the so-called nucleoli, or plasmasomes, I have been enabled to compare a number of different genera through the cooperation of D. H. Wenrich, a fellow student, who is giving especial attention to the growth period.

This work is based chiefly on Brachystola magna, a form already well known, so far as the general organization of the chromosomes is concerned, through the valuable researches of Sutton ('00, '02, '03). On this account, and in view of the fact that another paper by the same worker is soon to appear dealing with Brachystola, I shall restrict this paper strictly to the subject in hand, with the understanding that upon all other essential points my observations agree closely with those of Sutton.

While this chromosome does not come under McClung's original definition of a multiple, since it is not united with any of the others during the metaphase, it frequently forms a hexad during the prophase by uniting with one end of the accessory. It further separates itself from the ordinary chromosomes by having the spindle fibers attached to the free, instead of the synaptical ends, and in consequence, dividing transversely in the first spermatocyte when the other tetrads divide longitudinally.

OBSERVATIONS

1. *Spermatogonia*

Thanks to the accuracy of Sutton's drawings, but little remained in this stage for me to do. He found that the entire complex could be separated into two groups, one containing six small chromosomes and the other seventeen larger ones. Examination shows that his group of six small chromosomes is composed of five of about equal size and one decidedly larger. Two of his four spermatogonial figures show this clearly; in his first paper ('00), plate 33, figure 23, the largest chromosome of the small group, is apparently attached to the end of a member of the large group; in the second paper ('02) figure 2, the pair marked '*i*' are clearly unequal in size. In figure 1 the chromosomes are present in oblique or end views, and in figure 3 the real size of either '*i*' or '*j*' may be concealed by the overlying chromosomes. To appreciate properly the weight of this evidence, we must remember that Sutton believed that they formed pairs, the individual members of which were of equal volume. This is true, probably, except for the two that unite to form the unequal tetrad. Figures 1, 3 and 4 of this paper show typical polar views of metaphases, with the entire group of twenty-three present, and reveal the relative volumes of the six small chromosomes clearly. Figure 2 shows the six small ones and their relative volumes, although it does not contain the entire complex.

Polar views of complete spermatogonial complexes of Arphia also show a possible separation into a group of small and a group of larger chromosomes, with the difference here that the small group consists of but four members while the larger one has nineteen. Close observation of the four small chromosomes shows that three of them are of practically equal volume and the remaining one (figs. 5 and 6, *a*) is slightly smaller.

2. *Growth period and earlier diffuse stages*

These observations are based principally upon Arphia, for while the nuclei of Arphia and Brachystola are practically identical in size those of Arphia are much clearer, partly owing to

the smaller size of the polar granules, described by Pinney ('08) for Phrynotettix. This point is especially in favor of Arphia, since part of the chromatin which produces the unequal tetrad passes through the greater part of the growth period, and other stages of general diffusion, in a dense condition where it appears as nothing less than one of the so-called plasmasomes or nucleoli so often described for these stages. In this condition it is liable to be confused with the ordinary large polar granules of Brachystola.

My earliest study of the growth period impressed me with the strong probability that these bodies which at certain stages stain like chromatin, are really composed of chromatin. Further study convinced me that at least one of them is associated with the unequal tetrad, and this conviction led to the present study.

Apical cells contain at least one, perhaps more, clear, straw-colored, more or less spherical vesicles. Close inspection reveals a minute, deeply staining granule at one point of the periphery; and further that this point is always in connection with a mass of chromatin (fig. 7, *k*). These latter, the peripheral granule and the connection through it with the ordinary chromatin, are absolutely characteristic features of such vesicles wherever they are found. Since they may or may not be filled with chromatin, the staining capacity is a variable factor.

At least two of these vesicles are present in the cells surrounding the apical cell (fig. 8, *k*). In favorable preparations they may be traced in the several spermatogonial generations from early telophases (fig. 9 *k*) to later prophases (fig, 10 *k*). The one represented in figure 10 is connected to a chromatin thread with unequal arms, and, as telosynapsis of the elements which form the unequal tetrad of the first spermatocytes occurs at a stage even preceding this, as is clearly shown by figure 11, *c*, there is little doubt that this, also, is the precocious tetrad and its associated vesicle.

During the growth period there are three vesicles; two single and one double (fig. 13, *k*, *k*, *k*) (occasionally, one-half of the latter splits again forming a tripartite vesicle). For this reason, and especially since no thorough study of the earlier stages was undertaken, the largest number found at that period—two—

should not be taken as established. In early bouquet stages the vesicles are colorless and always occur on the distal part of their respective loops—that is, at the point farthest from the center of radiation of the chromatin threads. Figure 12, *k*, shows the double vesicle and its associated loop occupying a position in relation to the accessory, *x*, which is characteristic for them at this stage. The single vesicles behave in a similar manner; that is, they are colorless, except for a dense peripheral granule through which passes the spireme thread. Very soon all of the vesicles become densely stained (with Flemming's tricolor, iron hematoxylin or Auerbach's stains) but are still certainly recognizable on account of the granule which can yet often be made out. The halves of the double vesicle come to lie opposite each other, separated only by a dense granule common to both, through which the chromatin thread passes (fig. 14, *k*). This thread is thinner than any of the others and even a fragment of it separated from the vesicles can be identified. What the later history of this chromosome is has not yet been worked out. One of the single vesicles is associated with the unequal tetrad, as later evidence shows. Near the end of the growth period they begin very gradually to lose their staining power (fig. 15) until finally their outline becomes so faint that they can no longer be distinguished.

Figure 16 represents the latest stage of the growth period in which they are easily apparent in Arphia, though in some forms they persist till a much later period. Figure 23, *k*, shows one in Brachystola, associated with the unequal tetrad and persisting until a late prophase. Figure 29, *k*, shows the same vesicle still recognizable at the first spermatocyte metaphase. Since the plates were finished, favorable preparations have shown them in the second spermatocyte anaphases and in the spermatids. The term 'plasmasome' is self-evidently not applicable to these bodies, since their content is chromatin and they are distinctly concerned in the formation of the chromosomes. Winiwarter indicates ('12, fig. 25) a similar condition in human spermatogenesis. His drawing shows the peripheral granule and its connection with the spireme beautifully, although he does not mention either fact and calls the body a nucleolus.

Figure 11 represents an early secondary spermatogonium and is of especial interest on account of the loop shown at *c*. The dense granules at both ends of the dyads are very evident. This is a peculiarity that marks certain chromosomes as was noted by Miss Pinney ('08, p. 313). The curve of the free and of the longer dyad of the unequal tetrad (fig. 19 *c*) had long puzzled me, but in view of the earlier relation of these elements shown in figure 11 *c* it is evidently due to the separation, at this point, of the dyads which had previously been united at both ends. During the growth period this tetrad is associated with the accessory both in Arphia and Brachystola. This is probably merely a persistence of the conditions established at the formation of the composite granule. When the accessory breaks away from this body it carries with it several granules with their chromatin threads[1] —in Brachystola, for a time, forming a conspicuous second center of radiation. Figure 25 shows all three small chromosomes still adhering to it, but the relation with the unequal tetrad is the most persistent. Figures 18, 18 e and 19 are successive stages in Arphia. Figure 17 is from Brachystola.

3. First spermatocytes

Brachystola. As soon as the chromosomes have become sufficiently condensed to be recognized as distinct individuals, the accessory, which at first is in the form of a '*U*' with the arms approximated, is often seen connected with a small chromosome more dense than any of the others, except the accessory itself (fig. 24). There are at this time eleven individuals, counting the multiple as one (fig. 28). The larger dyad of this tetrad shows a transverse constriction, giving the whole a tripartite appearance. Either the larger or smaller end may be attached to the accessory (figs. 23 and 24.

In early metaphases the chromosomes appear as twelve separate individuals. Side views show the accessory in its characteristic position near one pole. The smallest chromosome frequently shows a constriction; the next in size rarely gives an indication of approaching division, while the third is entirely separated, only a

[1] This was first noted by Wenrich who also suggested the term 'composite granule.'

thin thread joining the two parts. A decided difference in the size of the two dyads proclaims this as the tetrad formerly united to the accessory.

While all the other tetrads are made up of quantitatively equal parts and follow the typical Orthopteran plan of division (longitudinal in the first spermatocyte and transverse in the second) already worked out for Brachystola by Sutton, this tetrad divides transversely in the first spermatocyte, as is evident from an examination of the prophases, where it is united with the accessory so that the longitudinal split between the chromatids of the tetrad corresponds with the division between the chromatids of the accessory. This involves the further exception that the spindle fibres must become attached to the free instead of the synaptical ends. The unequal size of the two dyads allows no mistake on this score; otherwise, we should have one large and one small chromatid passing to each second spermatocyte (a condition which Wenrich has actually found in a nearly related genus, Phrynotettix). Here we see that although the connection with the accessory is lost, this tetrad is still in the line of behavior described by McClung ('05) for the multiples of Hesperotettix and Anabrus, which remain associated with the accessory throughout the maturation divisions and are made up of quantitatively equal parts.

Three hundred cells were drawn under the camera lucida to determine the distribution of these dyads in relation to the accessory. Of these, 228 show the accessory and tetrad in the same section, and as this does not include any case in which there is any uncertainty, either in regard to the pole for which the accessory is destined or where there is the possibility that the dyads do not reveal their true size, there can, I think, be no reasonable doubt of the results. In 107 cells the smaller dyad was going to the same pole as the accessory, and in the remaining 121 the larger dyad occupies this position. In the other 72 the accessory and tetrad are in different sections, but great care was used to make sure that there was no mistake in identifying the cell or in labeling the drawings. The smaller is accompanying the accessory in 39 of the cells, and the larger in 33. This practically agrees with

the result in the case where no confusion was possible, owing to both accessory and tetrad appearing in the same section. As a net result, then, in the 300 cells drawn, the smaller dyad would have gone into the same second spermatocyte as the accessory, 146 times, or in 48.6 per cent of the cases; and the larger one, 154 times, or in 51.3 per cent of the cases. I might further state that 32 cells were drawn from one individual and a larger number from another, each giving substantialy the above results, before I saw the trend of the evidence.

Second spermatocyte figures are not striking in my material on account of the lesser difference in volume of the dyads, but early anaphases of the first spermatocyte, such as figures 33, 34, 41, 42, 45, 49 and 57 where the attachment of the spindle fibers is clearly evident, speak for themselves. The majority of my drawings are of early metaphases as represented in figures 30 to 32, 37 to 40, and so forth, but with a number of later figures to substantiate them, there seems to be no reason to doubt their reliability. More conclusive still, are polar views of late anaphases (figs. 58, 61 to 64). Figures 61 and 63 contain twelve chromosomes, including the accessory; in figure 63 one of the three small dyads is markedly larger than the other two—the larger dyad; while in figure 61 one is slightly smaller than the other two—the small dyad. Figures 58, 62 and 64 are eleven chromosome groups, the first two containing the larger, the last the smaller dyad.

One peculiarity is yet to be noted. In many instances the larger dyad has a constriction about one-third nearer the proximal than the distal end (fig. 57) corresponding to the tripartite appearance of the prophase. Usually it is very slight; sometimes no indication of it can be found, while in a few instances it is carried to the extreme seen in figure 29. Apparently this is an individual variation.

The unequal pair is comparable to any of the other tetrads and is not part of a sex group, such as has been described by Payne ('09), since the distribution of its parts is not related to sex, as indicated by the presence or absence of the accessory. As a corollary, it follows that its arrangement on the spindle is a matter

of chance. This is the first demonstrable case, so far as I know, showing that the maternal and paternal chromosomes do not pass collectively to given poles.

Payne, from his work on Gryllotalpa borealis Burm ('12) where he reports a large accessory and an unequal pair, the larger member of which always passes to the second spermatocyte which receives the accessory, argues that there is no haphazard arrangement, as is necessary for the explanation of Mendelian phenomena, that the chromosomes brought into the male by the egg pass into the female producing spermatozoon. A few lines later, in order to explain the transmission of characters from father to daughter, he says that a satisfactory explanation is found in the synaptic stage, assuming an interchange of the smaller units that make up the chromosomes. So, after all, he would not have the *material* (chromatin), which is the essential thing contributed to the male by the egg, pass into the female determining spermatozoon. Besides, as I shall point out more fully later, (p. 503) an interchange of material during synapsis could only affect the second generation and not the immediate offspring, though that is what he is attempting to explain.

Arphia. In early prophases the accessory is in the form of a *U* and well condensed (fig. 19*x*), and the unequal tetrad is nearly as dense as the accessory, the longer dyad having a curve at the free end as though it had been drawn over towards the free end of the shorter (fig. 19 *c*). One of the large tetrads is only slightly less precocious; a fragment of this chromosome is shown at *b*, figure 19. In late prophases the unequal tetrad has become straight and the accessory is but slightly curved (fig. 26). They are occasionally found associated end to end—a continuation of the growth period relation.

Twenty-five side views of metaphases and anaphases were drawn (figs. 52, 54, 55 and 56). Twelve contain the accessory and the larger dyad on the same side of the equatorial plate, and thirteen the accessory and smaller dyad in the same relation.

The complex differs from that of Brachystola, as may be seen by a comparison of figures 32, Brachystola, and 35, Arphia; (the latter lacks the accessory but contains the remainder of the com-

plex). The important point is that there are two small chromosomes in Arphia, instead of three as in Brachystola, and that one of these divides equally while the other divides unequally. These two small tetrads are very nearly the same size and might easily lead a hasty observer who was not familiar with both tetrads to the conclusion that they are homologous chomosomes when he observed them separated as they often are in sectioning and he would repeat the statement frequently seen that one of the small chromosomes sometimes divides unequally. The fact is that one particular small chromosome always divides unequally in first spermatocyte metaphases of Arphia simplex.

No indication of a secondary constriction of the larger dyad has been observed.

Dissosteira carolina. Figures 47, 53, 59 and 60 are typical first spermatocyte views. The larger dyad is constricted as represented (fig. 53), in numerous cases. In this genus it will be noticed, however, that the constriction is about the center instead of nearer the proximal end, as in Brachystola. One of the large tetrads shows a weakness in one chromatid of each dyad, as seen in figure 59. This has been found in several animals, and appears characteristic of that chromosome, though it does not always occur; in this way resembling the constriction found in the larger dyad of the unequal tetrad. That this peculiarity marks certain chromosomes and always occurs at the same point when distinguishable, is further evidence of the precise arrangement of their constituents, whether we consider it to be due to chemical, electrical or mechanical forces. A suggestive discussion of this subject is given by Agar ('12).

4. *Second spermatocyte*

Only a very brief period ensues between the first and the second spermatocyte divisions, but when the chromosomes become arranged in the equatorial plate, one of the small chromosomes is again sometimes found associated with the accessory, and, as one would expect from a knowledge of the first spermatocytes, varies in size, depending, evidently, upon whether the larger or

smaller dyad accompanied the accessory. Owing to the decreased size of all the chromosomes at this stage, the difference in volume of the smaller chromosomes is difficult to distinguish, and to be worth anything the figures compared must be not only from the same animal, but from the same slide, in order to avoid complications due to fixation and staining. Figures 65 to 68 comply with these conditions.

The result is clearly four sorts of spermatozoa. One-half contain the accessory, and of these again one-half contain one of the larger chromatids and one-half one of the smaller. Likewise, the spermatozoa without the accessory may be classed as those containing the large and those containing the small chromosome.

5. Spermatids

Spermatids of Arphia simplex, at the time when the ordinary chromatin has become quite diffuse, contain the three condensed elements; accessory, large precocious chromosome and one member of the unequal pair still in a dense condition. Figures 21 and 22 are drawings from such a stage of twelve chromosome spermatids, with the accessory, of course, present. One contains the large and one the small chromosome, *c*. A comparison of some of the features of these two spermatid nuclei with the nucleus at the end of the growth period shows the volume to be reduced about one-fourth. This, of course, is what might be expected, since four cells have been formed from the one with practically no resting period between the two divisions. The accessory is approximately one-half the volume of the accessory of the first spermatocyte which has undergone but one division (figs. 54 and 55). The large precocious element is beginning to diffuse, hence is more than one-half the size of the first spermatocyte dyad from which it is derived. The same applies to the derivative of the unequal tetrad.

SUMMARY OF OBSERVATIONS

1. The so-called plasmasomes or nucleoli are always associated with certain spireme threads or prophase chromosomes; in these species they never exist as free bodies. This connection is through a peripheral, dense granule.

2. During the growth period there are three of these vesicles, two single and one double.

3. They occur at the distal end; that is, opposite the center of convergence of the chromatin threads of the loops formed by the union of the spermatogonial chromosomes.

4. They have been found at all stages in the history of the germ cells, being visible even in the first spermatocyte metaphase, second spermatocyte telophase, and in the spermatids.

5. Their staining power varies; but in general they stain most densely when the chromatin is most diffuse and give up this power as the chromosomes condense.

6. The first maturation division is longitudinal for all the ordinary tetrads. This is proven by a knowledge of their structure gained from the prophase, by the point of attachment of the spindle fibers, and by the appearance of the rings during division.

7. One of the tetrads which is associated with the accessory chromosome during the growth period divides transversely in the first spermatocytes and longitudinally in the second, which the accessory also does in effect.

8. The dyads of this tetrad are unequal in size, hence the chromosome is easily recognized.

9. The different parts of this tetrad are distributed equally to both sorts of spermatozoa.

DISCUSSION

Sutton ('02) showed that the spermatogonial chromosomes occur in pairs, and his conclusion was that in the somatic cells and in the spermatogonia there is a double series composed of homologous maternal and paternal chromosomes. This theory was put forward by Boveri ('01) but it remained for Sutton's work to furnish direct evidence. These homologous pairs unite

in synapsis, and in the reduction division are separated into groups which are neither purely paternal nor purely maternal. This last suggestion, I believe, was pure theory advanced to meet the known experimental facts which show that either parent may transmit the characters of its ancestors of the opposite sex. It is strange that so careful an observer should have overlooked the very thing that was present (the unequal tetrad was first found on Sutton's slides) offering definite chromosomic proof for his theory. For, I think, there can be little doubt that the dyads of the tetrad described in the foregoing pages are distinct physiological individuals, representing respectively the paternal and maternal contribution to the formation of some character or characters; and, as each can be identified, they furnish an excellent means of tracing the process of segregation and recombination.

None of the female germ cells in maturation stages being available at present, definite knowledge of what occurs there is lacking. But it seems necessary to assume the presence of an unequal tetrad there also, for if its place were filled by an ordinary chromosome of equal parts we would sometimes find two large or two small united in the first spermatocyte, but in every one of the twenty animals studied the unequal tetrad was present. The only alternative would be to conclude that one-half the spermatozoa are not functional, for which there is not a shred of evidence.

If this assumption that after maturation one-half the ova contain the large and one-half the small dyad be correct, then selective fertilization becomes a necessity, since a spermatozoon containing the large dyad could only fertilize an ovum containing the small. This would in no way interfere with the ratio of Mendelian characters, for, owing to the abundance of spermatozoa, all the ova would be fertilized; nor does it necessitate the extension of the theory to those pairs which are quantitatively equivalent. But, whether further evidence shall show selective fertilization to be a fact or not, and regardless of the mode of origin of the inequality, its absolutely constant occurrence and the alternate distribution of the dyads in even one individual is sufficient for the essential part of this work—the segregation of at least part

of the paternal and maternal chromatin according to the law of chance.

The large dyad, which I shall arbitrarily designate as bearing characters of the male line, even when transmitted by the female, must represent characters of her male ancestors, either on the paternal or the maternal side. In like manner, the small dyad must represent characters of the female line, though half the time contributed by the male and, consequently, bringing in characters of his female ancestors. In other words, so far as this one pair of chromosomes is concerned, the spermatozoa may contain the small chromosome carrying factors from the maternal grandmother or from the paternal grandmother, or it may contain the large chromosome carrying characters of the paternal grandfather or the maternal grandfather. Likewise, the ova after maturation may contain, by hypothesis, the large chromosome inherited from either the paternal or the maternal male lines, or the small from the maternal or the paternal female lines. Eight combinations are, then, possible for this pair, as shown in the accompanying diagram. The possible combinations with various numbers of chromosomes has been worked out by Sutton ('03, p. 234, et seq.), so that further work on this line is superfluous. However, it may be well to emphasize the fact that the 68,719,476,736 combinations which he has shown to be possible in the zygotes of organisms possessing 36 chromosomes in the somatic series is amply sufficient to account for all observed variations, without the assumption of any interchange of material between the chromosomes.

The only question that remains to be considered is whether Mendelian phenomena are exceptional cases of heredity, or whether they represent the type form of all inheritance. Perhaps the strongest arguments against the latter view have been derived from blends, first crosses that breed true and mosaics.

In regard to the first, Hatai ('11) has shown that the series obtained from the square of the binomial $(X^2+2XY+Y^2)^n$ expresses the distribution of determinates for both Mendelian and blended inheritance, and that therefore the latter may be

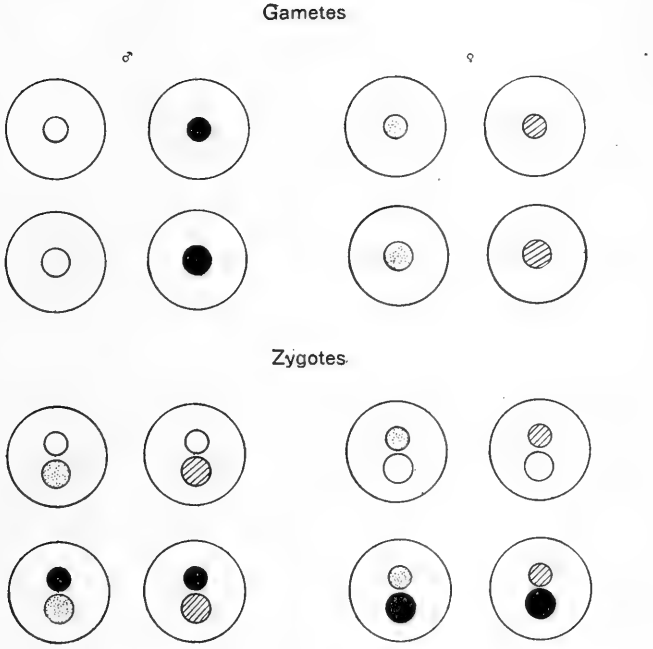


Diagram of Possible Combinations of Unequal Pair

considered as a limiting case of Mendelian inheritance where dominance is imperfect. In what are commonly recognized as cases of Mendelian inheritance the hybrid is indistinguishable from the dominant homozygote so far as gross characters are concerned, and the only means of sorting the pure from the hybrid offspring is by inter-breeding. But this is not necessarily the case, as shown by Punnett ('07, pp. 29–30). In the Leghorn breed of poultry white plumage is dominant to colored, but not perfectly so. To quote directly:

> When a white and a brown Leghorn are crossed together, all the resulting offspring are white, but almost invariably have a few colored feathers. The presence of these 'ticks' is the outward and visible sign of the heterozygous nature of the bird on which they occur. Such birds give off equal numbers of gametes bearing the white and colored characters. This is easily tested by breeding them together. It is found that from such matings one-quarter of the offspring are colored recessives, whilst the remainder are pure white, or white with a few ticks. The heterozygote resembles the dominant form much more closely than it does the recessive. Though we may speak of dominance in such a case it is necessary to remember the dominance is not perfect. This, however, makes no difference to the essential feature of Mendel's discovery, which is, of course, the segregation in the gametes of the factors corresponding to the dominant and recessive characters.

This differs only in degree from typical cases of blended inheritance. Yet it is clearly Mendelian, and accords perfectly with Hatai's idea of incomplete dominance. The segregation of pure colors in the gametes proves that there has been no interaction affecting the essential character of the determinants.

A case of complete blending in the first generation, followed by segregation in the second, is given by Castle ('11, p. 138). This is in regard to length of ear in maize, and has been worked out by East.

As to first crosses that breed true, it seems difficult to find such cases. The mulatto has been cited; but Davenport, handling the matter in a scientific manner, finds that there is segregation in the ratio of one to sixteen which can be brought into harmony with other Mendelian results by the assumption of four factors for black in the negro.

In regard to mosaics, Sutton says in part ('03, p. 245) "If each cell contains paternal and maternal potentialities in regard to each character, and if dominance is not a common function of one of these, there is nothing to show why as a result of some disturbing factor one body of chromatin may not be called into activity in one group of cells and its homologue in another." This view is supported by blends which later segregate out pure characters as well as the work of Tennent ('10) reversing the dominance in Echinoderm hybrids by changing the concentration of the hydroxylions in the sea-water in which they developed.

A consideration of the limited number of chromosomes and the large number of characters in any animal or plant, will make it evident that each chromosome must control numerous allelomorphs, or unit characters. It is to the individual dominance, either partial or complete of these unit characters, rather than to the dominance of the chromosome as a whole, that we may look for the explanation of Mendel's laws.

Since the rediscovery of Mendel's laws, increased knowledge has been constantly bringing into line facts that at first seemed utterly incompatible with them. There is no cytological explanation of any other form of inheritance; the long association of synaptic pairs during the growth period has suggested the possibility of some interaction between the chromosomes, but this association is between the chromosomes of the grandparents, directly. Let us suppose that these represent pure lines on both paternal and maternal sides, and that the character under consideration is a blended one. Now, the fertilized ovum resulting from a cross between these two pure lines at once develops an organism with the blended character without any long association of the chromosomes that produce it. In other words, if there were an interaction between chromosomes during the growth period, which would result in blended inheritance, it could not be manifested until the second hybrid generation. It seems to me probable that all inheritance is, in reality, Mendelian.[2]

[2] All observational work was done at the University of Kansas. Some drawings have been completed and the paper revised at the University of Pennsylvania.

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EXPLANATION OF PLATES

All figures are from camera lucida outlines. Original magnification 2400 diameters. Actual magnification of figures 1200 diameters.

Numbers 21 and 22, spermatids, are displaced from the position they would occupy in order of development for purposes of comparison; the others are arranged as nearly as convenient in that order.

All drawings are from B. magna unless otherwise indicated. Ordinary chromosomes are in outline for clearness of comparison.

Accessory at upper pole except figures 43 and 55.

PLATE 1

EXPLANATION OF FIGURES

1 to 4 Spermatogonial complexes of Brachystola magna showing division of entire complex into two groups; one containing six smaller chromosomes, the other seventeen larger ones. Figure 2 does not contain all of the larger chromosomes but shows the accessory, *x*, in recognizable form. Of the smaller group the probable pairs are designated *a*, *b*, and *c*, respectively. One of those marked *c* is obviously larger than any of the other five.

5 to 6 Spermatogonial complexes of Arphia simplex. Two groups with regard to size are again evident with the difference that there are four smaller and nineteen larger chromosomes. Three members of smaller group are practically identical in size; the fourth, *a*, is smaller.

7 Apical cell of Arphia simplex. Persistent chromatin vesicle *k* associated with flocculent mass of chromatin.

8 Nucleus from one of the cells surrounding apical cell, containing two persistent chromatin vesicles *k*, *k* with their associated chromatin masses.

9 Spermatogonial telophase of Arphia simplex showing vesicles *k*, *k* and associated chromatin threads.

10 Spermatogonial prophase Arphia simplex. One end of associated chromatin thread longer than the other.

11 Spermatogonial spireme, Arphia simplex, *c*, telosynaptic union of unequal pair; *x*, accessory; *p*, polar granules.

12 Early growth spireme, Arphia simplex. Position of persistent chromatin vesicle, *k*, on loop and relation of this loop to accessory, *x*, typical.

13 Same as above at a slightly later period as shown by splitting of threads. Chromatin in vesicles *k*, *k*, *k* forming definite chromatin bodies.

14 Fragment of nucleus, Arphia simplex. Chromatin bodies *k* still densely staining, associated thread thinner than ordinary threads.

15 Very slightly later stage, Arphia simplex. Bodies *k*, *k* less densely staining.

16 Still later stage, Arphia simplex. Vesicle *k* persisting though nearly colorless. Associated thread more dense than ordinary threads.

17 Part of cell of Brachystola magna near end of bouquet stage; *x*, accessory with precocious tetrad attached.

18 Part of cell Arphia simplex near end of growth period; *x*, accessory; *c*, unequal precocious tetrad attached to accessory. 18 e, *x* and *c*, later stage.

19 The same; early prophase; *x*, accessory; *c*, unequal precocious tetrad; *b*. fragment of another precocious tetrad.

20 Late prophase of Brachystola magna. Homogeneous accessory and associated precocious tetrad showing tripartite feature and greater density than ordinary tetrads.

21 Spermatid, Arphia simplex. The three condensed elements still intact; *x*, one chromatid of accessory; *b*, one chromatid of larger precocious tetrad; *c*, one of smaller chromatids of unequal tetrad.

22 The same except *c*, one of larger chromatids of unequal tetrad. Note that the nucleus of either spermatid is about $\frac{1}{4}$ that of the nucleus at end of growth period.

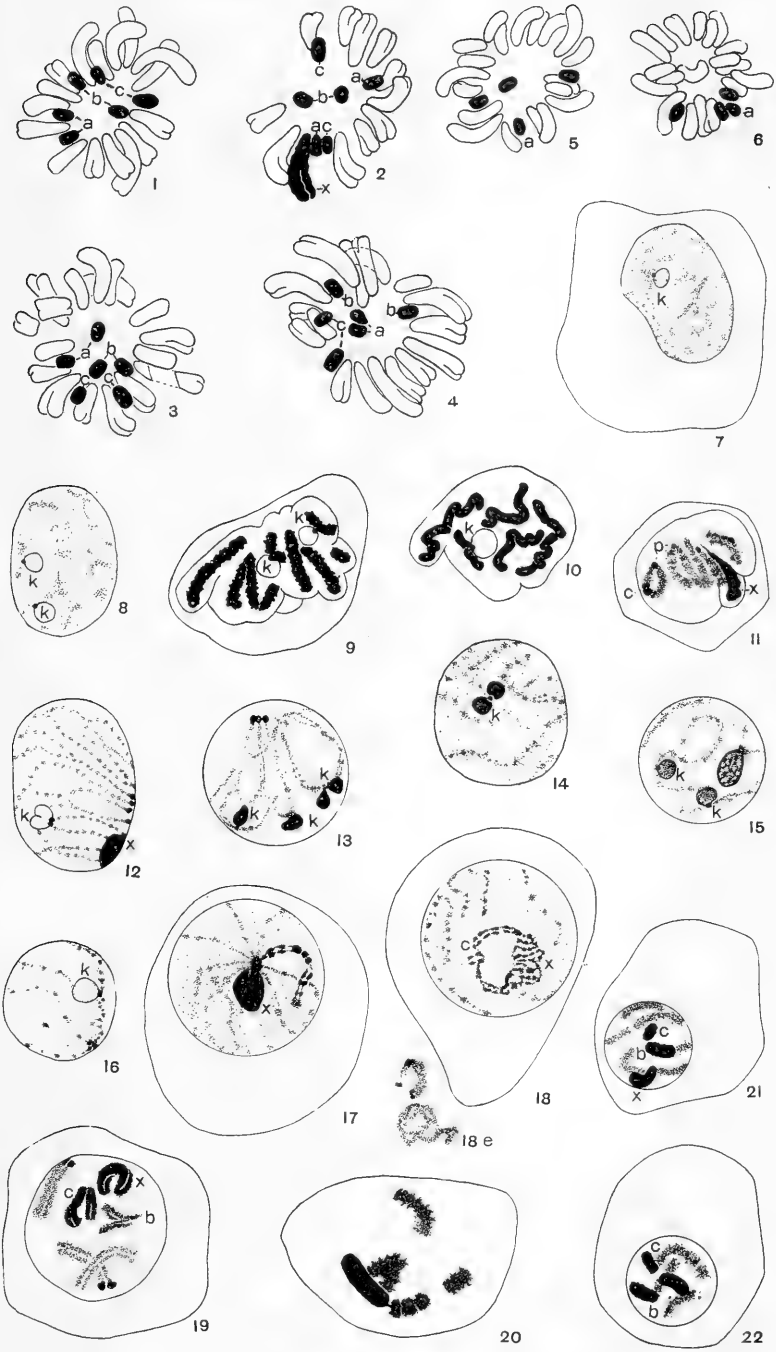
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PLATE 2

EXPLANATION OF FIGURES

23 Accessory and unequal tetrad forming a hexad multiple; *k*, remains of vesicle, smaller dyad attached to accessory.

24 The same; larger dyad attached to both ends of U-shaped accessory.

25 The same; all three small tetrads adhering to accessory.

26 Late prophase, Arphia simplex; unequal tetrad separate from accessory.

27 First spermatocyte metaphase; (this and most of the remaining figures are designed to show the distribution of the unequal tetrads in relation to the accessory).

28 Late prophase of entire first spermatocyte complex: at this time there are eleven separate elements; drawn from three sections.

29 Unequal tetrad with persistent vesicle, *k*, still distinguishable at first spermatocyte metaphase.

30 First spermatocyte metaphase from same animal as figure 39. Note that in the first instance the larger and in the last the smaller dyad accompanies the accessory.

31 and 32 Entire first spermatocyte complexes: 31 drawn from two sections, 32 from three.

33 and 34 Early anaphases of entire complexes. In figure 33 the smaller dyad accompanies the accessory while in figure 34 it is the larger one. Both drawn from two sections.

35 Entire complex of Arphia simplex except accessory. Note that there are only two small tetrads here corresponding to the four small chromosomes of the spermatogonial group; figures 5 and 6. Also note the general differences between the complexes of the two genera by comparing with figures 31 and 32.

36 First spermatocyte metaphase from same animal as figure 32.

37 and 38 First spermatocyte metaphases from another animal.

39 and 40 The same from a different animal.

41 and 42 Anaphases from still another animal illustrating the same feature.

43 and 44 From same animal as figures 39 and 40.

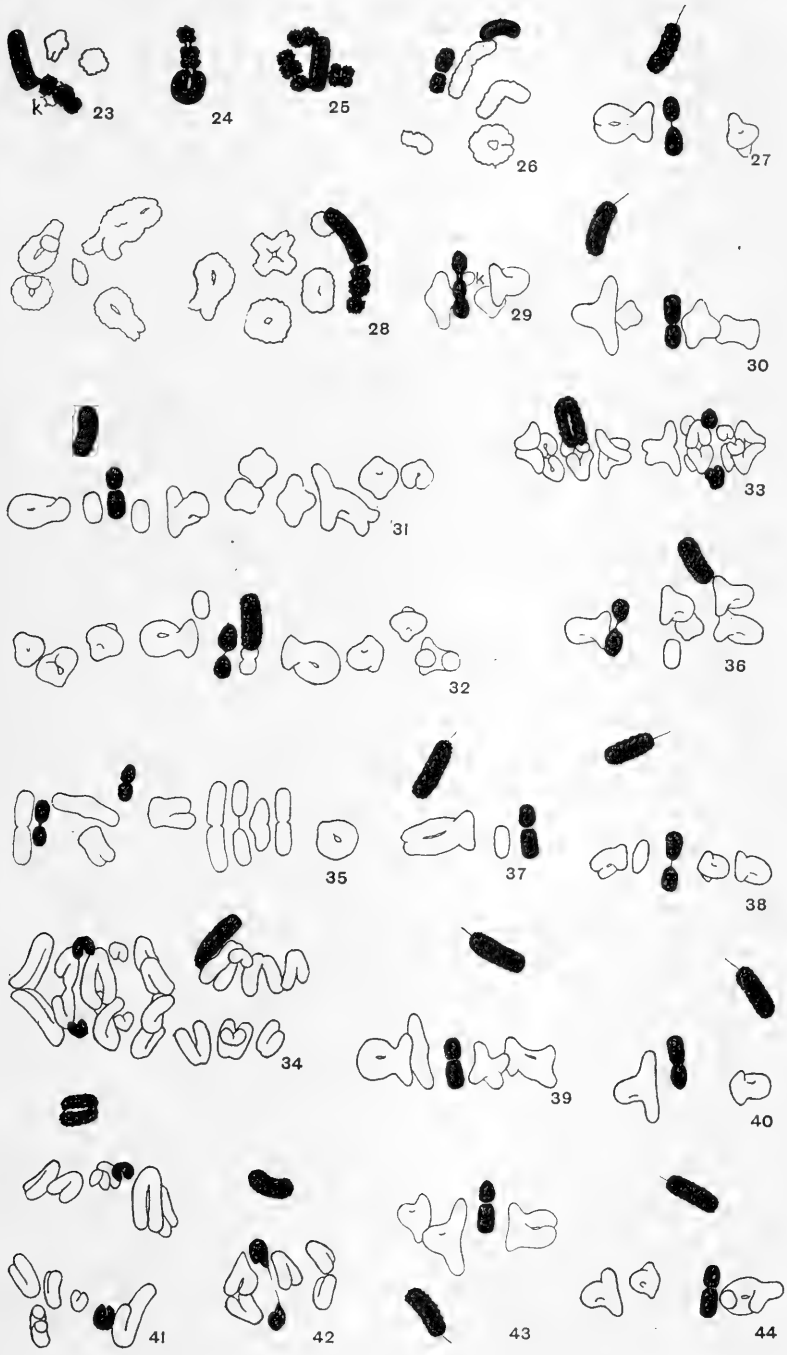
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PLATE 3

EXPLANATION OF FIGURES

45 Early first spermatocyte anaphase from same animal as figures 48, 50 and 57.

46 Late metaphase from yet another animal.

47 Metaphase from Dissosteira carolina.

48 Metaphase from same animal as figure 45.

49 Late metaphase or early anaphase from same individual as figure 34.

50 Late anaphase.

51 Metaphase from another animal.

52 Metaphase from Arphia simplex.

53 Metaphase from Dissosteira carolina; note secondary constriction of larger dyad.

54 and 55 Late anaphases from Arphia simplex.

56 Metaphase from Arphia simplex.

57 Late metaphases from same animal as figure 48. Note tendency to constriction of larger dyad.

58 First spermatocyte telophase, eleven chromosome group containing the larger dyad, *c*, and lacking accessory. (All telophases are not only from the same individual but also from the same slide.)

59 Late anaphase Dissosteira carolina showing weakness of one dyad of one of larger chromosomes.

60 Anaphase of Dissosteira carolina from same animal as figure 47.

61 Telophase of twelve chromosome group. Accessory and smaller dyad present; *e*, fragment of *e'* from another section.

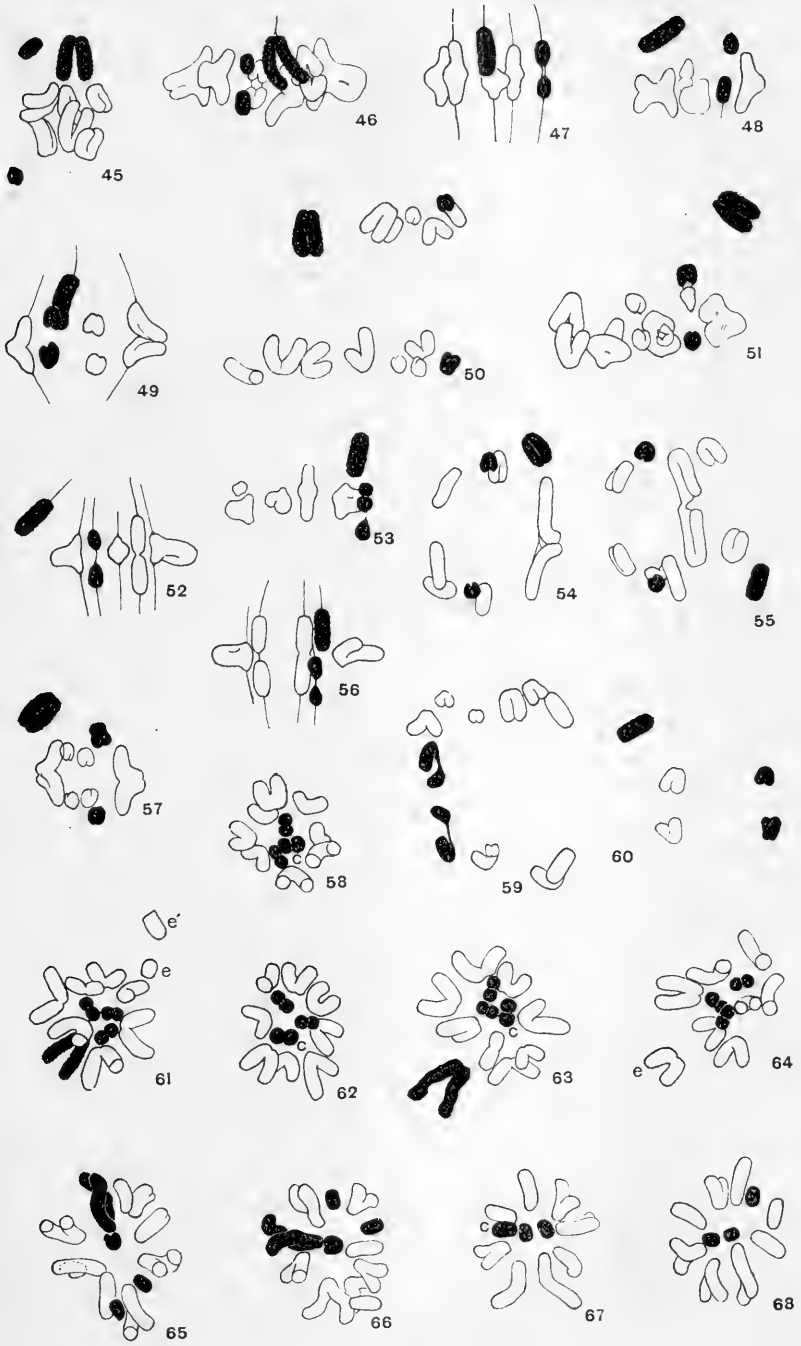
62 Similar to figure 58.

63 Similar to figure 61 except that larger dyad, *c*, is present instead of smaller one. Accessory drawn from another section.

64 Similar to figures 58 and 62 except that it contains smaller instead of larger dyad; *e*, ordinary dyad from another section.

65 and 66 Second spermatocyte metaphases containing accessory; and figure 65 smaller dyad, figure 66 the larger one.

67 and 68 The same as above, eleven chromosome groups, one containing the larger dyad, *c*, the other the smaller.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STUDIES ON THE HABITS AND DEVELOPMENT OF A HYMENOPTEROUS PARASITE, SPALANGIA MUSCIDARUM RICHARDSON

C. H. RICHARDSON

From the Entomological laboratory of the Bussey Institution, Harvard University

SEVENTEEN FIGURES

The present article contains the results of field and laboratory investigations upon the life history of Spalangia muscidarum Richardson,[1] a hymenopterous parasite whose embryonic and larval life is spent within the puparium of the house fly, Musca domestica L. To this has been added a fairly complete résumé of the literature dealing with hypermetamorphosis in the order Hymenoptera and a consideration of the various larval types known to occur in hymenopterous insects possessing supernumerary larval stages.

The writer wishes to express his sincere gratitude to Dr. W. M. Wheeler and Mr. C. T. Brues for their aid and advice throughout the course of this work.

Systematic relationships of the genus Spalangia. The genus Spalangia Latreille belongs to the Chalcidoid family Pteromalidae and is placed by Ashmead[2] in the subfamily Spalangiinae. This subfamily is clearly distinguished by the oblong shape of the head, the antennae consisting of eight to twelve joints and inserted close to the mouth, the long and depressed thorax, the distinctly petiolate abdomen and the length and narrowness of the costal cell of the fore wing. Of the four genera comprised in the subfamily Spalangiinae, the genus Spalangia is clearly separated by a number of characters of which the following are

[1] Psyche, vol. 20, no. 1, 1913, pp. 38–39 (1 plate).

[2] Classification of the chalcid-flies, or the superfamily Chalcidoidea. Memoirs Carnegie Mus., vol. 1, no. 4, 1904, p. 311.

most important: the normal non-tridentate head, the absence of distinct antennal furrows, the ten or twelve-jointed antennae and the presence of a transverse furrow in front of the posterior tip of the scutellum.

Description of Spalangia muscidarum.[3] Male: (fig. 1): Length 3 to 3.5 mm. Frontal aspect of head oblong-ovate, with numerous large depressions; eyes ovate, not emarginate in front; entire head covered with a short rather stout light-colored pile; ocelli present; labrum very small in proportion to length of head, the free border rounded, hairy; mandibles bidentate, length more than twice the width at base; antennae ten-jointed; scape as long as the three succeeding joints, covered with hair of the same texture as that on the head, second joint shortest; third joint almost as long as the succeeding two; the remaining seven joints except the last which is longer, of equal length; they are covered with fine light-colored hair; genae punctate like the face. Thorax above with the three divisions distinct; anterior narrowed portion of pronotum finely punctate and sharply marked off from the posterior part, which is sparsely and very coarsely punctate except for a median smooth space, widest posteriorly; a transverse row of deep umbilicate punctures near its posterior margin; mesonotum smooth and polished anteriorly, sparsely punctate posteriorly and laterally, leaving a smooth median space for its entire length; parapsides prominent with a few scattered punctures; parapsidal grooves deep, punctate; scutellum smooth, sometimes with several scattered punctures at sides; a distinct punctured line crosses it posteriorly; post-scutellum smooth; metanotum with two deeply punctate longitudinal lines separated by a smooth raised area; on either side of these lines of punctures is a smooth space bounded posteriorly and laterally by numerous deep punctures, smallest and most abundant on the extreme sides. Mesopleurae each with a single fovea; an aciculate depression below and behind the tegula. Abdomen smooth except petiole which is finely aciculate; third segment largest. Hind coxae swollen; first joint of tarsi not quite as long

[3] Richardson, loc. cit.

as the succeeding four. Wings hyaline, covered with short stout hairs. Venation piceous. Color of thorax deep bronze; abdomen aeneous; the tarsi yellow-brown except the last joint which is black.

The female is larger and of a more delicate structure than the male. The head is longer and narrower, the antennae are more slender and the abdomen is of different proportions.

The type locality of the species is Forest Hills, Massachusetts.

The following key, largely adapted from Ashmead,[4] will separate this from the other North American species:

FEMALES

1. Species blue black..... 3
   Species not blue black..... 2
2. Species bluish-green; tergum with a cupreous band at base, densely punctured; wings hyaline, slightly dusky.....polita (Say)
   Species black, more or less bronzed; thorax rugoso-punctate; wings dusky with brownish nervures.....aenea Prov.
3. Head punctured..... 4
   Head smooth the marginal vein two-thirds the length of the submarginal, wings hyaline.....haematobiae Ashmead
4. Whole prothorax with distinct punctures; wings hyaline, the marginal vein a little more than half the length of the submarginal..... 5
   Prothorax smooth, impunctured; wings hyaline, the marginal vein long .....drosophilae Ashmead
5. Mesonotum smooth medioanteriorly, punctate behind, scutellum above the punctured line very definitely punctate[5].....rugosicollis Ashmead
   Mesonotum smooth mediolongitudinally; scutellum above the punctured line impunctate or with a few sparse punctures..muscidarum Richardson

Geographical distribution. The species of the genus Spalangia are widely distributed throughout North America and Europe. A number have also been recorded from Central and South America and the Hawaiian Islands. They appear to be absent from Australia, Asia and Africa, but this may be due to the lack of diligence in searching for them.

[4] A synopsis of the Spalangiinae of North America. Proc. Ent. Soc. Wash., vol. 3, 1894, p. 35.

[5] Ashmead states in his description (loc. cit., p. 36) that the scutellum has "some sparse round punctures." I have examined the type, however, and find the scutellum to be quite heavily punctured when compared with that of S. muscidarum.

The twenty-eight recognized species inhabit the regions tabulated below:

NORTH AMERICA	
Spalangia aenea Prov.	Canada
Spalangia drosophilae Ashmead	Florida; Georgia
Spalangia haematobiae Ashmead	Virginia
Spalangia muscidarum Richardson	Massachusetts; Forest Hills Texas; Dallas; Gainesville Kansas; Wellington Louisiana; Addis
Spalangia polita (Say)	Virginia
Spalangia rugosicollis Ashmead	Missouri
CENTRAL AMERICA	
Spalangia chontalensis Cameron	Nicaragua
Spalangia impuncta Howard	Grenada
Spalangia nigra Latreille	St. Vincent Grenada
SOUTH AMERICA	
Spalangia bakeri Kieffer	Brazil; Pará
Spalangia braziliensis Ashmead	Brazil
Spalangia nigra Latreille	Galapagos Islands
HAWAIIAN ISLANDS	
Spalangia cameroni[6] Perkins	Oahu; Honolulu; Molokai
Spalangia laniaensis Ashmead	Lanai (2000 ft.)
Spalangia simplex Perkins	Oahu; Honolulu
EUROPE	
Spalangia astuta Förster	Germany
Spalangia erythromera Förster	Germany
Spalangia formicaria Kieffer	Germany; Luxemburg
Spalangia fuscipes Nees	Germany
Spalangia gonatopoda Ljungh	Sweden
Spalangia hirta Haliday	Great Britain Sweden
Spalangia homalospis Förster	Germany
Spalangia hyaloptera Förster	Germany
Spalangia leptogramma Förster	Germany
Spalangia nigra Latreille	Europe (Fere tota)
Spalangia nigripes Curtis	Great Britain
Spalangia rugulosa Förster	Germany
Spalangia spuria Förster	Germany
Spalangia subpunctata Förster	Germany
Spalangia umbellatarum Förster	Germany

[6] Perkins thinks that Cameron's S. hirta from Hawaii (Trans. Ent. Soc., 1881, p. 562) is referable to S. cameroni.

Hosts of Spalangia. Ashmead, writing in 1894,[7] stated that the members of the genus Spalangia are parasitic upon the larvae of Diptera. Since that time, the accumulation of additional data has shown that these Chalcidoids are by no means restricted in their parasitism to the Diptera, but as the accompanying list indicates, may even attack Lepidoptera, while several species have become myrmecophilous. However, a decided preference is shown for Diptera and especially for the house fly, Musca domestica.

SPECIES PARASITIZING DIPTERA	HOSTS
Spalangia drosophilae Ashmead	Drosophila sp.
Spalangia haematobiae Ashmead	Haematobia serrata Riley and Howard
Spalangia muscidarum Richardson	Musca domestica L. Stomoxys calcitrans L. Haematobia serrata Riley and Howard[8]
Spalangia nigra Latreille	Musca domestica L.
Spalangia fuscipes Ness	Lasioptera erynagii (Giraud)
Spalangia hirta Haliday	Musca domestica L.
SPECIES PARASITIZING LEPIDOPTERA	
Spalangia nigra Latreille	Coleophora giraudi (Giraud)
MYRMECOPHILOUS SPECIES	
Spalangia erythromera Förster	With Lasius fuliginosus
Spalangia formicaria Kieffer	With Lasius fuliginosus

Normal activities of the imago. Spalangia muscidarum is a very active insect, crawling and flying with perfect ease and considerable rapidity. Often when suddenly touched with any instrument, it will arch the body and draw its legs closely to its sides so that a quite lifeless position is assumed. This it may maintain for some time, or the spell may be only momentary, the insect returning to its normal poise almost immediately after the disturbance.

[7] Loc. cit., p. 28.

[8] Mr. W. D. Hunter informs me that Spalangia muscidarum was bred from this species in Texas on a number of occasions, but that Stomoxys calcitrans was a more common host.

Food. The natural food of Spalangia muscidarum was not ascertained. In the laboratory, however, it was observed to feed rather sparingly upon banana peel and sweetened water, showing a preference for the former.

Copulation. The male is sexually the more active. When he comes in contact with the female, he vibrates his wings rapidly, usually running around her once or twice before coitus. By some means not discovered but probably an olfactory sense, the female appears to detect the presence of the male when he is within close proximity. Upon his approach, she stops and drawing the antennae closely together, remains motionless and at the same time throws down the sternite which shields the genital opening. Coitus lasts but a few seconds after which the male clings firmly to the female with his forelegs and rubs the sides of her abdomen with his middle and hind legs. At the same time the female rubs her abdomen with her hind legs.

Oviposition. My observations show that S. muscidarum is exclusively a pupal parasite. The egg was invariably found upon the dorsum or sides of the host's abdomen within the puparium. Never more than one egg or a single larva was found on the same host. When ready to oviposit, the female, feeling her way with her antennae, crawls over the fly puparium. A suitable place having been found, she thrusts her ovipositor through the puparium either inter- or intrasegmentally and deposits an egg in from seven to eleven minutes. The parasite may occupy a nearly middle position on the puparium or one farther toward the posterior end. That a miscalculation may sometimes occur is evident from the fact that one female was observed to oviposit near the anterior end of a puparium. In such cases, it is highly probable that the active planidium larva could easily reach the abdomen.

Eclosion. The imago issues through an irregularly round hole made with its mandibles at or near the anterior (head) end of the house fly puparium. The insect is active almost immediately upon emerging.

Length of life of the imago in captivity. The imago when confined within a glass test tube in the insectary and fed daily upon

fresh banana peel lives about eighteen days. The extremes were eight and twenty-six days in a large number of cases noted.

The egg. The egg (fig. 2) is elongate, constricted at the anterior end somewhat widened below the middle and drawn out into a short, blunt petiole posteriorly. It is white in color and measures about 0.43 mm. in length by 0.14 mm. in greatest diameter.

Hypermetamorphosis in the Hymenoptera. Before considering the larval stages of muscidarum, it seems expedient to review briefly the known examples of hypermetamorphosis in other hymenopterous insects. The various types of larvae are discussed below and their distribution throughout the superfamilies is shown in table 1. It is impossible at this time to ascertain the extent of larval modifications within the different groups or to appreciate the phylogenetic value of the various larval types. Yet enough has been accomplished to suggest some very important characters for the more exact definition of families and genera as well as species. However doubtful such researches may be from the standpoint of phylogeny, their value in the study of parasitism and in taxonomy can hardly be questioned.

Ratzeburg ('44) figures the larval forms of the Ichneumonid, Anomalon circumflexum Linné, which passes through a remarkable series of stages. The first stage larva is found in the smallest lepidopterous larvae. Its caudal segment is greatly produced, equaling about half the body in length and is sharply pointed. The alimentary canal can be seen by means of the microscope, running out into the caudal appendage. The head is chitinized and is armed with a pair of mandibles. There is no trace of a tracheal system. In the second stage larva, the tracheal system begins to develop along the dorsum and the caudal appendage is reduced from one-half to one-fourth of the body length. Single-jointed antennae appear on the head. The third stage larva has a fully developed tracheal system. A pair of antennae, a pair of large curved mandibles, a pair of maxillae and a pair of smaller labial palpi are visible. This larva is inclosed in a peculiar sac. The fourth stage larva is entirely without a caudal appendage. The chitinous character of the

head has disappeared, the mouth parts have taken the form of the ordinary Ichneumonid larva and there is a great reduction in size.

Ratzeburg ('44) describes the larval stages of the Braconid Microgaster nemorum Hrt., an ectoparasite of lepidopterous larvae. The first stage larva is small, with twelve segments. The head and caudal appendage are not noticeably modified and no tracheae are present. The second larva is larger and the silk glands and mesenteron are conspicuous. The caudal segment bears a spiny knob-like swelling which Ratzeburg believes to be a respiratory organ, since there is no tracheal system at this stage. The mouth parts are very simple. The tracheae arise during the third larval stage and the silk glands extend into the caudal segment but not into the caudal swelling. Between this and the pupal stage there are two less distinctly marked transitional stages.

de Filippi ('51) has described the larval stages of an unidentified Pteromalid reared as a secondary parasite from the eggs of Rhynchites betuleti. The first larval form is of the cyclopoid type with a long furcated caudal appendage and a fringe of long spines at the juncture of the anterior with the following segments. The larva moves about in the egg by means of the furcated appendage which it lashes briskly. The second larva increases in size, due to the growth of an 'internal visicle' (undoubtedly an allusion to the enlarged mesenteron) and loses its mobility. Finally it is reduced to a mere sac with an anterior constriction. There may be some doubt as to whether the author really had a species belonging to the family Pteromalidae, since the type of larval development suggests strongly that of the Proctotrypoidea.

Metschnikoff ('66) and Ganin ('69) found the cyclopoid larva in a species of the Proctotrypoid genus Teleas. The second author also described the development of the Proctotrypoid, Polynema (species not given) and several species belonging to the genus Platygaster both of which showed a definite hypermetamorphosis. Three distinct larval forms were observed in Polynema. The first stage larva hatches without any visible

organs as a mere globular sac of cells. Following an ecdysis, the Histriobdella-like larva appears, having a superficial resemblance to the worm from which it has received its name. The third stage larva is also highly modified. Histoblasts of the mouth parts, antennae, wings, legs and ovipositor are visible, as well as a pair of lobate appendages on the sides of the last segment. The larval stages found by Ganin in Platygaster are comparable to the cyclopoid, intermediate and tertiary larvae described by Marchal.

Ayers ('84) studied the Proctotrypoid, Teleas (species not determined) a common egg parasite of the tree cricket, Oecanthus niveus. Two distinct larval stages were observed and an indication of a third, but the work was finished before this could be carefully elucidated. The first stage, the 'spindle-shaped larva,' has from five to eight segments along the equator of each of which is a series of spines. Ventrally, at the base of the caudal appendage, which is over half as long as the body, is a series of tooth-like projections. The head region is elongated in front to form a blunt process. There is a pair of hooked mandibles. This larva moves about in the egg by means of the rows of bristles, flexion of the tail and bending of the entire body. The second stage larva suggests the cyclopoid larva of Ganin and others. It possesses a pair of hooked mandibles with a peculiar beak-like labium lying beneath them. The abdomen terminates in a single long appendage, somewhat flexed upward like a telson. It is used in feeding. On either side of the body is a cuticular expansion the 'fin pad,' bearing numerous long bristles. The advanced second stage larva is very flat and has a large abdomen and smaller head region suggesting a third larval stage which is known in other Proctotrypoidea.

What is probably the larva of a species of Teleas has been inadequately described by Lemoine ('88).

Klapálek ('89) described two well marked larval stages in the Agriotypid, Agriotypus armatus Curtis. The first, which he called the 'larva,' is figured as being elongate, distinctly segmented and possessing a curious medial constriction, behind which the body is enlarged and finally tapers off toward the

caudal end. The second larva, 'the subnymph,' is larger, much less irregular in outline and the caudal segment is drawn out into a sharp point. Agriotypus armatus is parasitic upon the caddis fly, Silo and pupates within the case of this insect.

The larval forms of the Chalcidoid Leucospis gigas Fabr. have been studied by Fabre ('90) who found two distinct stages. The first is heavily chitinized, with a prominent head, antennae and a pair of small mandibles. The body is sparsely covered with spines and there are two longer spines, each situated on a basal process on the ventrum of all the body segments except the last. In general this larva resembles the planidium. The second stage larva is of the usual hymenopterous type, without visible traces of spines.

Kulagin ('98) found the cyclopoid larva in the Proctotrypoids, Platygaster insticator Say and P. herrickii Packard.

Seurat ('99) has described the young larva of the Ichneumonid, Mesochorus vittator Zetterstedt, which has an elongate caudal appendage and an undetermined species of Encyrtus with a similar elongate anal segment.

Marchal ('04) has found a larva in the Proctotrypoid Polygnotus minutus (Lindm.) which corresponds to Ganin's intermediate larva. One of these was seen with the partially cast off integument of a cyclopoid larva still clinging to it.

Ferton ('05) records two distinct stages in the post-embryological development of the Chrysidid, Chrysis dichroa, a parasite upon the larvae of Osmia versicolor which makes its nests in empty snail shells. The first stage larva has thirteen distinct segments; the head, heavily chitinized and distinct, bears a pair of blunt-pointed antennae and is armed with a pair of mandibles. The dorsal and lateral surfaces of each segment possess tufts of spines arranged in a single row across the body. The caudal segment is modified to form a short bifurcated appendage, the tips of which are bent inward. The second larva is of the usual hymenopterous type, devoid of spines and with a mere remnant of the furcated caudal appendage.

Marchal ('06) has given in detail the hypermetamorphosis of eight species within the family Platygasteridae (sens. Ash-

mead). The first of these to be mentioned, Synopeas rhanis Walker, possesses four larval forms, the cyclopoid, the intermediate, the secondary and the tertiary larvae. The cyclopoid larva resembles in superficial habitus the nauplius stage of certain crustaceans. The body consists of an enlarged cephalothorax composed of the head and at least the first thoracic segment, bearing a pair of short antennae, a pair of small chitinized first maxillae, which are mere tubercles, a heavily chitinized labium consisting of several rows of tooth-like points and below this a smaller chitinized ligula. The first thoracic segment has a pair of two-jointed appendages, which are probably sensory in function. The last abdominal segment terminates in two long serrated appendages. The intermediate larva shows a degeneration of the mandibular muscles and a contraction of the tissues of the appendages so that the latter now lie on a level with the body surface. The mesenteron enlarges enormously and invades the abdominal region. The intermediate larva undergoes an ecdysis and appears as the secondary larva. In this stage, the mesenteron is large and brown in color, the other tissues forming a clear zone around it. Metamerism is indicated by eight parallel muscle bands. The mandibles are now reduced to minute claw-like appendages. The first maxillae are represented by small oval tubercles behind the mandibles. The second maxillae are indistinct and have already fused with the labium, which is present in the form of a minute chitinized crest. Stigmatic openings and tracheae are present but the latter do not possess a lumen. The tertiary larva is characterized by distinct external segmentation. The mandibles are sharp and heavily chitinized. The stigmata are very distinct.

Trichacis remulus Walker agrees very well in its larval stages with the preceding species.

Inostemma piricola Kieffer possesses a cyclopoid, an intermediate and a secondary larva, the latter being segmented and homologous with the tertiary larva described for Synopeas.

In a species of Inostemma from Cecidomyia aenophila Haimh., Marchal has observed a cyclopoid larva comparable to that of Inostemma piricola Kieffer.

The larval stages of Platygaster lineatus Kieffer are comparable to those of Inostemma piricola, three forms only being present. Platygaster marchali Kieffer has the same type of metamorphosis as P. lineatus.

Platygaster ornatus Kieffer and a species of Platygaster from Cecidomyia oenophila Haimh. present a different type of metamorphosis from the preceding species in that the cyclopoid larva is wanting. The primary larva is ovoid, the mandibles are small and the last abdominal segment is without appendages. Segmentation is clearly indicated on the ventral surface. This larva passes gradually into the final segmented larva.

Matheson and Ruggles ('07) have figured the young larva of the Braconid, Apanteles glomeratus L. which is parasitic upon the common cabbage worm, Pieris rapae. In this larva the anal segment is enlarged into a globular swelling beneath which the hypodermis is greatly thickened. No later stages are described, but owing to the close resemblance between this and the first stage larva of Microgaster nemorum it is highly possible that such exist. Kulagin ('98) figures the caudal enlargement in the same species.

Wheeler ('07) found several interesting larval stages in the Chalcidoid Orasema viridis Ashmead, a pupal ectoparasite of Pheidole kingi Andre subsp. instabilis Emery. The planidium larva is less than 1 mm. in length and has a very definite segmentation, the anterior segments being longer and broader, the posterior smaller and often telescoped into one another. The head bears a pair of minute mandibles, the anal segment, a pair of hair-like cerci. The first three segments have on their dorsal surfaces each a pair of spines; there is also a pair of spines on the ventral surface of the seventh segment. The color of the larva in this stage is dark brown. The planidium larva moults, becomes lighter in color and increases rapidly in size. The anal cerci are lost and the larva enters what may be considered a second or intermediate stage. When this has attained complete growth, it undergoes an ecdysis, appearing as a thick-set 'semipupa.' Following an ecdysis, a third stage is entered upon. The 'semipupa' is now studded with large pustules arranged in

rows along each side of the body, but absent from the median dorsal and median ventral regions. The 'semipupa' here corresponds to the third larval stage in Perilampus hyalinus and Spalangia muscidarum.

Silvestri ('08) observed two larval forms in the Chalcidoid Encyrtus aphidivorus Mayr. The first, 800μ in length, is ovoid, with a well developed cephalic segment and mandibles and an elongate caudal segment quite comparable to that found by Ratzeburg in Anomalon. The second larval form closely resembles that found in Ageniaspis by Silvestri.

Two larval stages are described in the Chalcidoid Ageniaspis fuscicollis prasincola Silvestri by Silvestri ('08). The first stage 'larvette,' is small, 600 to 650μ long, elongate, with a well defined cephalic segment and a short pointed caudal segment slightly curved dorsalwards at the tip. The second stage, 'larve adulte' is 1 to 1.5 mm. in length, has the cephalic segment shortened and the caudal segment greatly reduced. In general appearance it agrees closely with the usual hymenopterous larval type.

Silvestri ('08) has recorded two larval forms in the Chalcidoid Oophthora semblidis Aur. The first stage larva is liberated within the egg of Mamestra brassicae L. and feeds directly upon the yolk. At this stage, it is of about equal dimensions anteriorly and posteriorly, the stomenteron and proctenteron being relatively large. The author does not figure mandibles in this larva. The second stage or adult larva is larger posteriorily, has well developed mandibles and an enormous mesenteron, while the stomenteron and proctenteron are relatively small.

Timberlake ('10) has made a study of the larvae of the Braconids, Praon simulans Prov. and Aphidius rosae Hal. (?) Three stages are present in both. P. simulans has a very unique first stage larva, the metathoracic and first to ninth abdominal segments of which are provided with a series of bristles on the dorsum and sides. The last abdominal segment bears a mediodorsal, cylindrical appendage nearly as long as the preceding segment and a pair of ventral appendages which are smaller. Sharp, chitinized mandibles are present. The second stage larva is intermediate in size between the other two. The last two

segments are fused and broadly rounded, the caudal segment being slightly indented at the tip. The head is small and the mouth parts appear as fleshy lobes. The integument is smooth and delicate. In the third larval stage the anterior end is much more pointed, the mouth parts, though not prominent, are represented by folds with chitinous plates. The integument is thick and chitinous, is thrown into large folds laterally and is everywhere roughened by fine granulations.

Aphidius rosae Hal. (?) has a somewhat simpler first stage larva than the preceding species. The head segment is quite large and bears a pair of long, curved mandibles. The last abdominal segment terminates in a single long, curved, bluntly-pointed process. The body is smooth and free from bristles. The second and third stage larvae are not distinguishable from those of Praon simulans Prov. except that the latter stage of A. rosae has a more pointed head.

Howard and Fiske ('11) have figured what is probably the first stage larva of the Braconid, Meteorus versicolor (Wesm.). It resembles that of Limnerium validum (Cress.) in the possession of a large, heavily chitinized head and an elongate caudal appendage. Judging from the figure, the mandibles are larger than those of Limnerium and are, according to the authors, like those of certain Platygasteridae. This species is in America a parasite of the brown-tail caterpillar (Euproctis chrysorrhaea).

Smith ('12) has described the remarkable larval stages of the Chalcidoid Perilampus hyalinus Say which is parasitic upon a number of other hymenopterous parasites and the Tachinid, Varichaeta aldrichi Townsend. The first or planidium larva is 0.3 mm. in length and about 0.06 mm. in greatest width. There are thirteen distinct body segments which are heavily chitinized and dark brown in color. The mandibles are well developed, hooked and crossed at the tips. The head bears a pair of stout antennal processes and back of these two stout spines. The ventrum is armed with spiny processes on each side, with larger single spines medially. These are probably ambulatory in function. The anal segment terminates in two long cerci. On the dorsum are a few scattered spines. The planidium is a free

moving form, running about on the body of the parasitized Hyphantria larva seeking for a place to enter. Having accomplished this, it attacks the larva of the parasite contained within. The planidium grows, becomes more robust, passes through a short resting period and after an ecdysis, emerges as the second stage larva. This differs greatly from the planidium, in its ovate shape and transparent integument, through which the tracheal system may be seen. The head is bent underneath the anterior portion of the body. Following an ecdysis, growth becomes rapid and the third larval stage is reached. The mouth parts are situated in a depression, beneath which are two bulb-like appendages, probably representing the maxillae. The antennae are represented by two large rounded elevations. The segments forming the head are somewhat constricted off from those that follow. Each of the first two thoracic segments bears a pair of medio-lateral tubercles and just above them another pair. Each of the three following segments has a larger pair of tubercles. The tracheal system is conspicuous at this stage.

Timberlake ('12) has described the larval stages of the Ichneumonid, Limnerium validum (Cress.), a parasite of the larva of Hyphantria cunea (Drury). The first stage larva has a heavily chitinized head and a long caudal appendage. The latter the author considers to be respiratory in function. The oral aperture is surrounded by a chitinized rim within which there is a broader rim. On the posterior inner margin of the latter is a chitinized plate bearing two teeth, separated by a median angular indentation. There is a pair of curved sharply pointed mandibles. During the growth, the proportions of the head and caudal appendage change, the body becoming more elongate and the folds of the integument, at first so prominent, are later represented by mere creases. The second stage larva appears after the first moult and may be distinguished from the preceding by the soft unarmored head, the slightly bilobed labium, the strong, curved mandibles and the large funnel-shaped mouth cavity. The caudal appendage is greatly reduced in size, but the larva itself is somewhat longer than that of the first stage. The segments are very conspicuous at this time. The third

stage larva is of the usual hymenopterous type. The mouth parts are very different from those of the preceding stages, and consist of a pair of strong mandibles supported by two sets of heavily chitinized ridges, the transverse ones reaching nearly to the lateral margins of the head. The mouth opening is hardly distinguishable. Below it is the circular labium with chitinized edges.

A very interesting paper by D. Keilin and G. de la Baume Pluvinel ('13) on the larval forms of the Cynipoid, Eucoila keilini Kieffer, has recently come to my notice. This hymenopteron has an extraordinary first stage larva which lives within the body cavity of its dipterous host, Pegomyia winthemi Meig. In outline it resembles somewhat the 'spindle-shaped' larva of Teleas, but the head does not have an anterior snout-like projection, nor are rows of bristles visible upon the dorsum. More striking however are the three pairs of long slender appendages on the ventrum of the thoracic region which easily distinguish this larva from any previously described. The mouth is circular and chitinized and mandibles are absent. There are two conical papillae on the ventral surface of the head which may represent either the maxillary or labial palpi. The body is conical in form with twelve visible segments. The posterior segment is produced into a single caudal appendage as long as the body, at the base of which a spinose appendage projects ventralward. The region about it appears to be covered with small chitinous scales. Circulatory and respiratory organs are wanting. The advanced larva is of the usual form common among the Cynipids and agrees very well with the prevalent hymenopterous type.

The authors were not able with the material at hand to discover the intermediate stage or stages which must surely intervene between the bizarre first stage larva and the more generalized advanced larva.

There are many striking resemblances in structure between the primary larva of Eucoila keilini and those of the Proctotrypids, Teleas and Platygaster, but the number and form of the thoracic appendages and shape of the head suggest another larval type which will be called the Eucoilaform larva.

THE LARVAL TYPES

The first stage larvae of the Hymenoptera in which hypermetamorphosis is known fall into ten quite distinct types. They are distributed as follows: one in the Vespoidea, two in the Ichneumonoidea, four in the Chalcidoidea, one of which also occurs in the Ichneumoniodea, three in the Proctotrypoidea and one in the Cynipoidea.

The Chrysidiform larva found by Ferton in Chrysis dichroa resembles the planidium type in its heavy chitinization, definite head segment and external feeding habit. But the curiously modified caudal segment, which is bifurcated with the tips of the two divisions bent inward, readily distinguishes it from any of the others.

The first stage in the post-embryonic development of Agriotypus armatus, has been called the agriotypiform larva. Its curiously irregular outline is well in keeping with the extraordinary aquatic habits of the adult and like none of the other hymenopterous larvae with which I am acquainted.

A larval type with a more or less elongated caudal segment has been named the caudate larva. Table 1 shows it to be well distributed in the families Ichneumonidae and Braconidae of the Ichneumonoidea and also to occur in the family Encyrtidae of the Chalcidoidea. The caudal appendage may be extremely attenuated as in Anomalon circumflexum, Limnerium validum, and Encyrtus aphidivorus, or very short as in Aphidius rosae and Ageniaspis f. prasincola. Again it may be enlarged into a rounded vesicle as in the young larva of Apanteles glomeratus and the second larval stage of Microgaster nemorum. Another larva which may represent a distinct type is found in Praon simulans. In addition to a short blunt, dorsal, caudal appendage it possesses a pair of shorter more delicate and more ventral appendages. The fourth to the thirteenth segments bear a single row of bristles posteriorly, much as in the cylindrical larva of Teleas.

The caudate larva is, so far as known, an internal parasite, without a functional tracheal system. Ratzeburg ('44) and Timberlake ('12) have suggested that the modified caudal appendage

may be a respiratory apparatus, but Kulagin ('98) was led to the conclusion through experimentation that its function is excretory. He injected a mixture of carmine and indigo-carmine into the body cavity of Apanteles (Microgaster) glomeratus and after two or three hours the presence of indigo-carmine could be detected in the cells of the caudal enlargement as well as in those of the Malphigian tubules, showing that the former possessed the power of removing waste substances from the blood. As is stated below, the larval stages of Spalangia are entirely without a functional tracheal system or any structure resembling a respiratory organ, yet it suffers no inconvenience through this deprivation. The caudate larva lives in a medium abundantly supplied with oxygen and there is reason to believe that it receives oxygen along with its food or directly through the cuticula without the aid of a specialized respiratory organ.

The name planidium, first suggested by Dr. Wheeler, has been used by Smith ('12) to designate the young larva of Perilampus hyalinus Say. This is an active, free moving creature, heavily chitinized, with a distinct head segment, antennae and mandibles and a few spines on the dorsum. There may be a pair of long cerci on the anal segment as in Perilampus hyalinus and Orasema viridis, or they may be absent as in Leucospis gigas. On the ventrum of Perilampus are spiny processes and rows of simple spines, while on the ventrum of Leucospis each body segment except the last bears a pair of long spines, each individual spine of which is situated on a short basal process. A less specialized type is seen in the planidium of Spalangia, described below, in which spines are wanting. The planidium larva appears to be restricted to the superfamily Chalcidoidea and to those species in which the first stage larva leads an ectoparasitic existence at least temporarily.

Another larval type occurs in the Chalcidoidea and has been found by Silvestri in Oophthora semblidis. I shall call it the oophthoraform larva. It is perhaps less distinct than the others, approaching more nearly the typical hymenopterous larva. Compared with the larval form into which it finally develops, it has a smaller mesenteron and a larger stomenteron and proctenteron.

According to the author's figure, mandibles are not present in this stage.

The simplest type yet observed is that found in Polynema by Ganin and here called the embryonic larva because of its very primitive structure. It is unlike any of the types above mentioned in that it hatches as a simple sac of cells without definite organization. During this stage its life is spent within the egg of Pieris brassicae.

Ayers has given the name 'spindle-shaped larva' to the first stage of a species of Teleas. The projecting cephalic process, the dorsal spines and the single long caudal appendage, as well as the embryonic internal structure render this larval type easily distinguishable. It lives within the eggs of other insects and up to the present time has been observed only in the genus Teleas.

The cyclopoid type is so well known through the researches of Ganin, Marchal and others that it hardly requires description. It exhibits considerable variation in structural details but the general scheme of organization is the same throughout. The body is divided into a large cephalothorax composed of the head and the first thoracic segment followed by a smaller segmented region. The caudal segment terminates in a bifurcated appendage which is variously serrated. Most striking is a pair of huge mandibles attached at the sides of the cephalothorax. The cyclopoid larva is an active entoparasite of very simple internal organization.

The name ovoid larva is given to a type discovered by Marchal in Platygaster ornatus and another undetermined species belonging to the same genus. The name is descriptive of the shape of this larva which resembles some of the intermediate larvae in the Platygasteridae. Caudal appendages are absent and the mandibles are small. Metamerism is indicated ventrally. This larva leads an entoparasitic existence.

The eucoilaform larva described by Keilin and de la Baume Pluvinel is superficially like the 'spindle-shaped' larva of Teleas, but lacks the dorsal bristles and has three pairs of ventral thoracic appendages. It is an entoparasite and probably represents

TABLE 1

NAME	FIRST STAGE	SECOND STAGE	THIRD STAGE	FOURTH STAGE
Vespoidea				
Chrysididae.				
Chrysis dichroa	chrysidiform	hymenopteriform		
Ichneumonoidea				
Agriotipidae				
Agriotypus armatus Curtis	agriotypiform	'subnymph'		
Ichneumonidae				
Anomalon circumflexum L.	caudate	intermediate	third stage	hymenopteriform
Limnerium validum (Cress)	caudate	intermediate	hymenopteriform	
Mesochorus vittator Zetterstedt	caudate			
Braconidae				
Praon simulans Prov	modified caudate	intermediate	hymenopteriform	
Aphidius rosae Hal (?)	modified caudate	intermediate	hymenopteriform	
Meteorus versicolor (Wesm.)	caudate	(other forms not known)		
Apanteles glomeratus L.	modified caudate	(other forms not known)		
Microgaster nemorum[1] Hrt		modified caudate	hymenopteriform	
Chalcidoidea				
Chalcididae				
Polynema sp	embryonic larva	histriobdella-like larva	modified hymenopteriform	
Leucospis gigas Fabr	planidium	hymenopteriform		
Perilampidae				
Perilampus hyalinus Say	planidium	intermediate	modified hymenopteriform	
Eucharidae				
Orasema viridis Ash	planidium	intermediate (?)	modified hymenopteriform	

[1] First stage larva inadequately described.

Encyrtidae				
Encyrtus aphidivorus Mayr	caudate	hymenopteriform		
Ageniaspis fuscicollis prasincola Silv	modified caudate	hymenopteriform		
Pteromalidae				
Spalangia muscidarum Rich	modified planidium	intermediate	modified hymenopteriform	
Trichogrammidae				
Oöphthora semblidis Aur	öophthoraform	hymenopteriform		
Proctotrypoidea				
Scelionidae				
Teleas (sp?)	"spindle-shaped" larva	cyclopoid	intermediate (?)	
Platygasteridae				
Inostemma pericola Kieffer	cyclopoid	intermediate	hymenopteriform	
Inostemma sp. from Cecidomyia aenophila	cyclopoid	(no other stages observed)		
Synopeas rhanis Walker	cyclopoid	intermediate	secondary	tertiary or hymenopteriform
Trichacis remulus Walker	cyclopoid	intermediate	secondary	tertiary or hymenopteriform
Polygnotus minutus (Linden)	cyclopoid (?)	intermediate	(only one stage studied)	
Platygaster lineatus Kieffer	cyclopoid	intermediate	secondary or hymenopteriform	
P. marchali Kieffer	cyclopoid	intermediate	secondary or hymenopteriform	
P. insticator Say	cyclopoid	(other stages not recorded)		
P. herrickii Packard	cyclopoid	(other stages not recorded)		
P. ornatus Kieffer	ovoid	hymenopteriform		
Platygaster sp. from Cecidomyia aenophila	ovoid	hymenopteriform		
Cynipoidea				
Eucoila keilini Kieffer	eucoilaform	hymenopteriform		

a distinctly Cynipoid type, since nothing like it has been observed in other forms.

There may be as many as four or as few as two larval stages in the development of the Hymenoptera in which hypermetamorphosis occurs. In general, as development proceeds the successive larval stages become less and less modified externally and more and more specialized internally. An exception to this rule, however, is seen in Polynema sp., in which the first larval stage is highly generalized and the greatest modification as well as internal specialization occurs in the last or modified hymenopteriform stage.

The end result of hypermetamorphosis is a larva resembling in external structure the common type found throughout the order, the hymenopteriform larva. The most extreme deviations from this type are found in Polynema, as noted above and in Perilampus, Orasema and Spalangia which have curious tuberculate hymenopteriform larvae.

Table 1 shows the distribution of the different larval types and the subsequent stages into which they develop before pupation.

HYPERMETAMORPHOSIS IN SPALANGIA MUSCIDARUM

The egg of Spalangia muscidarum hatches in the insectary in from two to three days. It is doubtful whether this represents the normal time of hatching, for the warm, moist natural habitat of the larval stages, that is, the manure heap, would undoubtedly bring about a greater rapidity of development than ordinary experimental conditions permit. Three quite distinct larval forms were found, first the minute planidium, second a larger atracheate larva and third and last a still larger tracheate larva.

THE PLANIDIUM LARVA

This larva is about 0.5 to 0.7 mm. long, its greatest width being about 33 per cent of the length. In color it is dull white, the region of the mesenteron showing as a median dark area. Thirteen segments are visible of which the anterior two are largest. The anterior segment bears a pair of short antennal

tubercles at the extreme anterior edge of the body and a pair of large projecting chitinized mandibles separated by the funnel-shaped mouth opening. The body is not heavily chitinized. The oesophagus is short and leads abruptly into the large sac-like mesenteron which fills the greater part of the body. The proctenteron appears as a tenuous cord of cells without a lumen and terminating in a small anal opening on the ventral side of the last segment.

The planidium larva is very active, moving rapidly over the dorsum of the host's abdomen, apparently searching for a place to insert its chitinous mandibles. In its habits it closely resembles the planidium of Orasema viridis Ashmead and the external planidium of Perilampus hyalinus Say, though I was not able to find ambulatory spines upon its body. It was never observed to enter the host's body as is the habit with Perilampus, but was found to be an ectoparasite throughout its entire life history. Nor have I ever seen it anywhere except upon the dorsum or dorso-lateral surfaces of the host's abdomen, and owing to the thick, chitinous integument of the fly pupa at every point except the abdomen, it is doubtful whether it could elsewhere gain access to its food. Having inserted its mandibles into the dorsal abdominal integument of its pupal host, it begins to grow and steadily increases in all dimensions. It is probable that an ecdysis takes place just before it passes over into the atracheate larval stage, but this was not definitely observed.

THE ATRACHEATE LARVA

Within three to five days after leaving the egg, the planidium reaches the atracheate stage. It is distinguished from the preceding by its larger size and the condition of the mandibles which are proportionally reduced and do not protrude beyond the mouth opening. The length varies somewhat, but is approximately 2 mm.; the greatest breadth is about 50 per cent of the length, showing a marked increase in bulk over that of the planidium. Thirteen segments are visible, the first being somewhat reduced, the second largest. The antennal tubercles can

still be seen on the anterior portion of the first segment. The reduced mandibles cross the mouth opening which is surrounded by a chitinous ring. The color of the larva is now light blue-white. The larger part of the body is occupied by the mesenteron which appears as a dark mass. The mouth opening communicates with a short, wide pharynx, into which the narrow oesophagus opens. This portion of the digestive tract is lined with a very perceptible chitin. Surrounding the food mass in the mesenteron is a very weak peritrophic membrane composed of but one loosely assembled layer of chitinous granules. The short, wide proctenteron ends blindly against the wall of the mesenteron. Dorsally to this lie the small paired ovoid gonads. The long salivary glands reach beyond the posterior end of the mesenteron. The oval adipocytes and the large larval oenocytes are present in this stage. The tracheal system is undifferentiated and the lateral tubercles so characteristic of the larval stage to follow are entirely absent. The name atracheate larva is given because of the lack of a tracheal system. No traces of a heart could be found.

The atracheate larva is a sessile parasite, never voluntarily changing its position upon the host and giving its entire attention to feeding and growth. Very slight movements of the body can often be detected, but the larva is quite unable to crawl. If it be carefully watched, deep peristaltic waves can be seen traveling from the anterior to the posterior end of the mesenteron. These are often so marked that the adipocytes can be seen shifting back and forth in the body cavity.

THE TRACHEATE LARVA

At the end of from thirteen to seventeen days, the curious lateral tubercles and rudiments of the tracheal system appear, marking the beginning of what I shall call the tracheate larval stage. This third larval form varies in length from 2 to 4 mm. and its greatest width is about 55 per cent of the length. The first segment is greatly retracted, especially in the larger individuals. The antennal tubercles and mandibles persist and a

chitinous ring still surrounds the mouth opening. The second segment bears a pair of very small tubercles; segments 3 and 4 are without tubercles, but a faint suggestion of them can be seen with high power binoculars; segments 5 to 12 each possess a pair, those on the posterior segments being noticeably smaller than those nearer the middle of the body. Of the imaginal structures which appear at this time, the most prominent are the thoracic legs and, in larvae destined to be females, a small group of appendages which will form the ovipositor of the adult.

As in the atracheate larva, the digestive system (fig. 7) consists of a wide pharynx, a short, narrow oesophagus which is slightly enlarged just anterior to its junction with the mesenteron, a large mesenteron and a short proctenteron now differentiated as the ileum, colon and rectum, the first ending blindly. The salivary glands are greatly enlarged.

The pharynx is an enlarged cavity separated by a slight constriction from the mouth opening in front and by a larger constriction from the oesophagus behind. A series of strong muscles radiate out from it to the integument. On the ventral surface of the mouth opening just in front of the pharynx is the small common opening of the salivary glands. Posterior to this opening, the salivary duct enlarges into a bulbous receptacle which receives the efferent duct of each gland at its posterior end. These ducts have a taenidial supporting structure resembling that found in the tracheae of the imago.

The salivary glands are voluminous, filling a considerable part of the body cavity and extending well into the region of the proctenteron. Anteriorly, they are thin walled with a large lumen which is filled with secretion. They are obviously reservoirs and not the active secretory parts of the organs. Posteriorly, the lumen becomes smaller and the walls thicker until at a point just in front of the proctenteron region, the glands become multilobed and of greater thickness.

The gland cells (fig. 8) are large, of an irregular shape with greatly ramified, granular nuclei, except in the thoracic region where they are narrow and elongate with elongate nuclei. The

cytoplasm contains many small vacuoles and numerous large and small canals. Intercellular canals are also present.

It is highly probable that the salivary glands are the most important digestive glands in the body of the parasite. This is discussed at greater length below.

The mesenteron constitutes by far the largest part of the digestive tract. Its length equals two-thirds that of the body and its greatest diameter two-thirds of the width of the body. Its cells are of the simple epithelial type, with ovoid, densely granular nuclei. The basement membrane is plainly visible, the intima very finely striated. The cytoplasm is filled with vacuoles of various sizes, generally smaller and more scattered toward the basement membrane. At the juncture of the oesophagus with the mesenteron, the cells are somewhat enlarged and produced into the lumen to form the peritrophogen. The cytoplasm of these cells is highly vacuolated, except near the periphery, where it is granular. The minute droplets of chitinous material secreted by the peritrophogen are carried backward by the peristaltic movements of the mesenteron to form a rather thick, homogeneous peritrophic membrane (fig. 9) which completely surrounds the food mass. From its delicate structure one is led to believe that it is vestigial in Spalangia.

The ileum consists of a thin strand of cells with a small lumen. Anteriorly where it comes in contact with the wall of the mesenteron, it is wider and there is a layer of several cells between its lumen and the basement membrane of the mesenteron. The Malphigian tubules appear as small evaginations of the ileum just behind the mesenteron. The ileum is constricted just before it reaches the colon, a large bulbous structure, possessing a somewhat ramified lumen. Beyond this is the long narrow rectum lined with chitin.

Although tracheal invaginations are formed during this stage, it is evident from their incomplete structure that they are not functional. The question naturally arises: How does the Spalangia larva obtain its oxygen supply? Our knowledge of the physiological processes in insects is at the present time extremely meager and will hardly admit of an adequate explanation in this

case. However, it is not improbable that a considerable amount of oxygen is absorbed directly through the thin cuticula, a known method of respiration in Collembola. Howard ('92) has expressed the view that oxygen is derived from the freshly aerated blood of the host which is ingested by the parasite. It may also be possible that a certain amount is liberated in a free state from the food during digestion in the mesenteron and that this is absorbed by the mesenteric epithelium.

No trace of a dorsal vessel or other structures for the propulsion of blood could be found. Such a system is quite unnecessary because of the strong and apparently continual peristaltic body movements which, as stated above, were sufficient to move the adipocytes within the body cavity.

The blood of Spalangia consists of a thin, colorless plasma in which float three types of cells, the leucocytes, the oenocytes and adipocytes.

The leucocytes are small round cells with a central nucleus and are of general distribution throughout the body cavity. My observations upon the oenocytes agree very closely with those of Weissenberg ('06) who worked upon the Chalcid parasite, Torymus nigricornis Boh. He found a larval and an imaginal generation of these cells, the former scattered about among the adipocytes without definite arrangement, while the latter, appearing shortly before pupation, originated from the dorsal imaginal discs on the fifth to the eleventh abdominal segments. Each group lay within a niche formed by the imaginal disc directly behind the developing stigma. During the pupal stage, the larval oenocytes underwent degeneration, their nuclei becoming crescentic and finally disintegrating.

The larval oenocytes of Spalangia (fig. 10) are distributed generally throughout the body cavity, from the anterior end of the mesenteron to and including the region of the proctenteron. Often they are seen in disconnected groups near the developing tracheal invaginations; they may occur singly, in groups, or in rows of four or five among the adipocytes. They are oval or somewhat polyhedral in outline and vary from about 40 to 46μ. in length. The cytoplasm is of a homogeneous structure, con-

taining vacuoles of various sizes which are quite evenly distributed. None of these vacuoles have been seen extruding from the periphery of the cells. The nucleus is also oval in outline and densely granular, varying from about 16 to 20μ in greatest diameter. The chromatin granules are of two sizes, the most abundant of which are small and irregular, while the less numerous ones are larger and rounded in outline.

Leucocytes were seen adhering to the periphery of the larval oenocytes only in a few instances and in very small numbers. It was certainly not as common a phenomenon as Weissenberg observed in Torymus.

The larval oenocytes were often so closely applied to one another as to suggest that they may have divided amitotically. However, there was nothing in the condition of their nuclei to warrant such a view and I am inclined to believe that this close association was due to the action of the reagents.

In the young pupae with a thin yellowish cuticula, the larval oenocytes were larger, but no signs of degeneration were visible. In the advanced pupae, the nuclei became crescentic or amoebiform (figs. 11 and 12), and the cytoplasm was often constricted off into definite lobes. At the same time, a garland of rather indistinct vacuoles appeared near the periphery as observed by Weissenberg. These agree perfectly with the degeneration stages in Torymus.

The imaginal oenocytes (fig. 13) lie in groups in cup-like depressions formed by the evaginated dorso-lateral imaginal discs which produce externally the lateral tubercles described above. We are thus given a clew to the meaning of these late-appearing larval structures. The imaginal oenocytes occur beneath the tubercles on the fifth to the eleventh segments, precisely those on which the tubercles are most strongly developed. The dorsal imaginal discs on the second and twelfth segments are but slightly thickened and bowed outward, so that the tubercles on these segments are weakly developed. Segments three and four are those upon which the histoblasts of the wings are found. These lie in pockets beneath the larval cuticula, but their outer surfaces are somewhat curved outward so that they simulate feeble tubercles.

That these larval tubercles are not ambulatory is evidenced by their dorso-lateral position. Neither is there anything in their structure to suggest a sensory function. They appear to be prepupal growth structures, corresponding in size with the extent of the evagination of the underlying histoblasts.

Similar structures have been observed in the mature larval stages of Perilampus hyalinus and Orasema viridis which may possibly be explained in the same way.

The imaginal oenocytes are polygonal or rounded in outline. The cytoplasm is of a homogeneous structure and the oval nucleus presents a granular appearance, but the granules are larger and less numerous than those in the larval oenocytes. In the tracheate larva, they lie closely packed together beneath the histoblast from which they arise and are of various sizes, those projecting farthest into the body cavity being the largest. During the pupal stage they become much more scattered but still may be found most abundantly in the stigmatic regions of the abdomen. After proliferation from the histoblast, there is a steady increase in size, but no instances of subsequent divisions were observed.

Nothing was discovered regarding the function of the oenocytes of Spalangia. Glaser ('12) found that the oenocytes of the leopard moth, Zeuzera pyrina L. secrete oxidizing enzymes which may activate the oxygen of the body. The oenocytes which he studied are found only in the abdominal segments just behind the tracheae and are apparently homologous with the imaginal generation of the Spalangia oenocytes. Whether the larval and imaginal oenocytes have a like function must remain an open question for the present.

The adipocytes are large single cells, spherical in outline, which float freely in the blood. In preparations stained with iron hematoxylin, the nucleus of each cell appears as a stellate mass of darkened chromatin granules. Outlines of the fat vacuoles are distinguishable, the contents, of course, being extracted by the xylol. Darkly stained globular bodies are abundant in the cells and are probably albuminous. Comparison of the adipocytes of the atracheate and tracheate larvae shows a steady

increase in the storage of these albuminous bodies so that just before pupation the cells in stained preparations are fairly blackened with them.

A careful search through the sections has failed to reveal urate crystals within the adipocytes and it is very doubtful whether these possess an excretory function in Spalangia. In fact no functional excretory organs were found in any of the larval stages. The absence of urate crystals in the adipocytes and the embryonic condition of the Malphigian tubules precludes the possibility of these organs possessing an excretory function. It would seem that any amount of excretory material that may be present must be retained in the mesenteron till its contents are ejected at pupation.

The nervous system presents no unusual features. The ventral ganglia are visible behind the subesophageal ganglion; posterior to the tenth, the ventral cord is attenuated and shows little differentiation.

The reproductive system consists of two gonads now lying to either side and below the proctenteron. Just prior to passing into the semipupa condition the unpaired reproductive canal is invaginated.

MUSCULATURE

The musculature of the tracheate larva does not differ in arrangement from the well known larval type. It is, with the exception of a series of rather powerful radiating pharyngeal muscles, very weakly developed.

Some attention has been given to the nature of the attachment of muscles to the integument in Spalangia and collateral studies have been made upon the following insects of other orders:

A Cucujid larva (probably Cucujus clavipes Fabr.)

Larvae of Porthetria dispar.

A Tabanid larva.

Larvae of Simulium sp.

Larvae of Calliphora erythrocephala.

Snethlage ('05) and more recently Törne ('11) have given excellent surveys of the literature so that it will be unnecessary to review the subject here.

Two views are current regarding the nature of muscle attachments in Arthropods; the first according to which the muscles are inserted upon the chitin of the exoskeleton, is upheld by Snethlage ('05), Tower ('06), Holmgren ('02, '07, '10) and many others; the second according to which the muscles are attached to the hypodermal cells which often elaborate a fibrillar supporting structure has found recent advocates in Maziarski ('03), Stamm ('04), Henneguy ('06), Wege ('11) and Törne ('11).

Törne has shown in his very exhaustive contributions, with which my observations accord, that there are three definite types of muscle attachments in insects. The first type can be seen in the attachment of the mandibular muscle fibers of Dytiscus marginalis. Here the 'indirect' insertion of the muscles is very evident, the hypodermal cells on which they are inserted having definite and characteristic nuclei. The second type of which the muscles of the larva of Yponomeuta padi Zll. are given as examples, shows the modified fibrillar structure of the hypodermal cells to which the muscles are attached. Many authors have used this type as evidence in support of the direct attachment of the muscles to the cuticula. A third type is seen in the wing muscles of many insects which show clearly that the muscles are inserted on the hypodermis.

The larva of Spalangia, owing to its weak muscular development and small size, offers rather unfavorable material for the study of the subject under consideration. Sagittal sections stained with iron hematoxylin-eosin show clearly, however, that the muscles are attached to the hypodermal cells. In figure 14, it can be seen that the fibrillae within the hypodermal cells are devoid of cross-striations and are separated by a definite line from the muscle fibers. This line is continuous with the basement membrane, and evidently is a part of it. This is a good example of Törne's second type of muscle attachment.

The wing muscles in the late pupa of Spalangia offer a much clearer example of muscle insertion than is seen in the larva. Figure 15 shows the hypodermal cells at the point of muscle attachment greatly elongated and definitely separated from the muscle fibers by a distinct line, the basement membrane. The

muscles present a very definite and regular series of longitudinal striae, but cross-striations are not visible at this stage of development. The hypodermal cells, on the other hand, do not possess such regular striae, but appear to be distinctly granular. To either side of the point of contact of the muscles, where muscular tissue is wanting, the hypodermal cells are of the same morphological structure though less attenuated and with the Giemsa-eosin stain give the same reaction. Almost the identical condition has been found in the wing muscles of the Chalcidoid, Monodontomerus, by Berlese ('09) and in the wasp Vespa by Törne ('11).

Large Tabanid larvae collected at Forest Hills, Massachusetts, proved to be excellent material for the study of muscle attachments. Sagittal sections 4μ in thickness, stained with iron hematoxylin-eosin show in a most conclusive manner the hypodermal attachment. At the point of insertion, the hypodermal cells are greatly elongated as in Spalangia. Their nuclei, which are not seen in figure 17, agree in structure with the adjacent hypodermal nuclei except that they are somewhat larger. A distinct line separates the muscles from the hypodermis, but I am not sure that the basement membrane is continuous between the muscle and hypodermis, although it can be traced in a little way on either side. Just within the hypodermis, where the two muscles come together, there is a mass of quite homogeneous substance which I believe to be an intercellular deposit.

Now, considering the nature of the dermal fibrillae which are seen in the dermis just above the elongated hypodermal cells, I believe that two facts preclude the possibility that they are fibrillar outgrowths from the muscles or hypodermal cells; first they do not take either the hematoxylin or eosin stains but remain slightly yellow-hyaline like the adjacent dermal substance; second, when sections are macerated in a strong aqueous solution of NaOH, they persist, even after the muscles have disappeared. With prolonged treatment, of course, the dermis disappears also.

Figure 16 offers strong support to the view that the hypodermal fibrillae are not continuous with the muscle fibrillae. When sections of the tabanid larvae were treated gently with

NaOH, the muscles often separated from the hypodermis, in which case the rupture always occurred along the base of the hypodermis at the point where it connects with the muscles. This happened so frequently that it could not be considered accidental.

In the Cucujid larva and larvae of Porthetria dispar, Simulium, and Calliphora erythrocephala, the muscle attachments are of the same nature as those in the Tabanid larvae, the insertion in every case being to the hypodermal cells which always possess hypodermal fibrillae within their cytoplasm. In none of these forms, however, could I detect dermal fibrillae.

THE SEMIPUPA

The semipupa marks a transition between the tracheate larva and the completed pupal stage. Still it belongs with the pupal stage, for the larval activities have ceased and the form of the imago has begun to develop beneath its old larval integument. Figure 6 shows the dorsal aspect of the semipupa, the circular areas marking the position of the abdominal tubercles which have collapsed, owing to the degeneration of the imaginal discs beneath them.

Just prior to pupation, the tracheate larva crawls toward the anterior end of the fly pupa, now reduced to a mere flattened mass of cuticle, so that its head lies near the end of the puparium. In this position, the semipupal stage is attained which soon completes an ecdysis, voids the dark meconial mass, and becomes a fully formed pupa.

THE PUPA

The pupa of Spalangia does not differ from the usual hymenopterous type and will not be described here.

EFFECT OF THE PARASITE UPON THE HOST

The parasite slowly consumes the blood plasma and its cellular content of the host, reducing the latter to a flattened mass of cuticle. Fat vacuoles, albuminous bodies, accumulations of blood plasma and what is apparently the cytoplasm of the adipo-

cytes have been seen in sagittal sections of the mesenteron. Recently ingested particles lying near the anterior region of the mesenteron are broken up and smaller than corresponding particles found in the abdomen of the fly pupa and I feel confident that a certain amount of digestion takes place outside the body. The salivary glands are hugely developed and well situated to secrete a ferment necessary for this.

HIBERNATION

The winter is probably passed in the pupal stage. I have found pupae and even semipupae late in February in fly puparia which were parasitized in October. The development is undoubtedly greatly retarded during the winter season.

ECONOMIC IMPORTANCE

This problem was undertaken too late in the fall to obtain definite results regarding the economic importance of this parasite. The highest proportion of parasitized house fly puparia was noted on October 5, 1912, when 9 Spalangia larvae and pupae were removed from 22 puparia. On another occasion, 5 larvae and pupae were taken from 101 fly puparia.

At Forest Hills, Massachusetts, I have reared this parasite with certainty only from puparia of the house fly. However, Stomoxys calcitrans was breeding quite abundantly with the house fly in a region infested with Spalangia muscidarum, and it seems reasonable to suppose that there was no discrimination shown between the two.

Bishopp ('13) found Spalangia muscidarum to be a parasite of Stomoxys calcitrans, Haematobia serrata and Musca domestica in Texas. In an examination of 2500 puparia of Stomoxys, 40 per cent were found to be parasitized by this and another undetermined Pteromalid.

In one instance, a large number of adult Spalangia were reared from puparia late in August. This leads me to believe that there are at least two regular generations and a third more or less regular one annually in Massachusetts. The first generation will emerge from the over-wintering pupae as early in the spring

as the weather permits. The second and undoubtedly the strongest generation will emerge during late July or August, while the third and somewhat weaker generation will appear late in September or early in October.

ADDENDA

A heretofore overlooked instance of hypermetamorphosis is given by Mr. H. J. Quayle[9] for the Chalcidoid, Aspidiotiphagus citrinus Craw. In the words of the author, "the egg is deposited within the insect and there hatches a very minute, white larva, with a tail-like appendage. This is afterwards lost and as the larva becomes mature it is about 0.85 mm. long and 0.35 mm. wide, tapering slightly toward the posterior end." The first stage larva appears to be typical of the caudate type, the mature larva of the usual hymenopteriform type.

A paper by the late Mr. Harry Pinkus[10] on the life-history and habits of Spalangia muscidarum as a parasite of the stable fly, Stomoxys calcitrans, has recently appeared. The author gives an excellent account of the habits and distribution of this hymenopteron. In addition to the localities listed above, he has reared Spalangia from puparia collected at Denison, Texas. The adults were found to be scavengers, preferring the remains of the hosts to all other food. The adult, egg, tracheate larva and pupa are figured. Apparently the author did not observe the minute planidium larva. In the concluding chapter, a method is given for the artificial propagation of these parasites and a special breeding cage is figured and described. By artificial breeding, the author believes that the period of development can be greatly shortened and that if the adults are liberated in the spring, they may be expected to be an important factor in the control of Stomoxys calcitrans.

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PLATE 1

ADULT AND EGG OF SPALANGIA MUSCIDARUM

EXPLANATION OF FIGURES

1 Dorsal view of the adult male (× 22).
2 The egg (× 80).

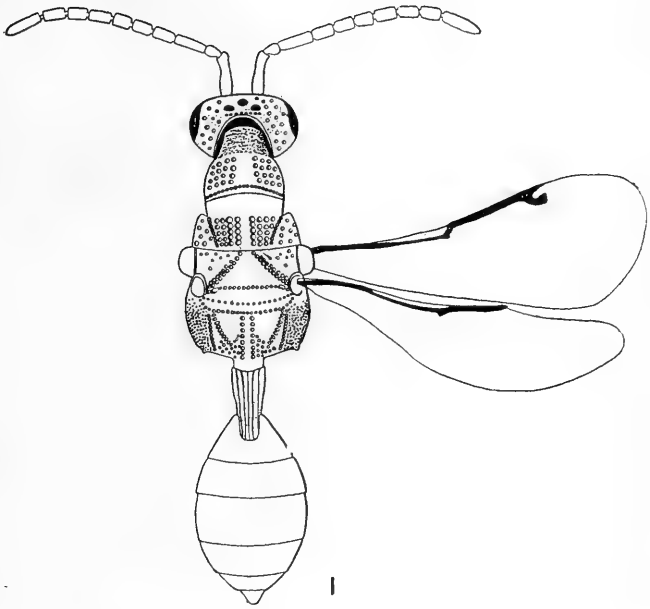

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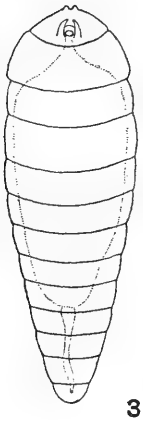
PLATE 2

LARVAL STAGES OF SPALANGIA MUSCIDARUM

EXPLANATION OF FIGURES

3 The planidium larva (× 86).
4 The atracheate larva (× 33).
5 The tracheate larva (× 26).
6 The semipupa (× 14).



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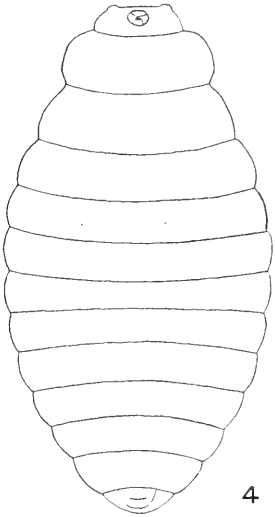

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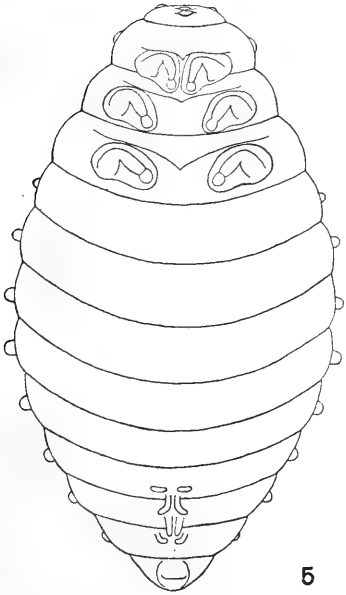

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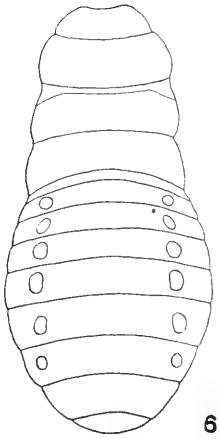

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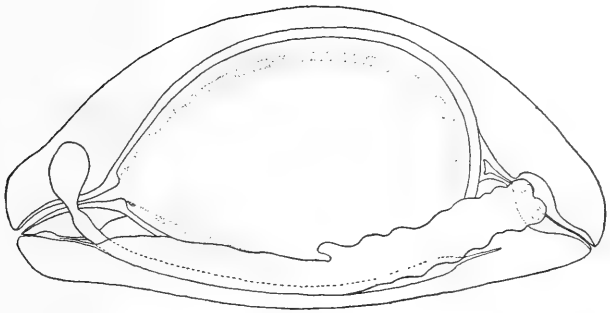
PLATE 3

SPALANGIA MUSCIDARUM

EXPLANATION OF FIGURES

7 Internal anatomy of the tracheate larva (diagrammatic).
8 Cross section of the posterior part of the salivary gland.
9 A portion of the peritrophic membrane showing its weak granular structure.
10 A larval oenocyte from the tracheate larva (× 1720).
11 and 12 Degenerating larval oenocytes from an advanced pupa.



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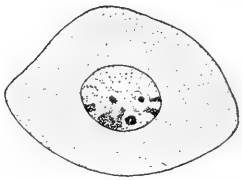

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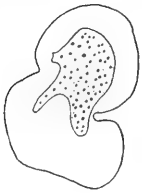

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PLATE 4

SPALANGIA MUSCIDARUM AND A TABANID LARVA

EXPLANATION OF FIGURES

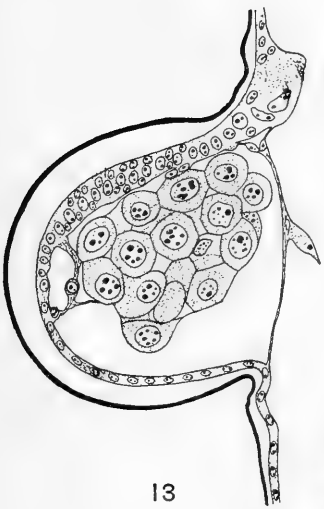
13 A section through a lateral tubercle of the tracheate larva with the group of imaginal oenocytes beneath. This section was cut a little to one side of the tracheal invagination.

14 Attachment of a pharyngeal muscle of the atracheate larva showing the hypodermal fibrillae.

15 Attachment of the wing muscles in a Spalangia pupa.

16 The point of insertion of muscles in a Tabanid larva. The muscles have been torn away, leaving the hypodermal and dermal fibrillae.

17 Normal relations of the muscles to the hypodermis in a Tabanid larva.



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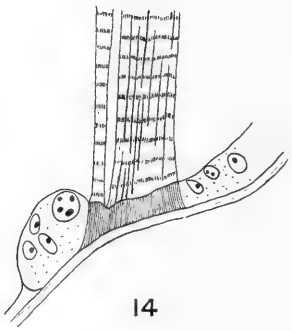

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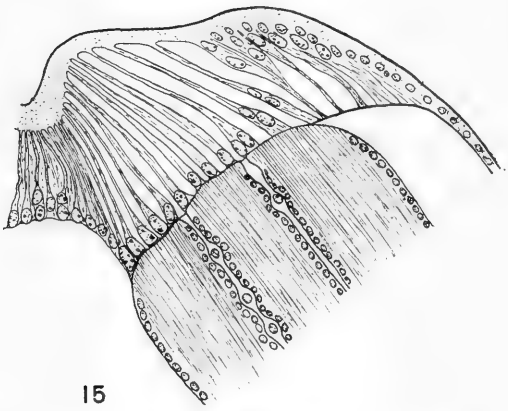

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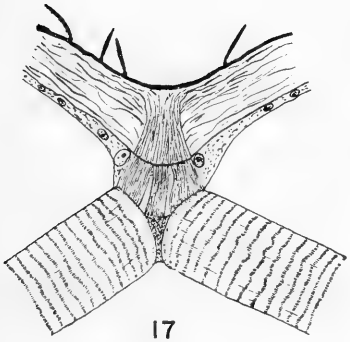

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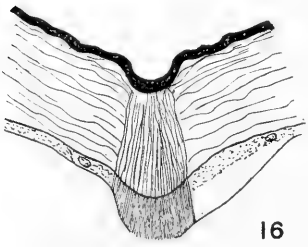

16

POLYEMBRYONIC DEVELOPMENT IN TATUSIA NOVEMCINCTA.[1]

J. T. PATTERSON

THIRTY-FIVE TEXT FIGURES AND ELEVEN PLATES

CONTENTS

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[1] Contributions from the Zoological Laboratory of the University of Texas. No. 115.

INTRODUCTION

The development of more than one individual from a single egg while not a rare phenomenon among animals, is nevertheless of much biological interest. It has become customary to classify such types of development as asexual or agamic reproduction; but obviously this term has come to include a variety of developmental phenomena, which are exhibited in animals ranging from the Protozoa to the mammals. Among the more common types of agamic reproduction are ordinary binary fission, budding, cyclical parthenogenesis, paedogenesis, and polyembryony.

Upon the basis of certain evidence which has been brought forward from a study of comparatively late embryonic stages, it has been correctly concluded that the type of agamogenesis which has become habitual in the Texas armadillo is that of polyembryony; but so far no one has succeeded in demonstrating the validity of this conclusion. The writer has in his possession a series of young stages which covers the period of early embryonic differentiation, and which represents the material upon which this paper is based. An outline of the more general features of the work has already been given in a preliminary paper (Patterson '12). The facts to be presented in detail are not without a certain interest and significance, not only because they raise to the dignity of an observed fact the claim for polyembryonic development in the armadillo, but also for the reason that they throw a great deal of light upon related phenomena in other mammals. It is unusual to find agamic reproduction in the highest class of animals, and a detailed study of the history of this process is greatly to be desired. Furthermore, there has been considerable speculation as to how 'identical twins' and similar types of development have arisen, and I believe that these studies on the development of the armadillo will at least indicate how these phenomena may have come about.[2]

[2] It is a pleasure to acknowledge here my indebtedness to my friend Mr. F. L. Whitney of the School of Geology for his able assistance in connection with the photographic work. I am also grateful to Mr. F. Pfeiffer, who has greatly facilitated this work by his many successful efforts in obtaining material.

METHODS

1. Technique

For all of the stages described in this paper, I have found Zenker's fluid to be the most useful fixing reagent. Kleinenberg's picrosulphuric acetic acid works well on attached stages, notwithstanding the fact that many embryologists have regarded this fluid as poor for preserving the finer structures. All of the material has been imbedded in paraffin, and the sections cut either 5 or 7 micra thick. Most of the preparations have been stained with acid hematoxylin, although Heidenhain's iron-hematoxylin has been used for some of the earliest stages. Whole mount preparations of the early embryonic vesicles, which were made by the glycerin jelly method, were found to be very useful in the interpretation of certain changes occurring in these young stages.

2. Method of securing early stages

In order to understand the methods employed for securing the young vesicles it is necessary to give a brief account of the structure of the uterus. The uterus of this armadillo is of the simplex type, like that of the primates, and gives no evidence, in its external appearance, of being in any way adapted to accomodate a litter of four. Viewed from either the dorsal or ventral sides, the uterus proper resembles a rather blunt spear-head, with the distal or fundus part representing the tip and the proximal or cervex part the shaft end of the instrument. The fallopian tubes, which lie in the plane of the broad ligament, enter the uterus at points lying approximately two-thirds the distance between the cervix and the tip of the fundus.

The external appearance of the non-pregnant uterus varies greatly, both in the virgin and in the old female; but in this connection we are concerned primarily with the condition of the internal surface. In the young female, two-thirds to three-fourths grown, the suface of the mucosa is rather smooth, and gives only faint indications of a folded condition, but in the adult virgin females of two years, and especially in the old females, the uterine mucosa becomes greatly folded. The folds have a general distribu-

tion over the lining of the uterus, except at the tip of the fundus, where there is a four-pointed, cross-shaped area of rather smooth mucosa. The arms of this cross meet each other at approximately right angles, and their common area is the extreme tip of the fundus. This cross also indicates the orientation of the uterus; for two of the arms mark the dividing line between the upper and lower halves of the fundus, and two form a rather broad, shallow groove extending from the mid-dorsal point around to the mid-ventral point of the uterine cavity. These facts are most clearly brought out in an everted uterus (fig. 21).

Each one of the arms forms a distinct furrow leading from the tip of the fundus to the uterine opening of the fallopian tube. As already indicated, the other two arms lead from the center of the fundus to the middle of the upper and lower surfaces, respectively. These usually form shallow furrows which end distally among the folds of the mucosa. For convenience we shall speak of the first pair as the right and left horizontal grooves, and of the second pair as the dorsal and ventral vertical grooves. On account of the fact that the uterine openings are situated slightly nearer the fundus than the cervix end, the horizontal grooves are somewhat shorter than the vertical grooves.

To the student of the early development of the armadillo, the significance and importance of this cross-shaped area can scarcely be over emphasized. The right and left horizontal groove forms the pathway along which the embryonic vesicle passes on its way from the fallopian tube to the tip of the fundus; and the center of the cross, or the central area of the fundus, is the attachment zone or placental area for the vesicle. The discovery of these facts has greatly facilitated the collecting of the early stages. Prior to 1911 attempts were made to obtain the young stages, and various methods were tried, such as that used with great success by English workers in collecting the early stages, namely, that of injecting the uterine cavity full of some killing fluid, and later examining the contents for the embryos. While this method works well where one is dealing with an animal which gives off several eggs at each ovulation, yet in the case of a single egg at each pregnancy the chances of its discovery are few. Furthermore, to any one who

has endeavored to study the fragile mammalian vesicle, the difficulty of preserving the delicate structure even after discovery, is indeed very great.

Early in the fall of 1911, however, the real significance of the cross-shaped grooves, and especially of the horizontal one, was first fully realized, and since then not much difficulty has been experienced in obtaining the free uterine or early attached vesicles. In the living or fresh uterus it can easily be demonstrated that the horizontal groove of each side is virtually a tube, and is therefore, a continuation of the fallopian tube. This is brought about by the depth of furrow and by the depressed condition of the uterus dorso-ventrally.

The best method to follow in seeking to obtain a young vesicle is to cut the uterus along each side in the plane of the broad ligament, beginning at the cervix and extending to a point lying a short distance from the opening of the fallopian tube, and then slowly to evert the fundus portion over the end of the small finger. By this procedure the horizontal furrows are spread wide open, and the vesicle, if present, will be revealed. The tip of the everted uterus is then applied to the killing fluid and the vesicle, if not attached, will float out on the surface of the fluid, and after a few seconds slowly sink to the bottom without distortion. In the case of attached vesicles, the everted uterus is placed in the fluid, and, after fixation and hardening is affected, a properly oriented block containing the vesicle is cut out of the mucosa. The process of obtaining these early free stages may be greatly facilitated by first determining which ovary holds the corpus luteum, and confining one's search to the corresponding horizontal groove.

NOTES ON BREEDING HABITS

1. General statement

During the past five years a considerable amount of information concerning the habits of this animal has been secured by the writer, and it may be worth while eventually to publish these observations; but here I wish merely to make a few remarks on certain phases of the breeding habits.

The breeding season extends over a considerable portion of the months of October and November, and thus any lot of embryos taken at a given time during the period of gestation will present much variation in development. This makes the determination of the length of gravidity difficult and quite uncertain. An exact determination could only be made by breeding animals in captivity. Since a majority of the young are born in the months of March and April, gestation is probably about one-hundred and forty days.

The old females breed first, mating in most cases before October fifteenth, while the second year virgin females continue to breed for some time after this period. Females of one year do not breed except in rare instances. My records for the past five years show just three cases of pregnancy among these young animals, out of at at least two-hundred examinations.

In connection with the collecting of material and this incidental study of habits, I have discovered a 'period of quiescence' of the embryonic blastocyst. The fact was first made apparent in 1911, when, after I had started collecting two weeks earlier than in the preceding year, I failed to obtain the cleavage stages, although judging from the condition of development in the vesicles collected in previous years, one would naturally expect to find these early stages during the period of my first collections in 1911.

Again in 1912, I began collecting material two weeks earlier than in 1911, and much to my surprise obtained blastocysts in almost exactly the same condition as those secured during the preceding fall. Practically all of these vesicles lie free within the uterine cavity, either in the horizontal groove or in the region of the attachment zone (placental area).

It is evident from these data that the embryonic vesicle remains for some time lying free within the uterine cavity. Just how long this period lasts, I am unable to state; for practically every old female taken at the earliest date (October 15) at which I have collected, possesses a free blastocyst. How long such blastocysts have been in the uterine cavity it is, of course, impossible to determine; but I should judge not very long, because two vesicles

taken from the fallopian tubes show a development almost as far advanced as that of some vesicles taken from the proximal parts of the horizontal grooves.

Taking all the facts into consideration, I estimate the 'period of quiescence' to last about three weeks; that is from about the middle of October to the third or fourth of November. There are exceptions to this, but not sufficient in numbers to modify the general conclusions. Of the thirty-four free blastocysts obtained in 1911 and 1912, twenty-eight of them were secured within this period.

There is another line of evidence which more or less supports the above conclusion. I refer to the condition of the corpus luteum. In all the females from which the free blastocysts were taken the corpus has attained approximately its maximum size. Unless we attribute a phenomenal rate of growth to the corpus, it is necessary to assume that quite a long period has elapsed since ovulation took place, in order to account for its large size. The short fallopian tube of the armadillo precludes the suggestion that the egg has spent a great while in traversing this passage, so that we must conclude that any arrested development in the egg takes place after it enters the uterine cavity.

So far as the writer is aware, the only other mammal in which a similar quiescent period in the development of the blastocyst occurs is the deer. According to Assheton ('98) Bischoff ('54) states that in this animal the embryo, upon reaching the so-called morula stage, enters upon a 'period of quiescence,' and remains unaltered for some weeks.

It is scarcely correct, however, to state that the blastocyst of the armadillos, during the entire period of quiescence, is in a state of arrested development, because it undergoes certain progressive changes. So far as one can tell from a study of sections, no mitotic divisions occur, but the vesicle increases in size, due to the accumulation of fluid within its cavity, accompanied by the attenuation of the trophoblastic cells. Furthermore, the differentiation of the ectoderm and entoderm from the inner cell-mass is completed while the blastocyst lies free within the uterine cavity.

2. Available material

The desirability of having at one's disposal a close series of early stages for a study of this kind is self-evident; but from what has been stated in the foregoing pages it is clear that many obstacles stand in the way of securing such a series. This is particularly true with reference to the cleavage stages. In another season this much desired material can probably be obtained by beginning to collect at a period still earlier than that of the preceding year. During the season of 1912 about a dozen uteri, with attached fallopian tubes and ovaries, taken from females in which signs of recent pregnancy were evident, were preserved. The study of some of these has led to the discovery of early blastocysts lying within the lumen of the fallopian tube. It is therefore highly probable that if a complete series of cleavage stages is to be had, it will be necessary to pursue the laborious method of making sections of the fallopian tubes from females showing signs of recent fertilization.

In this connection I should like to point out a possible source of error, and one that must be carefully guarded against. In sectioning the ovaries of females well started in pregnancy one occasionally finds undivided eggs in that part of the fallopian tube which is situated close to the ovary. Such cases are to be attributed to ovulations that have occurred after normal ovulation and fertilization have taken place. The fact that the nucleus in these eggs may undergo division does not signify anything of unique importance, since it must be regarded simply as an expression of the same tendency to parthenogenetic development which frequently is seen in matured ova still confined within the ovarian tissues of this animal.

In table 1 is given a list of all the free blastocysts which have been secured during the seasons of 1911 and 1912, including the two taken from the fallopian tubes. The first vertical column gives the catalogue number of the specimen, arranged chronologically; the second, the date at which the vesicle was taken; the third, the diameter of the vesicle in millimeters, measured in 70 per cent alcohol; the fourth, the ovary, right *R* or left *L*, from

which the egg came; the fifth, the age of the mother; the sixth, the number of the figure, in case the specimen is illustrated in the paper; the seventh, remarks. Unless otherwise stated, under remarks, the vesicle was taken from the right to left horizontal groove of the uterine cavity. In most instances differences in size indicate differences in the degree of differentiation among the several blastocysts, although there is some variation in vesicles showing corresponding differentiations.

The table brings out several interesting points, and to those that will not be specifically treated in subsequent sections we must direct a few remarks.

In twenty-nine of the thirty-two cases listed the ovary from which the egg came was determined; and the record shows that fourteen were derived from the right ovary and fifteen from the left, thus indicating that there is no tendency for one ovary to function more frequently than the other. In two of the remaining cases I failed to make a record on this point, but in the third (No. 297) both ovaries were enlarged. This would indicate that occasionally the two ovaries function simultaneously, although in this particular case I was not able to find a second egg. There is of course the possibility that the one ovary gave off an egg which failed to become implanted, and that a second ovulation, involving the other ovary, followed immediately.

It was stated above that the 'old' females, that is females which have previously borne young, breed first, and that the second year virgin females come on later. The table brings these facts out clearly. Thus, of the eighteen cases with complete records, which were taken in October, fourteen (Nos. 230, 287, 288, 291, 292, 295, 297, 300, 304, 305, 309, 320, 325, 326) were from old females, while only three were from virgin females, that is females that had never before borne young. Of the eleven cases involving second year females, eight vesicles (Nos. 243, 244, 245, 251, 258, 259, 261, 335) were taken between November first and eighth; while two of the remaining three were secured late in the month of October, both on the 26th (Nos. 311, 315). Finally, of the three vesicles obtained from the first year females, that is females that had been born during the previous winter or spring, two were taken

TABLE 1

Free embryonic vesicles

NO. OF SPECIMEN	DATE	SIZE IN MM.	OVARY	AGE OF MOTHER	FIGURES	REMARKS
230	10-30-11	0.319	L	Old		
242	11- 1-11	0.307	R	Old		
243	11- 1-11	0.495	?	2d year		
244	11- 1-11	0.263	L	2d year	9	From left fal. tube
245	11- 3-11	0.324	R	2d year		
249	11- 3-11	0.429	R	Old	14,41	Lying free on placental area
251	11- 3-11	0.420	L	2d year		
253	11- 3-11	0.306	R	Old		
258	11- 8-11	0.384	L	2d year		
259	11- 8-11	?	L	2d year		
261	11- 8-11	?	R	2d year		
268	11-14-11	0.330	L	1st year		
284	12-12-11	?	?	1st year		
287	10-15-12	0.312	L	Old	6	
288	10-15-12	?	L	Old		
291	10-18-12	0.404	R	Old		Lying free on placental area
292	10-18-12	0.480	L	Old		Lying free on placental area
295	10-18-12	0.380	R	Old		
296	10-18-12	0.376	L	2d year	10,37	From left fal. tube
297	10-19-12	?	R=L	Old		
300	10-19-12	0.656	L	Old	12,42	Lying free on placental area
304	10-22-12	?	R	Old		
305	10-22-12	0.348	L	Old		
309	10-26-12	0.432	L	Old		
310	10-26-12	0.360	R	?	7,36	
311	10-26-12	0.504	L	2d year	11,47	Lying free on placental area
315	10-26-12	0.380	R	2d year		
318	10-26-12	0.336	R	1st year		
320	10-28-12	0.387	L	Old	13,39,40	
325	10-28-12	0.290	R	Old		
326	10-28-12	0.339	R	Old		
335	11- 2-12	0.220	R	2d year	8,38	

very late in the breeding season, one (No. 268) on November 14, and the other (No. 284) on December 12.

In table 2 are listed all the early attached or implanted vesicles which are directly referred to in the subsequent parts of the paper. They are arranged, so far as possible, in the order of their degree of development. Vesicle No. 311 of the preceding table should probably be included in this list, and No. 300 should almost certainly be included. They were both lying free upon the placental area when first observed, and for that reason are included in the list of unattached specimens; but inasmuch as the early attached vesicles are so easily dislodged, it might well be that these two vesicles were loosened during the process of everting the uterus. Their size and structure most certainly point to this conclusion.

In determining the size of those vesicles which had gained a firm attachment to the mucosa it was found best to measure the base of the vesicle, as this gave the most trustworthy results. In everting the uterus, however, the mucosa of the placental area may become slightly stretched or otherwise distorted, thus spreading the base of the vesicle, and consequently giving a measurement that is abnormally large. This is the case in No. 256. Furthermore, all of the vesicles listed beyond No. 332 were measured from the microscopic preparations, and consequently after the shrinking effects of dehydration, clearing, and imbedding had come in. The sizes indicated for these vesicles are too small as compared with those listed above No. 332 (cf. 233 and 307).

DEVELOPMENT OF THE BLASTOCYST

1. The monodermic blastocyst

The transformation from the so-called morula stage to the hollow sphere, or 'monodermic blastocyst,' has not been observed in the armadillo. The youngest normal stage which I have at my disposal already shows the finished product of this transformation. However, I possess representative stages throughout the period of development which extends from this point on until the 'didermic blastocyst' is reached. In the present section we shall describe three blastocysts, which, although all of the monodermic

TABLE 2

Attached embryonic vesicles

NO. OF SPECIMEN	DATE	SIZE IN MM.	OVARY	AGE OF MOTHER	FIGURES	REMARKS
307	10–22–12	0.516	L	2d year		Came off after 2 minutes in fixing fluid
328	10–28–12	0.468	R	Old		Came off immediately in fixing fluid
339	11–15–12	0.425	L			Came off after 2–3 mins. in fixing fluid
340	11–15–12	0.438	L	2d year	15,48	Came off immediately in fixing fluid
316	10–26–12	0.449	L	Old	16,31 43–46	
332	11– 2–12	0.500	L	2d year	17,49,50	Measurement across widest points
233	10–30–11	0.340	R	Old	18,32,51 52	
329	11– 2–12	0.312	R	Old	53	
289	10–15–12	0.336	L	Old	54,55	
298	10–19–12	0.440	L	Old	56,57	
234	11– 1–11	0.598	R	Old	19,33,58	
256	11– 4–11	0.944	L	Old	20,59	
247	11– 3–11	0.722	R	Old	1,21,22 34,60,61	
255	11– 4–11	1.000	L	2d year		
290	10–15–12	1.490	R	Old	2,23–26 62–65	
175	11–14–10	0.931	R	Old	27–29,35 66–69	
257	11– 4–11	0.909	L	2d year	3,30 70–74	
170	11–18–11	1.533	L	2d year	4,75–77	
226	10–30–11	1.367	L	Old	80–84	
276	11–21–11	3 x 5	L	2d year	5,78	
216	1–11–11	90	L	Old	79	Advanced stage, containing embryos 10 cm. long.

type, show interesting and significant differences. These will be described in the order of their development.

Blastocyst No. 287. This specimen measures 0.312 mm. in diameter, and is therefore larger than several others, but in point of development it is the youngest specimen in the collection. The inner cell-mass appeared both in the living and in the preserved condition as an opaque spot, the inner surface of which showed many elevations, caused by the protruding embryonic cells. In section the mass is seen to be made up of embryonic cells with relatively large, distinct nuclei (fig. 6). For the most part each cell is delimited by a cell wall; but here and there may be found one in which part of the wall has disappeared, or at least the membrane can not be made out with certainty. Another point of interest is the fact that there is a considerable difference in the size of the nuclei. Thus of the eleven nuclei shown in the median section (fig. 6) six are decidedly larger than the remaining five. I am unable to detect any difference in the staining properties of these two types of nuclei, although the cytoplasm of some of the cells which possess the larger nucleus takes a much paler tint than that of the cells with the small nucleus.

The embryonic knob measures 0.026 mm. deep by 0.055 mm. wide. If we regard the trophoblast of the median section of the blastocyst as the circumference of a circle whose diameter is equal to the diameter of the blastocyst, and using the width of the inner cell-mass as a chord, then we may express the size of the mass in the terms of degrees and minutes, as measured on the circumference by the subtended arc. In this particular specimen the diameter was 0.312 mm., and since the diameter of the inner cell-mass was 0.055 mm., it covered an arc on the circumference equal to 20° 18′. The embryonic mass is composed of about 75 cells, as determined by a count of the number of nuclei. They are tightly pressed up against the trophoblast, from which, however, they are sharply cut off by the under surfaces of the trophoblastic cells.

Blastocyst No. 310 measured 0.360 mm. in diameter, and is therefore larger than the preceding, and that it is also further developed is evidenced by the fact that there are 136 cells in the embryonic

knob. In the whole condition the knob appeared as a conical-like elevation extending up from the trophoblast. The surface of the mass was uneven, due to the projecting embryonic cells (fig. 36).

In detail, the embryonic knob is composed of a central core of protoplasm surrounded by nuclei (fig. 7). Some of the nuclei lying towards the free surface of the mass are completely delimited by the cell-membranes, but in the majority of cases that portion of the cell membrane which is directed towards the center of the mass has faded out. Some of these incomplete cells have two nuclei. In the region of the trophoblast the cells have lost all traces of membranes, but Rauber's portion of the trophoblast retains its distinctiveness from the underlying embryonic mass.

While the embryonic knob of the preceding blastocyst presented when viewed from above, a perfectly sharp contour, the knob of this specimen, on the contrary, showed many ray-like protusions extending out from the base of the cone along the trophoblast. Thus the picture revealed in the upper view of such a mass is that of a many pointed star. The spreading of the inner cell-mass would seem to be accomplished by the migration of the cells from the base. They creep out with pseudopod-like processes, and can frequently be seen in the sections. On the left side of the median section (fig. 7) one of these processes is seen pushing out along the under side of the trophoblast, and on the right of the same section another cell is in the act of beginning a similar migration. In consequence of this spreading, the diameter of the embryonic mass has increased to 0.090 mm., as against 0.055 mm. in blastocyst No. 287; and the arc on the circumference covered by it is 28°, 57′.

Blastocyst No. 335. In many respects this is one of the most interesting of all the early blastocists in the collection. It is easily the smallest, since it measures but 0.220 mm. in diameter; and as compared with No. 310, is less than two-thirds the size. Nevertheless, the differentiation of the embryonic knob is distinctly more advanced than that of the preceding blastocyst. What explanation are we to offer then for this apparent disparity in size? Is this variation in size of the vesicles to be correlated with a difference in the size of the undivided egg? I think not.

The explanation is rather to be sought in the condition of the trophoblast. If the median sections of blastocysts Nos. 310 and 335, which have been photographed at the same magnification (figs. 36, 38) be compared, it is at once evident that, while the trophoblastic cells in 335 are unchanged, remaining relatively thick, as they must have been at the close of cell division, those of 310 are very much attenuated. The nuclei have become widely separated by the stretching of the wall, which in turn has become so thin in places that it appears as a delicate line in the photograph. Evidently this enormous increase of the blastocyst is the result of the accumulation of fluid within the cavity of the vesicle, and the consequent stretching of the wall. It is a fact worthy of note that one never finds the trophoblastic cells undergoing division while the blastocyst is free within the uterine cavity. The conclusion to which we must come is that the accumulation of fluid in the cavity of the vesicle must, at least in some cases, go on quite independently of the differentiation of the embryonic mass.

Turning to the detailed structure of the embryonic region one finds that the inner cell-mass is flattened out until it has become a lens-shaped structure measuring 0.024 by 0.115 mm., and covering an arc on the circumference equal to 63°, 1′. There has also been some increase in the number of embryonic cells, the knob now showing 166 cells.

The point of greatest significance and interest concerns the two types of cells of which the embryonic mass is composed. In the two preceding blastocysts there were two sizes of nuclei observable and here not only does this same disparity still exist, but there is also a corresponding difference in the cells themselves. Thus there are clearly two types of cells, a large cell in which the nucleus is large, and a smaller cell with a comparatively small nucleus. In this particular specimen the small cells have a general distribution throughout the mass, and appear either singly, or in groups of two or more. The ratio of the small cells to the larger is about as one is to three. Thus in the median section there are fourteen large cells and five smaller ones. The latter are in three groups: a single cell lying on the surface at the extreme left (fig. 8 *a*), a pair situated some distance to the right of this (*b* and *c*), and a second pair

lying about one-third the distance from the right end of the section (*d* and *e*). In the first pair one of the cells borders on the under surface of the mass, while the other is wedged in between two large cells. In the other pair one of the components also comes to the under surface and the other is in contact with the trophoblastic cells.

I interpret the condition here to mean that the nuclei of the small cells are identical with the smaller nuclei of the preceding stages, or else the progeny of such nuclei.

The size relation of the different cells is not the only evidence which indicates that the embryonic mass is gradually differentiating into two types of cells; for the small cells have begun to take on a slightly deeper tint of stain than do their larger fellows, and this difference in the staining capacity of the two types of cells becomes more and more evident as development progresses, until finally it becomes one of the most striking features of the armadillo blastocyst.

2. The didermic stage

The changes which we have just observed forshadow the transformation of the monodermic blastocyst into one of the didermic type; that is to say, the differentiation of the inner cell-mass into its two primary components, the embryonic ectoderm and entoderm. We have seen that the inner cell-mass gradually differentiates into two rather distinct types of cells, which differ from each other both in size and in staining properties. The bulk of the mass is composed of large, faintly staining cells which are more numerous in that part of the mass which is situated towards the trophoblast. The other type of cell is much smaller than the preceding, takes the stain much more readily, and has a sharply defined outline. These smaller cells are at first evenly distributed among the larger (except in a few cases to be noted later), but later become collected toward that side of the inner cell-mass which borders on the cavity of the vesicle. Subsequently they become split off from the lower side of the mass to form the entoderm. In speaking of these cells in my preliminary paper I make the following statement.

There are, however, two distinct types of cells. The bulk of the mass consists of rather large, ill-defined cells, the cytoplasmic and nuclear portions of which do not stain well. Scattered among these are smaller cells, which are found much more abundantly in that part of the mass lying towards the cavity of the vesicle. The smaller cells are characterized by their sharply defined outlines and by the ease with which they take the stain. There is considerable evidence to indicate that the smaller cells are gradually undergoing a segregation from their larger fellows to form the hypoblastic layer of the vesicle. At any rate the hypoblast upon its completion possesses cellular elements that very closely simulate the smaller cells of the earlier stages.[3]

This statement was made before I had had an opportunity of examining Hill's ('10) excellent paper on the early development of the 'native cat,' Dasyurus viverrinus. In this paper Hill shows in the clearest possible way that the entoderm arises in a manner quite similar to that in the blastocyst of Tatusia, and I shall therefore briefly state his general results on the point under discussion. It will be recalled that in the 16-cell stage the blastomeres are arranged in two superimposed rings of eight cells each. The eight upper cells, which are smaller than the eight lower, are destined to produce the formative or embryonal region of the blastocyst wall, while the eight lower cells will give rise to the non-formative or extra-embryonal region. In accordance with the characteristic mode of development in the marsupial, no morula stage is formed in the egg of Dasyurus, but the blastomeres proceed directly to form the wall of the blastocyst. This is brought about through the division of the blastomeres of each ring and their gradual spreading toward the opposite poles, on contact with the inner surface of the sphere formed by the zona and the shell-membrane. The daughter blastomeres continue to divide and eventually produce a complete cellular lining to the zona sphere, constituting the unilaminar wall of the blastocyst. The wall remains in this unilaminar condition until the blastocyst attains a diameter of 4 to 5 mm.

Hill here draws the most fundamental conclusions of his paper, pointing out that the formative or embryonal region, which from the first possesses no covering of trophoblast (i.e., Rauber's layer),

[3] Loc. cit., pp. 369–370.

is the homologue of the inner cell-mass of the eutherian blastocyst; and that the non-formative region is the homologue of the hypoblast of the eutheria and of the extra-embryonal ectoderm of the sauropsida and monotremata.

After the blastocyst has reached a diameter of from 4 to 5 mm., two distinct varieties of cells can be recognized in the unilaminar wall of the formative region. Hill regards this as the crucial stage in the formation of the primary germ layers, as it marks the transition from the unilaminar to the bilaminar condition. We may quote from Hill's summary such paragraphs as give a résumé of his account of the development of the entoderm.

> The formative region, unlike the non-formative, is constituted by cells of two varieties, viz.: (i) a more numerous series of larger, lighter-staining cells destined to form the embryonal ectoderm, and (ii) a less numerous series of smaller, more granular, and more deeply staining cells, destined to give origin to the entoderm and hence distinguishable as the entodermal mother-cells.
>
> The entodermal mother-cells, either without or subsequent to division, bodily migrate inwards from amongst the larger cells of the unilaminar wall and so come to lie in contact with the inner surface of the latter. They thus give origin to the primitive entodermal cells from which the definitive entoderm arises. The larger passive cells, which alone form the unilaminar wall after the inward migration of the entodermal cells is completed, constitute the embryonal ectoderm.
>
> The entodermal cells as well before as after their migration from the unilaminar wall are capable of exhibiting amoeboid activity and of emitting pseudopodial processes, by the anastomosing of which there is eventually formed a cellular entodermal reticulum underlying, and at first coextensive with, the embryonal ectoderm.
>
> The entoderm is first laid down below the formative or embryonal region of the blastocyst; thence it extends gradually by its own growth round the inner surface of the unilaminar non-formative region so as to form eventually a complete entodermal lining of the blastocyst cavity. In this way the blastocyst wall becomes bilaminar throughout.

Thus it will be seen that, contrary to the generally accepted view of mammalian embryologists, the entoderm of Dasyurus does not arise by delamination, but through an inward migration of differentiated entodermal mother-cells from among the ectoderm cells of the embryonic region. The independent discovery of a similar method of entodermal formation in the armadillo is of more than ordinary interest, and calls for a detailed account of the process in

this eutherian mammal. Before taking up this account, it should be pointed out that the blastocyst of Dasyurus presents an unusually favorable opportunity for the study of the origin of the entoderm. The large size of the vesicle, together with the unilaminar condition of its wall, makes possible the preparation of the embryonic region as whole mounts, by the means of which detailed observations can easily be made. The eutherian blastocyst, on the contrary, does not admit of satisfactory whole mount preparations, owing to the fact that the embryonic region is more than a cell thick. In the blastocyst of the armadillo this is particularly true, as the wall of the formative or embryonal region is about four cells thick. Nevertheless, the evidence gathered from the study of sections, which we shall now present, is convincingly in favor of the view that the entoderm arises by the means of migrating cells, and does not favor the commonly accepted idea that it arises by a delamination, that is, by the splitting off of the lower layer of cells from the embryonic knob.

Blastocyst No. 244 is next to the smallest blastocyst secured, measuring but 0.263 mm. in diameter, and, when taken, was on the point of entering the uterine cavity from the left fallopian tube. At this time it was distinctly spherical in outline, with an embryonic spot, having a slightly irregular margin and covering an arc 52° 58′. The specimen is described here, not only because it shows a continuation of the changes observed above, but also for the reason that it is remarkably well preserved, and consequently will give more than ordinary confidence to any interpretations which may be based upon its structures.

The embryonic spot, which measured 0.025 mm. deep by 0.100 mm. in diameter, was too thick to permit the determination of the details of structure from a study of the living specimen; but in the sections the details stand out brilliantly, and prove beyond a peradventure that there are two types of cells composing the embryonic mass. The entodermal cells, as in the case of specimen No. 335, are apparently quite generally distributed throughout the inner cell mass. The section passing through the center of the mass is typical in that it shows all of the essential features, and especially the relation which exists between the two elements con-

stituting the embryonic spot (fig. 9). There are six entodermal and thirteen ectodermal cells, or rather ectodermal nuclei, for in some cases two or more of these nuclei are included within a single cytoplasmic mass. No entodermal cells have been found which show more than one nucleus.

The entodermal cells in the section are located in three regions, similarly to those of the preceding figure. On the extreme left the single cell (fig. 9, *a*) is easily distinguishable from its adjacent, binucleated, ectodermal fellow. It gives evidence of beginning to spread out beneath the binucleated cell. To the right of this is a group of three entodermal cells (*b*, *c*, and *d*), one of which comes to the lower surface, while the other two lie one above the other, well within the mass. These cells are darker than the neighboring ectodermal cells, but the difference in the size of the nuclei that was so striking a feature of the preceding stage is here not so marked. In fact, the nuclei of some of the ectodermal cells are smaller than those of the entodermal ones. It must be kept in mind, however, that in these thin sections only the tip or at least a small portion of a large nucleus may be visible in a given section. A study of the preparation shows that on the average the entodermal nuclei are smaller than the nuclei of the ectodermal cells. The remaining entodermal cells constitute a pair situated about in the middle of the right half of the section (fig. 9, *e* and *f*). Both of these cells are on the surface of the mass. Their cytoplasmic and nuclear portions do not stain so deeply as in the other entodermal cells, but still more deeply than in the case of the ectodermal cells.

The affinity of the entodermal cells for the stains evidently lies in the nature of the protoplasm itself. In the ectodermal cells, both in the cytoplasm and nucleus, the structural configuration of the protoplasm is of a more open mesh-like character than that of the entodermal cells, in which it may assume a finely granular appearance.

The conditions observed in this and the other sections of the series is interpreted to mean that the more deeply staining cells are entodermal, all of which will eventually migrate to the lower surface of the embryonic mass to form the characteristic entodermal layer of the mammalian blastocyst. The cells marked *c*, *e*, and *f*

are in the act of migrating to the surface. The cells lying within the mass (*b* and *d*) are rounded in outline, while those in the act of coming to the lower surface are invariably elongated in the direction of migration.

The evidence obtained from a study of these sections indicates that each group of two or more entodermal cells has, in all probability, arisen from a single primary cell, or entodermal mother-cell (using Hill's terminology). Futhermore it suggests that the primary cell may, from the first, be situated on the surface; and consequently will undergo no migration; or it may migrate to the surface without having undergone division; or again, divisions may have come in before the migration occurs.

Blastocyst No. 296 came from the proximal part of the left fallopian tube, and measured 0.376 mm. in diameter. The embryonic spot had a very even outline and measured 0.161 mm. across. It is distinctly thicker than the last specimen, averaging about 4 cells, exclusive of the trophoblast (fig. 10). The arc covered by the embryonic spot is 50°, 42′.

In every way the blastocyst is more advanced than any we have so far described, It is not only larger, but also shows a higher state of differentiation. One feature in particular, although not entirely unique since it has been observed in one or two other cases, is nevertheless worthy of mention. This is the presence of faint structures on the outer surface of the trophoblast (fig. 37). These were observed in the preserved egg and were naturally taken to be the follicle cells, which sometimes adhere to the ovulated mammalian egg and persist for some time; but in section they are seen to be protrusions or exudations from the trophoblastic cells, and are probably formed at the time the egg is fixed.

The number of entodermal cells in the median section is thirteen as against sixteen ectodermal cells. This would seem to indicate that there has been a great increase of entodermal cells, but in several of the other sections they are very much less numerous, owing to the fact that the median section passes through the principal groups of entodermal cells.

The difference in size between the ectodermal and entodermal cells is very obvious (fig. 10), and, as compared with the preceding figures, stands in sharp contrast.

The distribution of the entodermal cells is interesting and instructive. Except for a single cell (fig. 10 *m*) the entire upper half of the embryonic mass is free from them; and certainly this suggests that these cells are gradually passing towards the lower surface. Already nine of the remaining twelve cells have reached the lower surface. The position of the twelve lower cells indicates that they probably came from five different sources (five entodermal mother-cells) as follows: cell *a* is in the act of migrating to the surface; cells *b–e* have had a common origin; likewise cells *i–k* have probably come from a single mother-cell; and finally, cell *l* gives evidence of once having occupied a position against the trophoblast between the two ectodermal cells situated farthest to the right in the section. It is now clearly in the act of migrating out from between these two cells.

I have already stated that cell *m* is the only entodermal element situated in the upper half of the mass. There is no way of determining whther this cell will also migrate to the lower surface. A pseudopodial-like process from its lower border is pushed in between two ectodermal cells, and suggests at least that it is about to move down. Furthermore, in the later stages, when the entoderm is completed as a distinct layer, no such cell as *m* is found within the ectoderm.

The cell which lies just above *e* has caused me considerable difficulty in attempting to determine to which of the two categories it belongs. Its relatively small, deeply staining nucleus closely resembles those of the entoderm, but its faintly tinted cytoplasm, together with its square-like outline, are sufficient to place it among the ectodermal cells.

A word here as to the changes occuring in the size and thickness of the inner cell-mass may be said. These changes can be seen by comparing figures 6 to 11. At first the inner cell-mass is composed of a group of spherical cells, but, as development progresses, the mass becomes flattened out against the trophoblast, until finally it forms a circular plate of cells about two deep. This period is then followed by one in which there is a distinct increase in the thickness of the mass, due evidently to the multiplication and growth of the cells. Growth and multiplication of the cells con-

tinues until the maximum thickness is reached in such specimens as No. 296 (fig. 10), when the embryonic spot again begins to spread and continues to increase in diameter until finally the mass is reduced to about two cells in depth (fig. 11).

This process of migration of the entodermal cells to the lower surface of the embryonic knob and their subsequent peripheral movement along the inner surface of the trophoblast must take place by amoeboid activity. Indeed, the evidence for this conclusion is irresistible. If one examine in the living condition a stage somewhat more advanced than No. 296, one finds that numerous pseudopod-like processes are radiating out in all directions from the embryonic spot. In many instances the connection of these processes with some of the outlying entodermal cells is clearly discernible.

In order that a photographic record of this phenomenon might be made, glycerin jelly mounts were prepared of several unstained blastocysts which exhibited it. A photograph of such a preparation is shown in figure 40. In the enlarged view of the embryonic spot of the same specimen (fig. 39), the pseudopodia are particularly clear and striking. The high power of the microscope was focused so as to bring the entoderm as sharply as possible into view and while the rather thick ectoderm of the central area obscured the true condition of the entoderm here, yet the peripheral portions are brought out sharply. A small area of these anastomosing processes from the lower border of the embryonic area is sketched in figure 13. Some of the pseudopodia have sharply pointed ends, others have rather blunt ends, and still others have flattened terminations. The processes from two or more cells frequently anastomose and form a fenestrated structure. In sections also the pseudopodia can be demonstrated, especially when the section happens to cut one of them lengthwise (fig. 14). However, it is from the study of the living material and glycerin jelly preparations that one obtains the most convincing evidence of these pseudopodia, and of the rôle they play in the formation and migration of the entoderm.

The segregation of the entodermal cells from the ectodermic mass is completed by the time the vesicle has attained a diameter

of about 0.430 mm. At this time the embryonic spot has not reached its maximum expansion, that is, it has not completely flattened out. Consequently in section the entoderm forms a slightly curved line, due to the bulging out of the mass of embryonic ectoderm (fig. 14). The ectodermal cells are large, relatively clear, and sharply cut off from the overlying trophoblastic cells. On the inner surface of the mass they are less sharply separated from the entodermal cells, which here and there send processes up between some of the bordering ectodermal cells. Such processes are undoubtedly the last remnants of the migrating entodermal cells to be withdrawn from the embryonic mass.

The condition of the entoderm is of much interest. It does not as yet form a complete sheet of cells underlying and coextensive with the embryonal ectoderm. In places the entoderm may be wanting for more than the width of a cell. Furthermore, on the right side of the sections of specimen No. 249 the entoderm is frequently wanting, indicating that it must have taken its origin from the left portion of the embryonal mass. In some of the earlier blastocysts this same fact was observed. The smaller, deeply staining cells, which give rise to the entoderm, were found to extend over not more than two-thirds of the embryonic mass. Finally, in the largest free blastocyst secured (No. 300) and one in which the entoderm is completely segregated, we find this same excentricity of the entoderm. It was so evident in this specimen that I have gone to the trouble of making a special preparation for the purpose of demonstration by a photograph. The vesicle in question was slightly stained in eosin and imbedded in paraffin. Under the high power of the binocular microscope the non-embryonic hemisphere was carefully pared away with a sharp razor. The remaining hemisphere was dissolved out of the paraffin, stained in hematoxylin, and mounted in balsam, with the cut surface uppermost. Thus it was possible to get an unobstructed view of the entoderm, and since the embryonic mass had become completely flattened, the entodermal layer lies in a single plane. The exposed sheet of entoderm was then studied and photographed (fig. 42).

The center of the embryonic spot lies about five millimeters below the center of the circular figure, and, since the entodermal cells are practically all in focus, it can be seen that, as a continuous layer, the entoderm covers only about the lower three-fourths of the embryonic spot. Over the other fourth only a very few entodermal cells are found, and these lie for the most part slightly to the left of the center. The other cells in this area which are slightly out of focus represent the exposed ectoderm, which is here only about one cell deep.

What does the excentric position of the entoderm mean? The observation of this phenomenon in some four or five blastocysts doubtless furnishes too meager evidence upon which to base any fundamental conclusion. Nevertheless one can not resist the temptation to suggest that we may have here a key to the much mooted question of gastrulation in eutherian mammals. For if it could be shown that the origin of the entoderm is confined to a definite area of the embryonic mass, the center of such an area might be regarded as corresponding to the region of a blastopore, regardless of whether or not this spot later became perforated by an actual opening or evanescent blastopore. Hubrecht ('02 '05 '08) has argued that the didermic stage of the mammalian blastocyst is to be regarded as a 'gastrula'. He further states that we must separate the phenomena of notogenesis from the phenomenon of gastrulation. He expresses himself very clearly and concisely on this point in the last of the three contributions mentioned above in which he makes the following statements:

> As soon as we separate the phenomena of notogenesis, such as we have found in all vertebrates—Amphioxus included—from the phenomenon of gastrulation, recognizing that the former follow upon the latter and bring about the formation of the notochord and the mesoblastic somites, the difficulties are considerably simplified.
>
> Gastrulation is thus terminated in the mammalia when the didermic stage of the embryonic shield has come into existance. We have seen that this takes place not in consequence of any process of invagination but by means of a most unmistakable delamination of the entoderm, out of the embryonic knob.
>
> This delamination gastrula of the mammalia generally enters upon the latter phases of ontogeny which will be described hereafter without the appearance of a distinct blastopore.[4]

[4] Loc. cit., p. 13.

Concerning the idea of separating the processes of gastrulation and notogenesis I believe that in the main we must agree with Hubrecht, although the close genetic continuity of these two processes must never be lost sight of, even in mammals, else the appearance of an evanescent blastopore in such forms as the hedgehog, Tarsius, rabbit, mole, shrew, and opossum would have little significance. But the unexpected discovery of migrating cells to form the entoderm in the blastocyst of the armadillo must deter us from speaking of the process of entoderm formation as one of delamination, for this term as used in mammalian embryology implies that the entoderm is differentiated from those cells of the inner cell-mass which happen to border on the cavity of the vesicle. So to regard the origin of the entoderm would be equivalent to accepting Driesch's ('93) aphorism; for it would amount to saying that the prospective value of one of these bordering cells "is a function of its position." Delamination is not therefore the correct term to employ in describing the mode of entoderm formation in the armadillo, for while it is true that the entoderm as a distinct layer is split off from the embryonic ectoderm, as we shall see later, yet prior to this so-called delamination there is an unmistakable migration of primary entodermal cells to the lower surface of the inner cell-mass, and this it seems to me is the fundamental step in the whole process of entoderm formation.

The final steps in the differentiation of the entoderm in the armadillo may be considered here, as we shall not have occasion again to refer to them. Following the conditions such as we have seen in figure 41 the entodermal cells spread out until they have formed a continuous sheet of cells beneath and coextensive with the embryonic ectoderm (fig. 47). During this change the entodermal cells become much flattened against the ectoderm. There is no evidence here, such as we have observed in the pseudopodia of earlier stages, to indicate that the entodermal cells at the margin of the embryonic area are pushing out further beneath the trophoblast. Even in the specimen shown in figure 42 the pseudopodia have practically all disappeared, except in the case of the upper marginal cells that border on the area of ectoderm still not covered by entoderm (fig. 12).

Measurements show that the entoderm has practically reached its maximum extension in such specimens as No. 311, in which the embryonic spot measures 0.020 mm. by 0.157 mm., and covers an arc on the circumference of 36°, 18′. It does not here reach much beyond the extreme limits of the ectoderm (fig. 11). This does not mean that the entoderm may not cover an actually great area, for it becomes attenuated through the expansion of the trophoblastic wall; but what is meant is that the entodermal cells do not push out any further along the trophoblastic wall. Consequently that part of the wall which lies beyond the limits of the embryonic area never becomes didermic, or bilaminar. Perhaps then it would be better to confine the use of the term didermic to the embryonic spot, and not apply it to the blastocyst as a whole.

It may not be amiss in conclusion to draw attention again to the very close similarity between the mode of origin of the entoderm in the armadillo and that of Dasyurus, as given by Hill. The similarity is indeed striking, especially if one consider the fundamental differences in the character of the walls of the two types of blastocysts. The thin wall of the formative region of the blastocyst of Dasyurus stands in sharp contrast to its homologue, the relatively thick inner cell-mass of the armadillo blastocyst, and yet in all essential features their modes of entoderm formation are practically identical.

ATTACHMENT OF THE BLASTOCYST

The implantation of the embryonic vesicle is a subject of much importance, and is treated elsewhere as fully as the material at hand warrants. At this point it is desirable to discuss briefly only the first step in implantation, or the attachment of the blastocyst.

A great deal of effort has been put forth to obtain the earliest attached stages, and, to date, four clear cases have been observed. Two other doubtful cases were seen. Unfortunately in each case the vesicle became detached from the mucosa upon placing the uterus in the fixing fluid or very shortly thereafter. In two of these the separation took place immediately, while in the other two from two to three minutes elapsed between the immersion of the

uterus and the loosening of the vesicle. However, these stages were studied under the high and low powers of the binocular miscroscope before the uterus had been placed in the fixing fluid, and it was therefore possible to make out most of the details of structure.

In general appearance these vesicles are not unlike the largest of those which were found lying free within the uterine cavity. The large polygonal trophoblastic cells are clearly discernible, and the embryonic area appears as a whitish spot lying directly in contact with the mucosa. In size, too, they are not larger than the more advanced free-vesicles.

These four vesicles were sectioned for microscopic examination, and in three of them a detailed study reveals nothing different from what we have already seen in the free stages; but in the fourth, which was one of the two that remained attached for the longest period, an important difference was observed. The change to which I refer involves the most essential part of the vesicle, the embryonic ectoderm, and consists of a thickening of that structure (fig. 48). It is certain that this increase is not due alone to a multiplication of cells, since mitotic figures are rarely found, but to a distinct rounding up of the entire embryonic ectoderm. In fact this change is the beginning of a process that will eventually transform the lens-shaped ectodermal mass into a ball-like structure.

In the study of these four vesicles particular attention has been paid to the area of trophoblast (Rauber's layer) which directly overlies the embryonic ectoderm, and which forms the seat of attachment. The trophoblastic cells of this area do not as yet betray any evident changes looking to the formation of the 'Träger.' We must conclude therefore that for a short time at least the vesicle is held to the mucosa by adhesion. It would seem that it simply 'sticks' to the uterine lining. Nor is there any evidence to show that there is a localized area within the attachment zone of the fundus to which the vesicle migrates before becoming attached. Any spot on the entire attachment zone may furnish a foothold for the vesicle, if one may judge from the collected date on the distribution of about twenty young attached stages. Apparently the vesicle adheres to the mucosa very soon after it

passes from the horizontal groove to the attachment zone; for it is almost invariably the rule that the vesicle is located on that half of the zone lying adjacent to the groove along which it had passed from the fallopian tube. Thus, in a pregnancy in which the left ovary holds the corpus luteum, the vesicle will be found to be attached at some spot on the left side of the attachment zone.

FORMATION OF THE ECTODERMIC VESICLE

In the preceding paragraphs we have referred to the change involving the embryonic ectoderm which takes place shortly after the vesicle becomes attached to the mucosa. This change is perhaps anticipated in the more advanced of the free blastocysts in which a few divisions of the ectodermal cells are taking place (fig. 47); but evidently does not express itself clearly until after the egg becomes quite well anchored to the uterine wall. As already intimated, the change consists in the transformation of the flat, circular plate of ectodermal cells into a spherical or ball-like mass (fig. 15).

The entoderm in this stage is recognized as a distinct layer, and, although no longer connected with the ectodermic mass by protoplasmic strands, yet is still in close contact with it. The entodermal cells which lie directly beneath the ectoderm become distinctly thicker than before, and accompanying this change is the appearance of mitoses (fig. 15).

The entodermal cells which lie beyond the limits of the embryonic ectoderm, differ from those lying beneath the ectoderm in two important respects. First, the nuclei of the cells remain small, thus indicating that the cells are not in an active state of division; and second, these cells are flattened out against the trophoblast, with which they are in very close contact. These cells form a narrow ring or annular zone around the margin of the ectoderm. This zone is of especial interest because it forms the axis about which the so-called inversion of germ layers revolves.

The transformation of the plate of ectoderm into a spherical mass results eventually in its entire separation from the trophoblast and its inclusion within an entodermal sac, thus leaving a

cavity between the Träger and the ectoderm. Material with which to follow the steps through which the blastocyst passes during this is not at hand, for there is here a slight gap in the series. However, I have been fortunate enough to obtain two blastocysts which show the condition immediately following the inclusion of the ectoderm. The two specimens are nearly of the same age, one being slightly more advanced than the other.

The younger blastocyst (No. 316) was found attached to a small leaf-like outgrowth from one of the folds of the placental mucosa. The outgrowth lay horizontal to the surface of the mucosa, and the vesicle had gained attachment to its under side, close to the edge. One side of the trophoblastic wall caved in during fixation, otherwise the vesicle is in an excellent state of preservation (fig. 16).

That portion of the trophoblastic wall which has caved in presents nothing different from what was observed in earlier stages, except that the cells are more attenuated, but the opposite wall of the vesicle has undergone a marked thickening, in addition to a great increase in its cellular elements. The cells have, therefore, changed in shape from a flattened squamous type to a distinctly columnar epithelium. All the thickened portion of the wall was originally in contact with the mucosa, but during the process of fixation the leaf-like fold of the mucosa has undergone a great deal of contraction and has shrunk away from the trophoblast, as is evident from its folded appearance. Evidently the thickened trophoblast owes its existence to contact with the mucosa, which in some way specifically stimulates the cells to a striking activity, as shown by the rate of division and change in shape. The specific reaction between the trophoblast and the mucosa must be limited to that portion of the uterine epithelium which covers the placental area, otherwise the wall of a free vesicle would give evidence of response before the blastocyst had reached this area.

It is also a point worthy of note that the columnar cells send out pseudopod-like processes, which eat into the mucosa, giving it a serrated appearance in section. That the placental trophoblast has therefore a specific action on the trophoblastic cells can

not be doubted, and this effect seems to be limited entirely to the region in direct contact.

The sections are cut somewhat obliquely, and hence the section passing through the middle of the embryonic ectoderm does not cut the region of the primitive placenta, or point of attachment (fig. 16). Consequently the exact relation of the entoderm to the other parts of the blastocyst can only be made out by referring to several of the sections of the series. In plate 2 is shown a series of four photographs which will demonstrate this relation.

In figure 43, which represents the section from which the drawing was made (fig. 16), the entoderm is a well organized layer passing around the sphere of ectoderm. On approaching the lower side of the ectoderm the entoderm at each side passes laterally and upwards to join on to the inner surface of the trophoblastic wall (fig. 16, *x*). The layer of entoderm lying directly beneath the ectoderm belongs to that portion which connects the entodermal sac with the left wall of the trophoblast. A few sections to the right in the series the lower layer of entoderm disappears, except a small group of cells (fig. 44). In this section the entoderm, on leaving the ectoderm, passes outwards and upwards as before, to join the inner surface of the trophoblast. Aside from the group of entodermal cells lying directly beneath it, the embryonic ectoderm is open below to a cavity which is bounded beneath by the placental portion of the trophoblast, and which represents what is later to become an extraembryonic cavity. In sections still farther to the right, at a point where the section cuts just the tip of the ectoderm (fig. 45), the extraembryonic cavity is almost free from cells of any kind. This section passes through the center of the Träger (fig. 31). Beyond the limits of the ectoderm (fig. 46) we see nothing but a line of entodermal cells, which represents the right lateral portion of the entodermal layer as it passes outwards to unite with the trophoblast on this side of the blastocyst.

To sum up: The conditions revealed in the series of photographs shows that the ectoderm, upon assuming a spherical form pushes up into the cavity of the blastocyst, carrying before it the well established layer of entoderm, and creating behind a cavity which gives rise to the extraembryonic cavity, and which will

subsequently become lined with a layer of mesoderm. The condition here presented reminds one somewhat of that found in a corresponding stage of the blastocyst of Pteropus edulis (Selenka and Göhre '92), except that in the case of the latter the entoderm continues around the inside of the trophoblast, forming a complete, inner layer to the blastocyst.

In the older of the two blastocysts (No. 332) the sections pass exactly parallel to the median axis of the vesicle, and consequently the relation of the different parts of the embryo is clearer than in No. 316. The general conditions of the trophoblast are much the same in the two specimens, except that at one point on No. 332 there is a knot of cells (fig. 17, *k*) which, in the living condition, fitted into a corresponding crypt in the mucosa. In later stages we shall see further evidence of similar knots, which represent points on the trophoblast that have been specifically stimulated to cell proliferation.

The relation of the entoderm to the embryonic ectoderm is remarkably clear in this preparation (fig. 17). In contrast with the preceding blastocyst, the entoderm of this specimen has undergone one important change, in that it has folded in beneath the ectoderm, forming all but a closed entodermal sac. Only a small pore-like opening (fig. 17) remains to place the extraembryonic cavity in communication with the cavity of the entodermal sac. On the right-hand side (or lower side, owing to the inclination to the right of the blastocyst) a fusion has taken place between the two layers of the entoderm and a portion of the Träger; but this fusion, especially with the Träger, covers a very small area, as it no longer exists in the sections a short distance to either side of this one. The loop of entoderm which lies between the fused area and the pore (fig. 49) is probably comparable to the group of cells situated in a similar position in the other specimen. The loop of cells is especially clear in figure 17.

Returning to a fuller consideration of the ectodermal sphere, we see that even in so young a stage as that of No. 316 it is no longer a solid mass of cells, as must have been the case at first, but in the central portion there are three relatively large and distinct besides several smaller, less distinct vacuoles. In fact, the

entire core of the ectodermal sphere becomes honeycombed by these vacuoles, which later unite. In blastocyst No. 332 the union of the vacuoles has already made considerable progress (figs. 49, 50). Eventually there is produced a large distinct cavity within the ectodermal mass, thus transforming it into a true vesicle.

I obtained one vesicle which clearly represents a further advance in the progress of vacuolization, and yet one in which the completed stage of the vesicle has not been attained. Unfortunately the specimen became slightly crushed in the course of transportation from the field to the laboratory, after it had been fixed and partially hardened. I therefore deem it unsafe to base any definite conclusions upon its structures; but simply give a photograph of one of the sections (fig. 53), which, in a measure at least can be understood after we have considered a normal specimen of a little later stage.

In figure 51 is shown a section of a stage at the completion of the ectodermic vesicle. The specimen consists of the following structures: (1) an outer layer of trophoblast, which on the lower side has become modified into the primitive placenta; (2) an incomplete entodermal sac which is connected laterally with the trophoblast; and (3) an ectodermic vesicle.

The trophoblast is composed of a single layer on the upper or free surface (fig. 18), but towards the base it thickens into two layers of cells, especially on the right side of the figure. The trophoblast, now in contact with the mucosa, has greatly extended its area, as compared with that of specimens Nos. 316 and 332. Since it is intended to devote an entire chapter to the subject of placentation, we shall not give here any further consideration to the lower layer of trophoblast, which is of course concerned with placenta formation.

The entodermal sac is, as already stated, incomplete, remaining open on the side turned towards the mucosa. The cells at the point where the layer turns out to join the trophoblast are relatively thick, while those of that portion of the entoderm which passes over the ectodermal vesicle have undergone no important change. In the extraembryonic cavity are found a few scatter-

ed cells which are, for the most part, of entodermal origin, but which will soon disappear.

The entodermic vesicle is completely developed. Its upper or embryonic side is two or three cells thick, while its lower side is but a single cell deep. On this side is a small opening or pore which places the amniotic cavity in communication with the extraembryonic cavity. This pore is found in but four sections, and no other vesicle shows it, thus leading one to suspect that its existence is more or less accidental, due to the manner in which the vacuolization took place. In blastocyst No. 332 the vacuolization occurs towards that side of the ectodermal mass which is nearest to the mucosa, and in the present specimen we may suppose that the excentric position of the vacuolization has resulted in perforating the lower side of the vesicle.

There is, of course, another very plausible explanation, namely, that the opening appears in all of the vesicles, but soon closes thus accounting for the fact that it is never seen in the older stages. This view receives support from the work of Fernandez ('09) on Mulita. The youngest stage secured by Fernandez is about in the same state of development as this specimen of the Texas armadillo, and presents the same structural relations. Fernandez states that the cavity of the ectodermal vesicle and what he calls the 'Träger cavity' are connected by a small pore which he compares to the 'Verbindungsröhre' in the mouse (Melissinos '07). His 'Träger cavity' represents the same space that I have called the extraembryonic cavity in T. novemcincta. I have so named this cavity because later, when it becomes lined with mesoderm, it is recognized as a true exocoelome. It should be pointed out here that what Fernandez terms the Träger cavity in his second youngest state (Fernandez '09, text figure 2) I have regarded as an artifact (for reasons to be presented later) and consequently it can not be compared with the similarly named cavity of his youngest stage.

In this mode of producing a vesicle of the ectodermal sphere is to be recognized an amnion formation through a process of vacuolization; for the entire subsequent history of development demonstrates that the cavity thus formed is an amniotic cavity.

The cavity has been previously termed the 'common amniotic cavity' (Fernandez '09, and Newman and Patterson '10), because it is common to the amniotic connections of the four embryos which later take their origin from the ectodermal vesicle.

It is evident from this that in the mode of amnion formation the armadillo is to be classed with that group of mammals in which the amniotic cavity is from the first an enclosed space, and never has a free communication with the space outside the trophoblast. The cavity is always intra-trophoblastic (Hubrecht '08), and consequently no folds ever arise to deliminate it, for this is not necessary. The armadillo can therefore be added to Hubrecht's ('08) compiled list of mammals, in which the amniotic cavity arises within the ectoderm and remains a closed vesicle. His list is as follows: "Cavia and other rodents, Pteropus, Galeopithecus, Erinaceus, Gymnura, monkeys and man.[5]

ORIGIN OF THE EXTRAEMBRYONIC MESODERM OR MESOTHELIUM

The origin of the mesoderm in mammals is a problem which has given rise to much difference of opinion among embryologists, but it is not necessary here to enter into this controversy. The first appearance of the mesoderm in the armadillo blastocyst is seen in connection with the cavity which lies between the ectodermal vesicle and the placenta, or what has been termed above, the extra-embryonic cavity.

The entoderm, at the angle where it parts company with the ectodermal sphere to join the trophoblast (fig. 51), gives rise to a few cells which frequently become scattered throughout the extra-embryonic cavity. There is good reason for believing that most of these cells undergo disintegration pari passu with the development of the ectodermal vesicle. At any rate there comes a time when the exocoelomic space is essentially free from such entodermal cells (fig. 54). In some of the sections of this series a few of these cells are still found within the cavity. Thus in figure 55 there are four of them, two lying some distance below the ectoderm and slightly to the left of the center, and two situated against the

[5] Loc. cit., p. 71.

under side of the ectodermal vesicle, at a point where the embryonic and amniotic portions of the vesicle meet on the left side. All such straggling entodermal cells show signs of decadence, and without doubt play no rôle in the development of the mesoderm.

The first evidence of mesoderm formation is also found in this same vesicle, and is indicated by the beginning of a process of proliferation involving the ectodermal cells which lie at the point or angle where the entoderm parts from the ectodermal vesicle. In figure 55 one of the proliferated cells, which has just been set free, is seen on the right, slightly removed from the angle. Only a very few such cells are found about the edge of the vesicle, but the presence of mitotic figures in this general region of the ectoderm indicates the approach of a rather profuse proliferation of mesodermal cells.

In another vesicle, somewhat larger than the preceding, the formation of the mesoderm has made rapid progress. The cells are proliferated in clusters, and soon develop into small vesicular structures (figs. 56, 57). In this particular specimen there are about eight small mesodermal vesicles, but it was not possible to determine whether their origin was confined to one or more localized regions of the ectodermal vesicle, or centers of active proliferation. The preparation shows every stage in the formation of vesicles. In most instances the cluster of cells is set free from the ectoderm before a cavity appears within their midst; in others, the cavity arises while the cells still retain a connection with the ectoderm. In all cases the smaller mesodermal vesicles gradually fuse together to form larger and larger cavities, until finally the entire space lying below the ectoderm is lined with a mesodermal layer.

While I was unable to determine any definite localized regions of mesoderm proliferation in this specimen, in all the older stages the mesodermal vesicles fuse in such a way as to produce two main vesicles. For example, specimen No. 234 has two well differentiated mesodermal vesicles, which are unequal in size (fig. 19). The larger one on the left-hand side is composed of a typical mesodermal layer. In this region it is free from any connection with the ectodermal vesicle, but in other sections it not only has such connections, but is also united to other smaller mesodermal vesi-

cles, with which it is undergoing fusion. The smaller vesicle is similar in its general structure to the larger one, but retains a distinct conection with the ectodermal vesicle.

The relation of the mesodermal vesicle to the rest of the chorionic vesicle is of great interest, and can be illustrated by explaining the manner in which the sections have been cut. In each of the specimens represented in figures 51 to 61 the sections have been cut so that their plane is parallel to the plane passing through the 'horizontal grooves' of the uterus and perpendicular to the surface of the mucosa (fig. 21). We thus see that the right and left mesodermal vesicles lie on those sides of the blastocyst which are turned towards the right and left openings of the fallopian tubes, respectively.

In figure 19 the mesodermal vesicles lie to the right and left of the center of the blastocyst, and, as we shall see later, hold the same orientation as do the two primary buds of the embryos.

Figure 59 shows a median section of a vesicle in which the two mesodermal vesicles have expanded until they occupy the entire extraembryonic cavity; but the double partition made by the approach of their adjacent sides does not allow a communication of the two cavities (fig. 20). However, this condition does not exist throughout the entire series of sections, for in many places a portion of the partition has already broken down. Eventually it will entirely disappear. This final condition in the formation of the extraembryonic mesoderm is soon reached, and the single layer of flattened mesodermal cells then completely lines the space below the ectoderm, conforming to the various irregularities of its bounding walls (fig. 22). The new cavity thus formed and lined with the mesodermal epithelium may now be called the extraembryonic coelome.

COMPARISON WITH OTHER FORMS

At this point it is well to compare briefly the armadillo blastocyst with that of other forms. The early development of the armadillo parallels most closely that of the mammals in which the so-called inversion of germ layers is found. Fernandez ('09) has pointed out that certain stages of the South American armadillo

Mulita are comparable to corresponding stages of the mouse, as figured by Melissinos ('07); and, while a similar comparison may be made between the mouse and the Texas armadillo, nevertheless, a closer similarity exists between the early stages of the frugiverous bat Pteropus and this armadillo. Thus one of the youngest stages of Pteropus figured by Selenka and Göhre ('92 pl. 41, fig. 4) is strikingly like the blastocyst shown in figure 17 of this paper; for in each case the embryonic ectoderm has separated from the trophoblast to form a spherical mass, which has become included within the entoderm.

The principal feature in which they are dissimilar is seen in the extension of the entoderm. In Pteropus the entoderm completely lines the cavity of the blastocyst, forming an epithelial lining for the yolk-sac. In the armadillo the entoderm extends out along the inner side of the trophoblast for only a short distance from the ectoderm, and at most never covers an area of over 80° on the circumference of the blastocyst. Consequently a closed epithelial sac of entoderm is not formed, and the yolk-sac cavity is bounded on the non-embryonic side by a single layer of trophoblastic cells, or chorionic ectoderm (fig. 19).

The similarity between Pteropus and the Texas armadillo is not confined to this early period, but is also seen in later stages; especially is this true with reference to the formation of the amniotic cavity. In each, the solid sphere of ectoderm becomes hollowed out through the disintegration or vacuolization of the core to form the primary amniotic cavity (*cf.* fig. 18 with fig. 6 of Selenka and Göhre). Finally, in the condition of the mesoderm the two forms show several points of similarity.

In the blastocysts of the mouse and of the armadillo are also to be seen many points of similarity, though the resemblance is here less striking than in the preceding case. The figures of Melissinos ('07) are very similar to several of the stages shown in this paper. His figure 31 shows a stage directly comparable to our specimen No. 311 (fig. 11); and his figures 33 and 34 illustrate the manner in which the embryonic ectoderm is pushed out into the general cavity of the blastocyst, carrying before it the visceral layer of entoderm. In the armadillo I have not succeeded in ob-

taining a stage which corresponds exactly to the stage of the mouse shown in figure 33 of Melissinos, although specimen No. 340 (fig. 15) may be compared with it. Figure 34 of Melissinos and my figure 19 can be directly compared, especially if it be kept in mind that the parietal layer of yolk-sac entoderm is incomplete in the armadillo. In the mouse the embryonic ectoderm is borne upon a mass of cells conecting it with the ectoplacental plate, and the separation of these two embryonic structures does not take place for some time. In the armadillo, on the contrary, the ectodermal mass early parts company with the Träger or ectoplacental region, thus giving rise to an extraembryonic cavity at a very early stage. It is this difference in the time of appearance of the extraembryonic cavity which renders difficult an exact comparison between the two forms throughout the subsequent history of development; and in support of this view we may cite the case of mesoderm formation.

We have seen that the mesoderm does not make its appearance in the armadillo blastocyst until after the extraembryonic cavity has arisen, and that upon arising from that portion of the ectodermal vesicle which is turned toward the placental region, it immediately develops into an epithelial lining membrane for this cavity, which is thereby transformed into a true extraembryonic coelome.

In the mouse, according to the account of Melissinos, the mesoderm is very early recognized as a mass of cells lying in a position somewhat similar to that of the mesoderm in the armadillo; that is, immediately ventral to the ectodermal mass, at the point of constriction between the embryonic ectoderm and the group of cells connecting it with the ectoplacental plate. Later, when the embryonic ectoderm and the ectoplacental plate become entirely separated, a cavity lined with mesoderm appears between these two embryonic structures. This cavity becomes then an extraembryonic body cavity (the 'mittlere Höhlung' of Melissinos). When this process is completed we are presented with a condition quite similar to that of a relatively late but corresponding stage of the armadillo (*cf.*, fig. 43 of Melissinos and my fig. 22). The essential difference lies in the fact that in the armadillo blastocyst there is no ectoplacental cavity; but even this difference later be-

comes lessened upon the formation in the armadillo of a distinct Träger epithelium, with a potential cavity lying between it and the invaded mucosa (fig. 23).

Other comparisons might be drawn between the armadillo blastocyst and those of other mammals, but this is not necessary. Sufficient evidence has been presented, I believe, to establish the fact that the early stages of the armadillo give a history corresponding in its general outlines to the development of an egg which in other forms produces but a single individual. The differences noted are no greater than would be expected to exist between mammals as widely separated as the armadillo and the bat or mouse.

ORIGIN OF PRIMARY BUDS

In the late stages of development it has been demonstrated (Newman and Patterson '10) that the four embryos of an armadillo litter are arranged within the single chorion in two pairs, which hold a very definite orientation with reference to the uterine axes. Thus it was found that one embryo always occupied that portion of the chorionic cavity lying adjacent to the dorsal wall of the uterus, one holds a ventral position, and the other two lie to the right and left sides, respectively. The heads of the embryos are always directed toward the cervix end of the uterus, and consequently point in a direction exactly opposite to that of the head of the mother. It was further found that the ventral embryo (I) is paired with the right-lateral (II) and that the dorsal (III) and left-lateral (IV) embryos are members of the other pair.[6] This relation was apparent, not only from the very close hereditary similarity existing between the two individuals of a pair, but also from certain foetal connections, notably the union of the amniotic canals of each pair in comparatively early stages.

The question now arises as to how such a striking relationship between the embryos has come into existance; and in seeking

[6] The terms 'right-lateral' and 'left-lateral' refer to the position of the embryos within the blastocyst, and not to the right and left sides respectively of the uterus. For example, the right-lateral embryo lies on the left side of the uterine cavity. Throughout the paper I use the Roman numerals (I to IV) to designate the embryos, so that the reader will find no difficulty in locating any embryo to which reference is made.

answer to this question we are at once confronted with the much more important problem of the origin of the multiple embryos from the single fertilized egg. In the paper cited above the position was taken that the embryos belonging to a pair were probably derived from one of the blastomeres of the two-celled stage, and that each embryo could therefore be looked upon as a lineal descendent of one of the blastomeres of the four-celled stage. In the present contribution the view is held that the four embryos do not owe their origin to a spontaneous blastotomy, but rather that they are the product of a form of agamogenesis belonging to the general category of budding.

It was considered then of the utmost importance to determine at just what point in the development of the armadillo blastocyst evidence of its quadruplicity first appeared. Consequently a sharp lookout was maintained in the study of all the early stages for signs of the first expression of polyembryony. The earliest observed evidence which could be interpreted as representing the beginning of multiple embryos comes in the formation of the mesothelium—not in the manner in which the elements of this layer arise, for localized centers of proliferation were not found, but in the early formation of the two large mesodermal vesicles through the fusion of several smaller ones. The development of two mesodermal vesicles would not in itself be so significant, as it might be merely an expression of a bilateral arrangement of mesoderm similar to that of many other vertebrate embryos, were it not for the fact that they hold a position corresponding exactly to the two primary ectodermal buds; that is, they lie on the sides of the vesicle which are directed towards the openings of the fallopian tubes. However, it may be that these two mesothelial vesicles have no general significance with reference to polyembryonic development, for it must be kept in mind that they have arisen by the fusion of numerous smaller vesicles, and later they in turn fuse to form a single vesicle.

Whatever may be the significance of the position of the two mesodermal vesicles, certain it is that the first indisputable evidence of the differentiation of the four embryos from the blasto-

cyst, appears in the formation of two diverticula from the ectodermal vesicle. In the preliminary paper these diverticula were termed the 'primary buds,' and I shall continue so to designate them. The buds appear on the opposite sides of the vesicle, and, with respect to the orientation of the blastocyst within the uterus, on the right and left sides, respectively, that is, on the sides of the vesicle that face the openings of the fallopian tubes. In accordance with the statement made above, the right primary bud faces the left fallopian tube opening, and similarly the left primary bud faces the right opening.

The primary buds do not develop for some time after the completion of the ectodermal vesicle, although their appearance is anticipated soon after this period by certain easily detectable changes in the walls of the vesicle. It will be recalled that immediately after the ectodermal sphere has become transformed into a vesicle, that portion of the wall of the vesicle which is turned toward the free pole of the blastocyst is of a relatively uniform thickness (fig. 51). Very shortly thereafter one can detect a tendency in this region of the wall to become less thick (figs. 55–58). The thinning out may be due in part to an increase in size of the vesicle by the accumulation of fluid within its cavity, but undoubtedly in the main it is brought about through the shifting of cells from here to the lateral portions of the wall, for these show an increase in thickness (fig. 59).

The shifting of the cells from the pole of the vesicle results in the formation of a thickened zone adjoining the thin or endothelial-like portion of the ectodermal vesicle (figs. 59, 61). The zone is not uniformly thick, but is thickest at the two regions corresponding respectively to the right and left sides of the vesicle. One can therefore correctly speak of these thickened areas as lateral plates.

The primary buds arise from these lateral plates, and appear as two broad, blunt processes protruding from the sides of the ectodermal vesicle (fig. 21). Each bud involves the greater portion of the side of the vesicle, covering an arc of approximately 80 degrees on the circumference. These points can be made out in specimen No. 247, which will now be described.

In the preserved condition, the chorionic vesicle measured 0.722 mm., from right to left, at the base, and the ectodermal vesicle 0.443 mm. in the same plane; while in the antero-posterior plane it measured 0.866 mm., and the vesicle 0.312 mm. The ectodermal vesicle is, therefore, approximately a third wider in the right-left plane than in the antero-posterior plane, and this difference is due to the presence of the primary buds. In

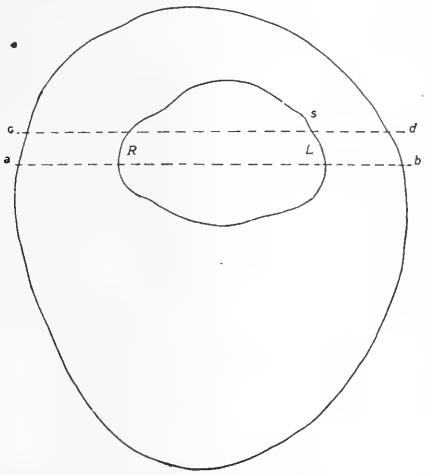

Fig. 1 Outline reconstruction of specimen No. 247. This shows the well formed 'primary buds,' situated on the right and left hand sides of the vesicle. The broken line shows the plane of the two sections illustrated in plate 6, figures 60, 61. × 95.

preparing the specimen for microscopical study the sections were cut so as to pass parallel to the long axis of the ectodermal vesicle (fig. 1).

In the median sections of the series the primary buds are instantly recognized on the right and left sides of the ectodermal vesicle. They appear as outgrowths from the vesicle, and their cavities (fig. 22, *R* and *L*) are seen to be extensions of the common amniotic cavity. The wall of each bud is three or four cells thick, while the roof of the vesicle has thinned out to a single layer in thickness. Furthermore, the cells in the walls

of the buds are undergoing a rapid increase, as is evident from the numerous mitotic figures seen here.

On the lower side of the ectodermal vesicle the wall is composed of a single, thin layer of cells, and below this is the layer of mesoderm. It will be recognized that these two layers together constitute the true amnion, out of which the amnion of each embryo will eventually arise.

The mesoderm of the amnion is a portion of the mesothelium, the origin of which was described in a preceding section. The extraembryonic body cavity occupies the entire space lying between the amnion and the mucosa, and only traces (fig. 22) of the partition of the two original mesodermal vesicles remain to tell the history of this large cavity.

The outer (upper) surface of the chorionic vesicle consists of entoderm. This unique condition has been brought about through the disappearance of the chorionic ectoderm (hinfälliges Ectoderm of Fernandez '09), which has sloughed off. Its disappearance may occur prior to this period (fig. 20), or it may persist even until the four embryonic rudiments are established (fig. 23). The ectoderm breaks off just beyond the point where the entoderm unites to its inner surface (fig. 22, *x*), and thus exposes the entire outer surface of the entoderm to the cavity of the uterus. This condition persists throughout the entire period of gestation, in a manner to be described later.

Prior to the formation of the primary buds, the ectoderm and the adjacent entoderm remain distinct from each other, as seen in such specimens as those shown in figures 58 and 59; but, upon the development of the buds, these two layers are brought into intimate contact, which at certain points amounts practically to a fusion between the two layers. These points are situated just above the central area of each primary bud, and therefore mark the general region of the primordia of the future embryos.

No important changes in the extraembryonic mesoderm appear to be taking place at this time. It retains its epithelial-like character and no cell proliferations are found. A few mesoder-

mal cells are found just beyond the outer margin of the embryonic ectoderm, in the space lying between the entoderm and the extraembryonic mesoderm, but such cells are undoubtedly given off from the marginal cells of the ectoderm.

ORIGIN OF THE SECONDARY BUDS

The formation of the secondary buds immediately follows the establishment of the primary diverticula, and three or four specimens in the collection show the main steps in the process. However, it will be necessary to secure a closer series through this period of development before a detailed account can be given of the origin of the secondary buds. Each primary bud gives rise to two secondary buds, and consequently there are four secondary diverticula. Each secondary bud carries the rudiment or primordium of an embryo. The first step leading to the development of the secondary diverticula consists in the formation of two thickenings in the wall of each primary bud. One of these areas lies at the tip of the bud, while the other appears slightly to the left (as viewed from above) of the tip. The secondary buds then arise from these areas as blind diverticula, which extend down along the inner surface of the yolk-sac entoderm. In specimen No. 247 the beginning of the secondary buds can be seen in the left-hand primary bud (fig. 1). At the point marked *s* is seen a slight protrusion which will form secondary bud No. III.

The secondary buds soon become recognizable in surface views of living specimens, and appear as four blunt processes from the sides of the ectodermal vesicle. Upon the upper surface of each bud an embryonic rudiment appears, in the form of a white, opaque spot. It is somewhat difficult to make out the exact limits of the different parts of the ectodermal structures, owing to the fact that the entoderm (and the chorionic ectoderm, if still present) tend to obscure the view. This difficulty was obviated by making an outline reconstruction from the series of sections of the blastocyst.

The reconstruction of the specimen, which in point of development comes closest to No. 247 (fig. 1), is represented in figure 2, in which the outer, circular line marks the margin of the

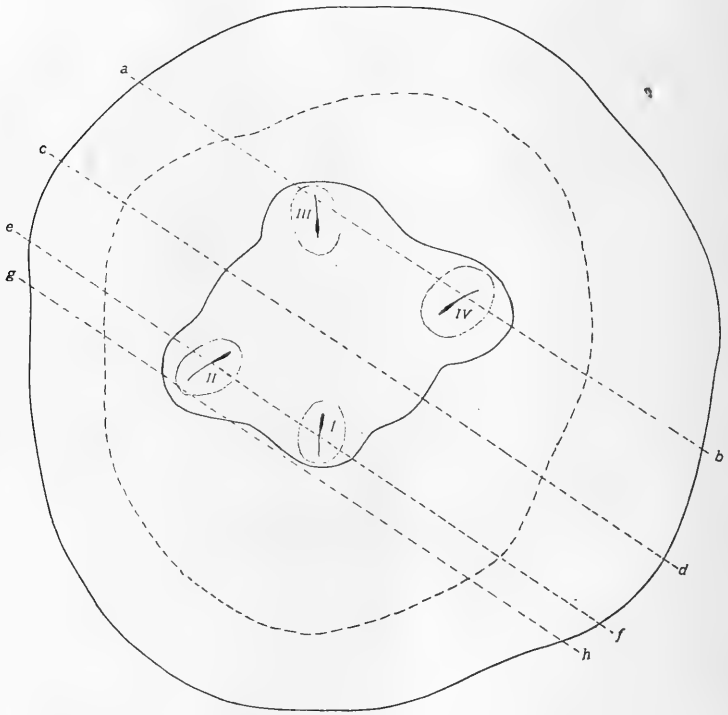

Fig. 2 Outline reconstruction of specimen No. 290. The outer circular line marks the margin of the blastocyst; the broken line indicates the upper limit of the Träger; and the diamond-shaped central figure is the outline of the ectodermal vesicle. The four oval figures lying within the ectodermal vesicle are the embryonic areas. The parallel broken lines represent the planes of the sections shown in plate 7. × 63.

blastocyst, the broken line the upper limit of the Träger, the central irregular line the limits of the ectodermal vesicle.

Our attention must naturally be centered on the ectodermal structure. Its general outline is much like that of a diamond, in which the points are occupied by the secondary buds, or em-

bryos. The 'right-lateral' (II) and 'left-lateral' (IV) embryos lie at the acute angles of the diamond, while the 'dorsal' (III) and 'ventral' (I) embryos occupy the obtuse angles. The same condition prevails in two specimens but slightly older than this one; hence we may conclude that it is the normal arrangement of the secondary buds.

It is certain from the evidence that Embryos I and II are the product of the right-hand primary bud, while Embryos III and IV have come from the left-hand primary bud. It might appear from an examination of figure 2 that the secondary buds at II and IV had alone come from the two primary buds of such a specimen as No. 247 (fig. 1), while those at III and I had arisen de novo from the dorsal and ventral sides, respectively, of the ectodermal vesicle. But the entire genesis of these secondary buds argues against such an interpretation, as will become clear after a complete account of their development has been given. Nevertheless, it is an interesting fact, and one I believe not to be without significance, that the secondary buds II and IV occupy positions on the periphery similar to those of the two primary buds. Furthermore, it is probably correct to regard Embryos I and III as having arisen as outgrowths from the left sides (assuming that the observer is situated in the center of the free surface of the blastocyst) of the right and left primary buds, respectively.

The oval figure in each bud indicates the area over which the ectoderm and entoderm have come into intimate contact or have fused, while the solid line within each oval area shows the position and extent of the primitive groove. The head of the future embryo will, in each case, be directed towards the center of the vesicle.

The plane of the sections is indicated by the broken parallel lines, which also show the position of the four sections from which the photographs of plate 7 were made.

The section passing through the plane *e* to *f* will be taken for detailed account of the different parts of the chorionic vesicle. The outer membrane of the chorionic ectoderm (fig. 23) shows signs of breaking away. It has usually disappeared before this

time; thus exposing the entoderm to the uterine cavity. The entoderm is connected at the margin to a thickened portion of the chorionic ectoderm, which is recognized as the Träger (*Tr.*).

The mesothelium which lines the extraembryonic cavity (*E. E. B. C.*), has undergone no important changes; but there has been organized just beneath it a distinct Träger epithelium (*Tr. Ep.*), which in its relation to the uterine mucosa is particularly clear in the photograph (fig. 64).

The most important changes involve the ectodermal vesicle, and chief among these is the one affecting the organization of the embryonic primordia. On account of the inversion of germ layers, the lower side of each embryonic primordium is uppermost in the figure, that is, the lower side of the future embryo will be on the outside of the ectodermal vesicle. The section under discussion shows the rudiments of Embryos I and II, which are cut across at a slightly oblique angle (fig. 2). Each embryonic spot is characterized by (1) a fusion between the ectoderm and the entoderm, and (2) by a rather shallow depression, or primitive groove, which is situated on the lower or inner side of the embryonic ectoderm (fig. 23, *P. G.*). Between the two embryos the entoderm is entirely free from that portion of the ectoderm which joins the adjacent sides of the two embryonic rudiments. The cavity lying below the embryos is the bay of the original right-hand primary bud, and is a portion of the general amniotic cavity. The thin lower wall of this cavity is, therefore, the ectodermal layer, which, together with the adjacent mesodermal epithelium, constitutes the true amnion (fig. 23, *Am.*).

The primitive groove of each embryo measured about 0.095 mm., and is rather shallow throughout the greater part of its length. There is, however, a pit-like depression at one point which undoubtedly corresponds to a primitive pit. This is especially distinct in Embryo I, in which it lies about 0.032 mm. from the anterior end of the groove (fig. 24, *P. P.*).

Beneath the primitive groove there is the typically early primitive streak region, from which cells are being proliferated laterally to form the true embryonic mesoderm (fig. 24, *E. Mes.*). Throughout the extent of the primitive streak there is no

evidence that the underlying entoderm takes part in this proliferation of the mesodermal cells, but directly anterior to the primitive streak the entoderm is found to be actively dividing to form a group of cells, which can be traced throughout successive stages to mesodermal tissue. Undoubtedly this center of proliferation corresponds to the protochordal plate of Hubrecht (fig. 25, *P. Pl.*).

The conditions which we have here described for the pair of Embryos I and II also hold for the other pair, or Embryos III and IV. The relation of the various parts is identical in the two pairs. The only point in which they do differ is the fact that the two secondary buds III and IV are more widely separated than I and II, and hence the ectodermal vesicle appears much wider (*cf.* fig. 62 with fig. 64).

Following the sections through from the anterior limits of either pair of embryos towards the center of the vesicle, two important changes are noticeable: First, the entoderm becomes entirely separated and distinct from the ectoderm; that is, these two layers have never become fused; and second, the roof of the ectodermal vesicle thins out to a single layer, while to either side its wall remains from two to three cells thick (fig. 63). These lateral thickenings are found prior to the appearance of the embryonic rudiments, when the roof of the vesicle undergoes the general reduction in thickness.

Toward the posterior ends of the embryos the posterior grooves fade out, completely disappearing before the sections which cut the tip of the primary buds are reached (fig. 26). In the case of Embryos III and IV the posterior ends of the embryos extend well back into the primary buds, which are seen to be sharply separated from each other, especially in the last three or four sections which cut the ectodermal vesicle (fig. 26).

The embryonic entoderm or gut-entoderm of each embryo is differentiated from the primary yolk-sac entoderm. Apparently any region of the yolk-sac where the embryonic buds happen to impinge against its inner surface will differentiate into gut-entoderm.

The conditions which we have just recorded may be further chronicled by a brief account of specimen No. 175, which is the

next oldest blastocyst in the collection. In the entire blastocyst the primary buds with their accompanying embryonic rudiments stood out clearer than in the preceding specimen (No. 290), mainly because the chorionic ectoderm had already sloughed off from the upper side of the vesicle, thus giving a better view of the ectodermal vesicle.

The general arrangement of the secondary buds is identical in these two specimens (*cf.* fig. 2 and 28), but No. 175 is distinctly further developed. This becomes especially evident in a detailed study of the sections. In the section which passes through the place *a* to *b*, figure 29, bud IV is seen to be well defined, and the accompanying embryonic rudiment extends down into the tip of the bud, showing a well defined primitive groove (fig. 66). Anteriorly the groove becomes very pronounced, due in a large measure to the elevation of the medullary folds (fig. 67).

In the central region of the ectodermal vesicle (fig. 68) the lateral walls have become distinctly thinner, and will soon reach a state in which the entire wall, exclusive of the embryonic portions, will become but a single layer thick. When this condition is once attained the ectodermal vesicle undergoes no further growth or expansion, but remains a small inconspicuous structure (common amniotic vesicle) to which the embryos remain connected by means of amniotic canals or tubes. These canals are developed through the extension of the secondary buds, which rapidly push outward and then downward along the under side of the entoderm, carrying with them the embryonic rudiments. At this point we are concerned with the beginning only of the tubes, and this can be seen not only in figure 66, but also in figure 69, especially in the case of Embryo II.

It remains to say a word about the cavity lying just beneath the Träger epithelium in blastocyst No. 175. This cavity extends throughout the greater part of the left side of the chorionic vesicle (fig. 27, *Cav.*). If these figures are compared with text figure 1 of Fernandez ('09) it will be found that there is much similarity between them as regards this cavity. Fernandez designates it the Träger cavity (or Ectoplacentarplatten-

höhle) in Mulita; but in the Texas armadillo the enlarged space is clearly an artifact. One can easily observe in the sections that it has been produced by lifting up the Träger epithelium from the subjacent emaciated mucosa, probably through the action of the fixing and hardening reagents. It has not been observed in any other specimen, either older or younger, and this leads one to suspect that it is likewise an artifact in the blastocyst of Mulita, especially as it does not appear in the older vesicles of this animal.

In plate 9 is shown a series of five sections from a chorionic vesicle presenting a further advance in the development of the secondary buds and their accompanying embryos. The specimen is one of the finest in my collection, not only because of its excellent state of preservation, but also for the reason that it remained turgid while undergoing fixation, and thus gives us a picture in the sections which most closely resembles that of the living vesicle.

Figure 3 is an outline reconstruction of this series, and shows that the buds have made considerable progress. Buds II and IV are still larger than I and III, but this inequality gradually grows less and less as the buds extend outward and downward beneath the entoderm.

In the section passing through plane *e* to *f* of figure 3, the general relation of the various parts is well shown. The large extraembryonic cavity, lined with mesoderm, is conspicuous. Above this, and separated by the thin amnion, is the amniotic cavity of the ectodermal vesicle (fig. 72). The section passes a little to the left of the center of the left-lateral bud, which appears in section as a prolongation of the left side of the ectodermal vesicle. The outer covering of the chorionic vesicle is the entoderm, the chorionic ectoderm having already disappeared. The point at which it has broken off close to the base of the vesicle is clearly seen in this and the other photographs.

The other sections illustrated in the plate present special parts of the several embryos. Thus figures 70 and 74 show transverse sections of Embryo III and Embryo I, respectively. In each case the primitive groove is distinct. In figure 71 the

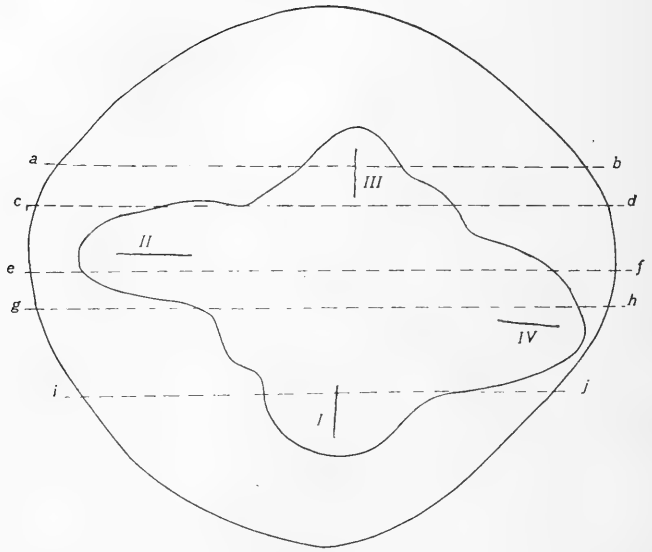

Fig. 3 Outline reconstruction of the left half of specimen no. 257. The five sections from this specimen are illustrated in plate 9. × 83.

section passes through line *c* to *d* of figure 3, and thus cuts across the angle lying between Embryos II and III. Finally figure 73 passes a little to the left of the center of Embryo IV, and shows the structure of that embryo.

At the tips of the buds the cells are in active division (fig. 30), indicating that the extension of the buds, during their early existence, is to be accounted for in this way. Their subsequent extension is due to other factors, which will be considered in the next sections.

EXTENSION OF THE SECONDARY BUDS AND ORGANIZATION OF THE EMBRYOS

The further extension of the secondary buds from the sides of the ectodermal vesicle very rapidly follows such conditions as we have described in connection with specimen No. 257. A rapid growth or increase in size of the vesicle also follows or accompanies this extension of the buds. Each bud grows down along the inner side of the wall of the vesicle, between the entoderm and the meso-

thelium, eventually establishing a placental connection with the extending Träger, thus forming a sort of 'belly-stalk' through which the placental blood vessels run, and into which the rudimentary allantois later extends. In their growth from the vesicle the buds do not pass out as four distinct rays from a common center, the common amniotic vesicle, but extend out in pairs, the individuals of each pair retaining a common connection with the ectodermal vesicle. The paired condition is but a further expression of the same relation which was noted in connection with the account of the origin of the secondary buds.

The earliest phase of this condition is very clearly brought out in one of the specimens of the series. This specimen has almost completely collapsed and is inclined to the left. Hence Embryos I and II are in part folded beneath the wall of the blastocyst, making their study difficult.

In plate 10 are shown photographs of three sections which pass through Embryos III and IV at different levels. Figure 75 is taken across the common bay of the two embryos, about half way between the common amniotic vesicle (remains of the old ectodermal vesicle) and the point of departure of the two secondary buds. The chorionic ectoderm has disappeared and consequently the entoderm is the uppermost layer. It is entirely distinct from the embryonic ectoderm.

Figure 76 represents a section which passes through the point where the secondary buds arise and diverge. The bud on the left contains Embryo IV, and its width is almost twice that of its paired mate on the right, or number III.[7] The difference between the two buds exists throughout their entire length. Thus in the section cutting the middle of the buds (fig. 77) the difference in size is particularly striking. Each embryo consists of the following parts: (1) the entoderm, which is in contact with the primitive streak mesoderm; (2) the primitive streak mesoderm, which is being proliferated from the ectoderm; (3)

[7] In the preliminary paper (figs. 8 and 9) these embryonic buds were incorrectly labeled. This was due to the fact that the sections had inadvertently been reversed in mounting them on the slide, a fact not discovered until after plate 10 of the present paper had been made up.

the thick embryonic ectoderm, curved upward on each side; (4) the thin amniotic ectoderm above; and finally, (5) the mesoderm of the false amniotic or extraembryonic cavity.

Briefly stated then, each embryo consists of a tube-like outgrowth from the ectodermal vesicle, with which it retains a

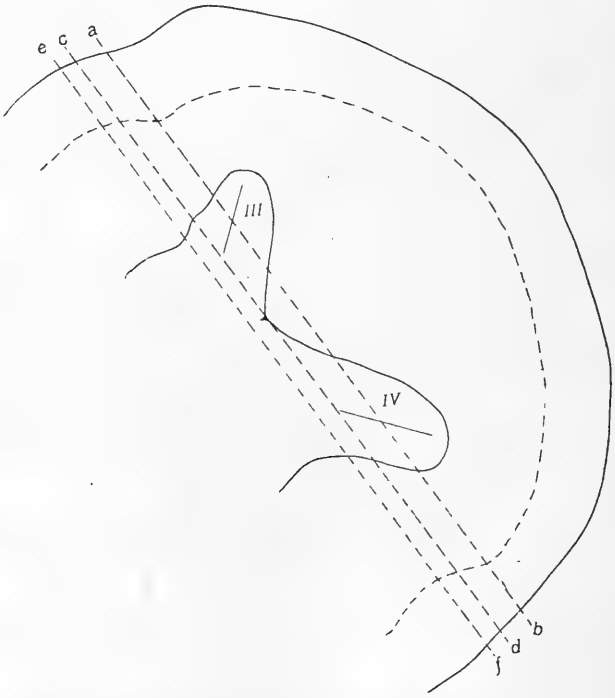

Fig. 4 Outline reconstruction of the left half of specimen No. 170. Three sections from this specimen are shown in plate 10, figures 75 to 77. × 62.

connection in the form of the proximal part of the secondary bud. As a result of the manner in which the secondary buds arise from the primary ones, this connection is common to two embryos, which always constitutes a pair.

Aside from the differentiation or organization of the embryos, the final stages in the extension of the secondary buds presents very little of special interest. We may therefore refer to them briefly. First of all, it should be stated that following a stage

such as we have just considered the vesicle grows rapidly in size, quickly filling up the entire lumen of the uterus. The first noticable change in growth affects the free or distal portion of the wall, and the chorionic vesicle soon becomes bulb shaped (fig. 5).

The expansion and growth of the wall of the blastocyst gradually carries distally the common amniotic vesicle, which by this time has ceased to grow. As a result, the embryonic rudiments are gradually separated from the amniotic vesicle, and their organic connections with the vesicle are drawn out into small tube-like structures, the amniotic connecting canals. These canals lie against the under or inner surface of the entoderm, and each consists of an inner layer of ectoderm, surrounded by a layer of mesoderm.

In plate 11 are shown five sections, taken at different levels, from a specimen in which the amniotic vesicle was just beginning to be drawn away from the embryonic rudiments, through the expansion of the wall. The sections are not quite transverse to the axes of the four embryos.

The section represented in figure 80 cuts the tip of the chorionic vesicle and passes through the lower portion of the common amniotic vesicle. The anterior parts of three of the embryos are seen in the section. These are the dorsal, right-lateral, and ventral embryos. The position of the other embryo, or the left-lateral, is indicated by the evagination at the left side of the vesicle.

In figure 81, which is three sections further down, the same relation with reference to the embryonic rudiments still exists, but the section passes through the extreme lower limit of the amniotic vesicle, and the right-lateral embryo becomes entirely separated from the others.

In figure 82, which is five sections lower down on the chorionic vesicle than figure 81, the dorsal and right-lateral embryos are both free from any connections with the amniotic vesicle. In section each embryo appears as a section of a tube. The chief interest in this section lies in the condition of the ventral and left-lateral embryonic rudiments. Only the anterior tips of

these two embryos are shown, and they lie each at the end of a common bay by means of which their amniotic cavities are placed in communication with the cavity of the common amniotic vesicle. This condition is of the greatest significance, since it indicates the common origin of the pair of embryonic tubes from the left-hand primary bud.

Figure 83 shows all four of the embryonic rudiments in section lying on the sides of the wall of the blastocyst, and facing on the inner side of the large extraembryonic cavity.

Figure 84 is taken about half way between the posterior ends of the embryonic tubes and the base of the chorionic vesicle. Here the wall of the blastocyst is composed of the typical structures, entoderm on the outside and mesoderm within. The latter is rapidly becoming vasculated, especially in the regions lying directly posterior to the embryonic tubes.

Soon after the stage just referred to, the chorionic vesicle begins a very rapid expansion, and, in doing so, first assumes a bulb-like shape (fig. 5). The vesicle is united to the mucosa by an annular zone of thickened trophoblastic ectoderm, the so-called Träger (fig. 23), and from the edge of this the yolk sac entoderm extends upwards to form the outer layer of the chorionic wall, the chorionic ectoderm having long since disappeared. The inner layer of the chorion everywhere consists of mesoderm.

The embryonic portion of the vesicle consists of the relatively small, common amniotic vesicle (fig. 5, *C A. V.*), from the right and left sides of which spring a pair of small connecting canals. Each canal places the cavity of the amniotic vesicle in direct communication with the amniotic cavity of the embryo. It will be recognized that these canals are the elongated proximal parts of the original 'secondary buds', and that the embryonic rudiments are the distal portions of such buds. This is the reason why the canals spring in pairs from the vesicle. However, there is considerable variation in the relation of the pair of canals to the vesicle. The one presented in figure 5 is rather unusual, in that the amniotic vesicle is greatly elongated, instead of spherical, and the two canals of a pair arise very close together from the end of the vesicle. The usual condition shows

the pair of canals united at a very short distance from the vesicle, and thus entering it as a single short tube. Variations from this are seen in those cases in which the union takes place further and further away from the amniotic vesicle. In one of the most extreme cases observed the two canals on one side were united for a distance of about three millimeters from the vesicle, or more than half way between the anterior end of the embryo and the amniotic vesicle.

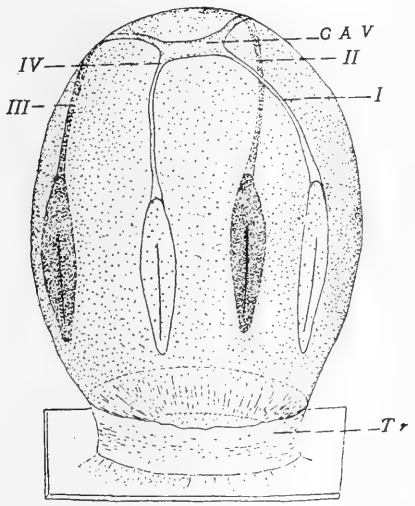

Fig. 5 A free hand drawing of specimen No. 276, in which the embryonic rudiments are well started. Each embryo is connected with the common amniotic vesicle (*C.A.V.*) by means of a slender tube-like canal. In the living condition the vesicle measured about 5 mm. high by 3 mm. at the widest point. × 11.

The important point is that in all of these cases the paired condition of the embryos is unmistakably clear, and I know of no other way to account for this except to assume that it rests upon the manner in which the pair of secondary buds arises from the primary buds. If the secondary buds start soon after the primary buds are formed, than a condition similar to that seen in figure 5 might readily result; but if the primary bud has made considerable progress in its extension from the vesicle before the secondary buds arose from it, then we should expect

to find a common canal for the two embryos developing out of the proximal part of the primary bud.

Each embryonic rudiment is a slipper-shaped structure lying at the terminus of a canal (fig. 5), and is in a relatively late primitive streak stage. At the anterior end of the embryo the medullary plate is well formed, although as yet the medullary folds have not become elevated. Posteriorly the embryo ends as an irregular, blunt process of mesodermal tissue.

Fig. 78 shows a transverse section of one of the embryonic tubes from specimen No. 276. The section passes through the embryo at the level of the extreme anterior tip of the primitive streak, and hence cuts the thickened medullary plate, which curves upward and inward to become the thin ectodermal layer of the amnion. Beneath the medullary plate, and between it and the entoderm, are seen the scattered mesodermal cells which have been proliferated from the primitive streak.

Lateral to the embryo is a loose mass of mesodermal cells which lie between the entoderm and epithelial-like mesoderm of the extraembryonic cavity. In the whole condition of the chorionic vesicle the mesoderm was seen to fringe each side of the embryonic tube, extending throughout the entire length of the embryo proper. It already shows a rudimentary net-work of blood-vessels, which represents the beginning of the area vasculosa. I have not worked out the detailed history of the vascular area, but undoubtedly it arises in each embryo in a manner similar to that in the typical mammalian ovum from which but a single embryo develops.

The embryonic mesoderm is thickest at the posterior end of the embryo, where it gives rise to a series of enlargements which extend for some little distance behind the extreme posterior tip of the embryonic tube. This is the portion of the mesoderm into which the umbilical vessels and the rudimentary diverticulum of the allantois later extend; that is, it forms the basis for the belly-stalk.

The further development of each embryo is very similar to that of the ordinary mammal, and therefore calls for a brief description only. The reader is referred to an earlier paper in

which is found an account of the late stages of development (Newman and Patterson '10).

The differentiation of the embryo within the tube is soon made evident by the elevation of the neural folds to form the neural tube, and by the cutting off of paired mesodermic somites. As these changes in the embryo are progressing, the tube-like amnion is rapidly undergoing modification. First, it develops a finger-like prolongation which extends back beyond the posterior limit of the embryo. Then there follows a rapid expansion of that portion of the amniotic tube which is occupied by the embryo, the anterior portion of the tube remaining small, and, together with the common amniotic vesicle, is destined to degenerate and disappear.

During the expansion the amnion at first assumes a cigar-like shape, and the amnia of the four embryos soon come to fill the entire cavity of the chorionic vesicle, each embryo and its membranes occupying one quadrant of the vesicle. In the mean time, each embryo has become constricted from the extra-embryonic parts by the development of the characteristic head, tail, and lateral folds, and retains its connections with the chorion by means of a typical umbilicus. It thus floats quite freely within the amniotic fluid.

During all of these changes the paired condition of the embryos is perfectly distinct, and, in the final stages of development, expresses itself in the arrangement of the embryos into right-hand and left-hand pairs, as is evidenced by the umbilical connections with the placenta (fig. 79).

IMPLANTATION OF THE OVUM AND PLACENTATION

The more general features of the late phases of placentation have been presented in another paper, but most of the material for the study of the early development of the placenta has but recently been secured. The nature of this paper does not call for a detailed account of this process, and even though it did, it would not be possible to write it in full at this time, since one or two of the critical stages have not yet been been obtained.

Particularly is this true of the changes which immediately follow the attachment of the blastocyst. It is therefore proposed briefly to describe some of those changes involved in the early history of implantation and placentation which are essential to a clear understanding of the embryology of the armadillo. This account has been deferred until now, because it will be somewhat easier to follow after the description of the development has been read.

A statement as to the manner in which the embryonic vesicle migrates along the horizontal groove of the uterus and eventually attaches itself has been given above. The principal facts are as follows: After the blastocyst has reached the placental area, which lies at the extreme anterior tip of the fundus, it adheres to the mucosa, apparently for some time before the actual placentation or intimate union with the uterine membrane is consumated. Four clear and two somewhat doubtful cases of early adhesion between the ovum and the mucosa have been observed. In every instance the ovum freed itself either immediately or very shortly after the application of the fixing fluid, thus indicating that the implantation proper had not really taken place. Fortunately, these vesicles were studied in salt solution under the binocular microscope before fixation and it was possible to make out several important points. It was observed that the ovum always comes to rest upon the uterine surface in such a way that the embryonic spot or germinal area is turned toward the mucosa. Hence, the foetal contribution to the early placenta arises from that portion of the trophoblast which overlies the inner cell-mass; that is, from the so-called Rauber's layer.

It is to this region of the trophoblast then that one must look to detect the first indications of placentation. In five of the blastocysts to which we have just called attention no evidence of importance in this connection was observable, but in the sixth unmistakable indications of early placentation are present. In this specimen Rauber's layer is seen to have undergone important modifications. Several of the cells are in mitosis, and all of the cells of this region have lost their original attenuated

appearance, and now show a decided increase in thickness (fig. 11). There is also a distinct tendency for some of the cell walls to disappear, thus transforming Rauber's layer into a syncytium. Furthermore, the surface of Rauber's layer presents a 'fuzzy' appearance, the outer wall of some of the cells actually being broken as though ruptures had resulted when the blastocyst was freed from its insecure moorings to the mucosa.

That all of these facts are evidence of the beginning of implantation is clearly indicated by the act that the trophoblastic cells which lie beyond Rauber's area are still unchanged, and show the mosaic-like arrangement of polygonal cells so characteristic of all of the free blastocysts. Unfortunately, there is here a slight break in the series, so that we are not able to follow up this clew through the obviously critical period of implantation. We are therefore obliged to pass directly to an account of the modifications which are occurring both in the mucosa epithelium and in the wall of the blastocyst in a vesicle which has already become firmly anchored to the maternal tissue.

Blastocyst No. 316 represents the youngest firmly attached stage that has been secured. A series of sections from this specimen is shown in plate 2. In the living vesicle it could be seen that, in addition to the small area which had established an intimate union with the mucosa, almost the entire left side was lying in contact with the uterine wall, and that as a result the trophoblastic cells here had greatly increased in thickness (fig. 16). Whatever may be the nature of the stimulus which causes the trophoblastic cells to react whenever brought into close relation with the uterine epithelium, it is certain that the influence is not confined to Rauber's layer, but any portion of the trophoblast, upon coming in contact with the mucosa, will respond. It does not necessarily follow that such thickened trophoblast establishes eventually a fusion or placental union with the uterine wall, although a study of the stages more advanced than this one, suggests that some of it may so unite. However, it should be pointed out that only in rare instances does a blastocyst become attached in a manner such that an

extensive area of the trophoblast is brought into close contact with the mucosa. Whenever the blastocyst happens to become anchored at the bottom of a furrow or on the side of a fold, as in the case of No. 316, it follows that a larger area of contact is possible than when its union is established on the top of a fold or on a perfectly smooth surface.

Although there is an extensive area of contact in the specimen under discussion, yet the place of fusion is indeed small, and does not occupy a space any greater than that previously covered by the Rauber's layer. The chorionic vesicle rests upon a mass of trophoblastic tissue which is in the form of a concave disc, with the concave side directed upwards, and with the upper margin of the disc passing insensibly into the free trophoblastic portion of the vesicle (fig. 31).

The disc or primitive placenta is comparable to the attachment mass of rodents, and has been termed the Träger. In the armadillo the Träger arises through the formation of the syncytium in Rauber's portion of the trophoblast, followed by a fusion of this syncytium with the surface layer of the mucosa. The fused mass thus forms a bridge across which the embryonic nuclei can pass from the syncytium into the maternal tissues, portions of which are soon destroyed by these invading nuclei, doubtless as a result of their phagocytic or histolytic action.

In specimen No. 316 the fusion is firmly established and several embryonic nuclei have already penetrated well into the mucosa epithelium (fig. 31, *Em. N.*). Several other nuclei are on the point of passing into the mucosa. Evidences of the histolytic properties of these foreign nuclei are everywhere present in the maternal portion of the fused region, and the nutritive substances which result from the breaking down of the maternal tissues must serve as an embryotrophe. The embryotrophic phase of placentation must last throughout a relatively long period of the early development, because neither the maternal nor foetal circulation is established in the placenta until the embryonic rudiments are well formed.

In the next stage, which is but slightly older than No. 316, a somewhat larger number of nuclei have passed over into the uterine wall. They have almost completely destroyed the epithelium, and have also affected the sub-epithelial layers (fig. 17). The syncytium and adjacent thickened trophoblast together form a layer which is coextensive with the germinal spot, that is, they extend to the margin of the entoderm. In addition there is found on the left wall, and somewhat removed from the rest of the thickened extragerminal ectoderm, a distinct knot of cells which must have resulted from the contact of a very small spot of the trophoblast with the mucosa (fig. 17, *K*).

The next important change is a spreading of the base of the vesicle. Instead of having but a small area of contact or point of union, as in specimen Nos. 316 and 332 (figs. 31 and 17), the blastocyst soon acquires a base, often greater in width than that of the entire free portion of the vesicle (fig. 18). As a result, the wall of the chorion, upon approaching the mucosa, instead of curving inward, now slopes gradually outward, and merges into the uterine tissue with which it has established a very intimate union. The base forms an annular zone of thickened trophoblast, which in width extends from the margin of the entoderm to the surface of the uterine wall. In its more distal part the annular zone consists of thickened cells (two or three cells thick), but its proximal part is much thicker and one finds here a tendency to form a syncytium, continuous with the syncytial-like mass lying directly beneath the chorionic vesicle.

In the latter region are found numerous embryonic nuclei, which have destroyed the mucous epithelium, the connective-tissue stroma, and even portions of the uterine glands. These nuclei may be divided into two groups, one of which occupies a superficial position; that is, its elements border on the extra-embryonic cavity and apparently are inactive in so far as the destruction of the mucosa is concerned; and the other constitutes the deeper lying nuclei which are instrumental in dissolving the mucosa. At first this distinction is not very evident, (figs. 18 and 32), but as development progresses the protoplasm surrounding the surface nuclei becomes more and more dense

(fig. 33), and there is gradually evolved a distinct epithelial layer (figs. 33, 34, 64). Following the suggestion of Fernandez ('09) in his paper on Mulita, we may designate it the 'Träger Epithelium,' on contrast to the Träger zone, or thickened annular portion of the trophoblast which forms the base of the attached chorionic vesicle. The Träger epithelium, together with the layer of extraembryonic mesoderm which directly overlies it, forms the lower wall of the chorionic vesicle.

In the meantime the deeper-seated nuclei continue to migrate farther into the uterine tissue, which, as already stated, soon becomes destroyed. During these changes the upper portion of the syncytium begins to degenerate, as is evidenced by the appearance of numerous vacuoles (figs. 34, 35). The vacuoles flow together and eventually produce a shallow cavity which lies just beneath the now well-organized Träger epithelium. The final condition in the development of this 'sub-Träger cavity' is well illustrated in the series of photographs in plate 7. All of these show the condition just referred to, but figure 64 is particularly clear. Here the sub-Träger cavity is sharply defined above by the Träger epithelium, but on the lower side it is connected with numerous spaces which lie between the remains of the partly dissociated mucosa.

Occasionally during the process of fixation the Träger epithelium becomes pushed up into the extraembryonic cavity, carrying before it the layer of mesoderm. Under such conditions it appears as though the Träger epithelium was normally arched (figs. 66–69). I am inclined to believe that a similar artificial condition in the chorionic vesicle of Mulita has lead Fernandez ('09) to conclude that the Träger epithelium is normally pushed up into the extraembryonic cavity, and that consequently the cavity lying just below it is to be regarded as a true Träger cavity. In view of the fact that Fernandez had only a few scattered stages at command, it was easy for him to be mislead into drawing this conclusion. But the evidence which I have just presented from a study of the development of this cavity makes it doubtful whether it should be regarded as a Träger cavity, in the sense in which that term is used in rodents. For that

reason I have preferred to call it by the name of sub-Träger cavity.

A further examination of the figures on plate 7 will show that at this stage of development the blastocyst is held to the mucosa by the Träger zone alone, so that in order to free it from the uterine wall it is merely necessary to cut this band of tissue at a short distance beyond the edge of the vesicle.

The transition from the Träger stage or primary placenta to that of the secondary placenta, or a condition in which distinct villi are present, is very gradual. The first villi arise from that portion of the chorionic wall which corresponds to the Träger zone; that is at the attached margin of the chorionic vesicle. From here they successively appear further and further toward the central area of the Träger epithelium, until its entire surface becomes studded with them. However, the villi in the central region do not become so long or so highly developed as those that are situated towards its peripheral parts.

Each villus starts as a thickening in the Träger epithelium, and soon becomes a mass of cells protruding from the epithelial surface. At first it is flat or disc-shaped and seems to serve as an adhesive pad, but later it elongates into a true Träger cord, which may become very much branched. These cords later become invaded by a stroma-like mesenchyme, developed from the mesodermal epithelium which directly overlies the Träger epithelium.[8]

In order to understand the changes which take place in the upper free portion of the chorionic wall it is necessary to recall that all of the trophoblast lying above the margin of the entoderm sooner or later sloughs off, leaving only a fragment-like base, which in section can often be seen protruding from the side of the vesicle at a short distance above the uterine surface. This fragment is clearly shown in all of the figures of plate 9.

The portion of the trophoblast which thus breaks away corresponds to the chorionic ectoderm in the vesicle of other mam-

[8] For a more detailed account, and for figures of the structure of the villi, see Newman and Patterson, '10.

mals, but in the blastocyst of the armadillo its loss results in bringing the yolk-sac entoderm to the outer surface. From now on the increase in size of the blastocyst is evidently brought about through the growth of this entodermal portion of the wall of the ovum, as can be determined by comparing successive stages. But after the vesicle has attained a diameter of from 4 to 5 mm. (fig. 5) the entodermal portion does not expand to any great extent, and the chorionic vesicle owes its further growth to the extension of the Träger or placental portion of the chorionic wall.

As the Träger area increases in extent the yolk-sac is carried farther and farther toward the cervix end of the uterus, and in the advanced stages of gestation it forms a cap at the tip of the cervix end of the chorionic vesicle. However, it becomes partly covered by an overgrowth of Träger tissue, which arises at the boundary line between the yolk-sac and the Träger, and extends posteriorly as a free margin which later becomes fused with the wall of the cervix. In vesicles which contain 30 to 35 mm. embryos, the yolk-sac portion of the chorion protrudes through the thickened ring-like, placental overgrowth as a clear transparent membrane.

If a chorionic vesicle from which the chorionic ectoderm has already disappeared be examined, it is found that the entoderm is attached to the upper edge of a mass of Träger tissue which lies just inside the basal fragment of trophoblast (figs. 70–74). In tracing back the origin of this mass one finds that it arises as a thickening on the inner surface of the Träger zone, just below the margin of the entoderm. It can be recognized in very early stages as a small mass of actively dividing cells which lie in the angle formed by the Träger zone and the potential Träger epithelium (fig. 55, on left). The thickening gradually increases in volume (figs. 33, 34, *m*), and apparently involves the entire inner surface of the Träger zone, and thus comes to form at its crest a fusion with the margin of the entoderm (fig. 23, on right). At the same time the outer layer of cells of the Träger zone becomes split off from the mass (fig. 23, on left), and thus forms the basal fragment when the chorionic ectoderm breaks away.

The mass of Träger tissue constitutes the material upon which the further growth of the chorionic wall depends, and after the 4 to 5 mm. stage is reached it rapidly extends upward, becomes thinner, and carries before it the cap of the yolk-sac entoderm, in the manner already described. It also forms the basis for the formation of the villi which appear upon this region of the chorionic wall. At first the villi have a general distribution over the surface of the modified Träger zone, but in blastocysts which have attained a diameter of 25 to 30 mm., the long branched villi become restricted to four distinct areas or patches. These patches are situated just below the boundary line between the Träger and the yolk-sac, and each villous area lies opposite the point at which an umbilical cord arises from the inner surface of the chorionic wall. The four villous areas give rise to the four placental discs of later stages, and, corresponding to the position of the embryos, are arranged into two pairs. In the final stages of gestation the two discs belonging to a pair become closely associated together, thus forming two large double-discoidal placenta, which occupy respectively the right and left sides of the chorion.

In the formation of the placenta, as well as in the general development of the blastocyst of the armadillo, there are many opportunities for comparison with the developmental stages of other mammals, but such comparisons can be more safely drawn after we have had a chance to work out a detailed history of placentation.

GENERAL DISCUSSION

1. Theories of polyembryony

A great many different views have been expressed in explanation of polyembryonic development. Most of these are pure conjectures, and as such hold no place in any serious attempt at a scientific treatment of the subject. Exceptions are made here to those theories only which seem to hold a grain of truth and which have gained a certain number of adherents.

a. Theory of polyovular follicles. An attempt has been made to account for polyembryony on the basis of polyovular follicles.

Since it was known that the mammalian ovary occasionally possesses such follicles, it was natural to suppose that this fact might furnish a clew to the problem of polyembryony. In fact, Rosner ('01) not only made this claim, but he also attempted to prove his point by a study of the ovaries of the South American variety of the very animal with which this paper deals, namely, the nine-banded armadillo. Rosner studied a single pair of ovaries from a pregnant female sent him by von Jhering. He found fifty-two polyovular follicles, as follows: one with seven ova, one with five, two with four, seven with three, and eleven with two. Rosner believes that the condition of multiple embryos in the armadillo was to be explained by assuming that four young, adjacent follicles fused together, and that the four ova thus brought within a single cavity were later ovulated and fertilized, and held together in such a way as to produce eventually the quadruplex foetal structure characteristic of the armadillo pregnancy.

Aside from the fact that only two out of the fifty-two polyovular follicles observed by Rosner possessed the requisite number of ova to account for the four embryos of each armadillo litter, it has since been abundantly proved (Cuenot '03, and Newman and Patterson '10) that the pair of ovaries studied by Rosner was quite exceptional. Further, sufficient literature has accumulated to show that the phenomenon of polyovular follicles occurs in several widely separated species of mammals, and consequently can have nothing to do with polyembryonic development.

Schrön ('63) seems to have been one of the first to record the occurrence of polyovular follicles. He observed them in the ovaries of the cat. Since then they have been reported in various Eutheria, as follows: in the human by Nagel ('88), Schottländer ('93), Stoeckel ('98), Rabl ('99), and Arnold ('12); in dogs by Waldeyer ('70), Wagener ('79), Bouins ('00) and Smyth ('08); in cats by Rabl ('99); in bats by Van Beneden ('80); in rabbits by Wagener ('79) and Honoré ('00); in the armadillo by Rosner ('01). In addition to these records on the Eutheria, O'Donoghue ('12) has very recently added the Marsupial, *Dasyurus viverrinus*.

These citations will suffice to indicate how widespread is the occurrence of polyovular follicles among mammals; but, although they have a distribution among widely separated forms, their occurrence in a given species seems to be rare. Thus O'Donoghue ('12) found them but twice in forty-five individuals of Dasyurus; and Schrön ('63) only twice in the ovaries of four hundred cats and but once in eighty dogs. The writer has examined in all probably more than fifty pairs of ovaries from the Texas armadillo without finding a single case.

In the light of these data it is impossible to associate the occurrence of polyovular follicles with the causation of polyembryonic development in mammals. The fact that Rosner has found a single armadillo with such follicles can have no greater importance than have the similar sporadic cases in certain other mammals. The significant fact is, rather, that in the ovaries of fifty individuals belonging to a species which reproduces by specific polyembryony alone not a single case of polyovular follicles was found.

It is well to emphasize the fact that the polyovular follicles do not lie at the basis of polyembryony, for to accept Rosner's theory would be equivalent to a denial altogether of the phenomenon of polyembryonic development in the Mammalia. A multiple gestation from ova which have accidentally become associated together during a part of the ovarian history, through the fusion of adjacent follicles, may have no more interest or significance than a similar gestation resulting from ova from uniovular follicles, but simultaneously ovulated, as occurs in many mammals that are normally multiparous. One cannot hope to throw much light on such fundamental biological problems as those of sex determination, the limits of hereditary control, and others, by the study of this type of development. It is only to those cases in which the several embryos of a multiple pregnancy have taken their origin from a single fertilized egg that we must look for facts with which to elucidate these problems.

It is not intended here to underestimate the biological importance of polyovular follicles and of multiple gestations other than those of polyembryony; but rather to point out that these

phenomena have an interest lying along a different line. In fact there is considerable evidence to indicate that polyovular follicles and such multiple gestations are to be correlated as cause and effect. This applies not only to cases of multiple pregnancies among forms that are normally uniparous, but also occasionally to cases in animals that are normally multiparous. According to Wilder ('04) von Franque ('98) was the first to start the discussion which has led up to the conclusion that polyovular follicles bearing two eggs might result in the origin of twins—not compound monsters or duplicate twins, but to fraternal twins, as Wilder points out, since the eggs must be fertilized by different spermatozoa.

The theory that polyovular follicles may account for 'fraternities' of this sort receives considerable support in the case of a dog reported by Smyth ('08). In 1906 he obtained a young setter pup from a litter of fourteen pups (four dogs and ten sluts) born to a Gordon setter. When the pup was ten months old she was spayed for reasons of convenience, and upon preparing the ovaries in sections it was discovered that not a few of the ripe follicles held double and triple ova. The ovaries from one of the other pups were also sectioned, and they too possessed polyovular follicles, one containing as high as seven ova. In 1907 one of the sluts from this same litter gave birth to nine pups, three dogs and six sluts. It is a great pity that the ovaries from this bitch were not studied, as well as those from her mother. But, even though the data are not as complete as might be desired, still they point unmistakably to the fact that a tendency to polyovular follicles was inherited in this family of dogs; and, further more, they suggest that the unusually large litters of the mother and her daughter might in part be accounted for on the basis of compound follicles.

It is a well-known fact that other normally multiparous animals sometimes show a tendency to bring forth very large litters. While it is of course possible that in such cases all of the ova belonging to a given litter may come from simple follicles, yet it is not improbable that some of them may come from polyovular follicles. There is but one way in which it would be possible to

settle this question satisfactorily, and that is to study histologically the ovaries of several generations in the family of some animal belonging to a species normally bearing a single young, but showing a tendency to multiple births. If polyovulated follicles were present in those individuals which had had multiple births and if the number of corpora present in the ovaries after a given pregnancy were found to be fewer than the number of young in the litter, it would be reasonably certain that the multiple gestations were the result of ova from a compound follicle. Some of the cases in cattle recently cited by Pearl ('12) would have made excellent material for such a test.

I have discussed the topic of polyovular follicles somewhat in detail in order to emphasize the necessity of keeping it entirely distinct from the subject of polyembryony, for not to do so will most certainly lead to great confusion. This applies not only to the problem of sex heredity, but also to other questions in heredity that are capable of elucidation through the study of polyembryonic development, particularly the one dealing with the limits of hereditary control. It is here essential, as I have already pointed out, that all of the individuals of a litter of embryos to be studied should have the same germinal constitution; but this condition would never be fulfilled in multiple gestations resulting from ova from a polyovular follicle, even if this could be proved beyond question, and even though all the ova came from a single mother cell in oögenesis; because in that event each egg must be fertilized by a different spermatozoon.

b. Theory of blastotomy. According to this theory each embryo is looked upon as the lineal descendant of one of the early blastomeres. In the case of two embryos arising from a single egg (identical twins) it has been supposed that each individual is the product of one of the blastomeres of the two-celled stage, while in the case of four embryos (armadillo quadruplets) it has been assumed that each embryo arises from one of the blastomeres of the four-celled stage. And so it has been argued for those cases in which even a greater number than four come from one egg.

The idea of an early spontaneous blastotomy lying at the basis of polyembryony has been very persistently urged by a number

of investigators, and in the earlier work on this armadillo the same view was held. This idea undoubtedly has its inception in, and has received most of its support from the results of certain experimental studies involving the mechanical or semi-mechanical separation of early blastomeres. For it has been demonstrated that an early isolated blastomere of the two-celled or four-celled stage of the eggs of the echinoderm (Driesch '92), medusa (Zoga '95–6), Amphioxus (Wilson '93), and teleost (Morgan '93) may develop into a complete but small larva, and even in the egg of the amphibian a blastomere of the two-celled stage, under certain conditions (Schultze '95, Morgan '95, and Herlitzka '97) may also develop into a complete organism. What then seemed more logical then to conclude that in the case of polyembryonic development the early blastomeres had in some way become displaced or isolated, and that each cell thus separated formed the center for a single individual. Moreover, this inference seemed all the more plausible in the light of certain studies on twins in the human species. Wilder ('04) in particular, in his extensive paper on duplicate twins and double monsters, advocates the theory that each member of a pair is the product of one blastomere.

The evidence that has been presented in the first part of this paper makes it certain that polyembryonic development in the armadillo cannot be explained on the basis of a spontaneous blastotomy, in the sense that each embryo is the lineal descendant of a single blastomere of the four-celled stage, and it causes one to view with some doubt the conclusions of this same nature that have been drawn by those who have worked on other polyembryonic forms. In this connection it should be kept in mind that, although an equipotentiality seems well established for the early blastomeres of the eggs of the echinoderms, medusa, amphioxus, teleost, and others, yet there are many forms in in which a blastomere does not have the power to develop into a whole individual. Crampton ('96) on gasteropods, and Chabry ('87) and Conklin ('05, '06) on ascidians have conclusively demonstrated that a blastomere of the two-celled or four-celled stage

in these forms develops essentially in the same manner as though producing a part of the whole embryo.

It is not intended to deny that influences of a mechanical nature may not, in certain cases, lie at the basis of multiple-embryo formation. Any one advocating such a theory may bring to his support not only the facts of artificial blastotomy, but also those derived from experimental studies on later development, like those of the pioneer work of Haeckel ('69) on the blastulae of Crystallodes and of the more recent and well-known studies of Spemann ('01, '03) on the triton egg. This rather simple mechanical or semi-mechanical explanation might hold in the sporadic cases of polyembryony, like those of duplicate twins and double monsters, but what evidence have we that blastotomy operates in the case of specific polyembryony in higher forms? As yet we know very little about the details of the early development in such cases. It is a significant fact that such evidence as we do possess does not support the theory, and it is certainly true that these studies on the armadillo—a form in which we have a most striking case of specific polyembryony—have not revealed any evidence which tends to support the blastotomy theory.[9] On the contrary, the evidence points unmistakably to a different explanation, namely, that a type of budding lies at the basis of polyembryony in this form.

c. The theory of budding. The process of budding is a very common method of reproduction among organisms. In plants it is practically universal, and in animals it is frequently met with, especially among the lower forms. In many cases asexual reproduction by budding occurs late in the life cycle, as for example among coelenterates. In such forms as the common Hydra it is customary to regard the organism as an adult when budding begins. But the appearance of budding is by no means confined to adult organisms, or even to late stages of development, for it may appear very early in the life cycle.

[9] The term 'spontaneous blastotomy' has been used by Bugnion and Marchel to describe the process of polyembryony in the parasitic Hymenoptera, but not in the sense that each embryo can be traced to a single blastomere. Brandes ('98) has suggested the term 'Germmogonie' in lieu of polyembryony.

In the earthworm, Kleinenberg ('79) has described a gemelliparous development occurring in the gastrula stage and initiated by a sort of fission or budding. In the Cyclostomatous Bryzsoa (Harmer '93, Robertson '03) a primary embryo, prior to the formation of germ layers, buds off a large number of secondary embryos which differentiate into larvae. In the parasitic Hymenoptera (Marchal '04, and Silvestri '06) the differentiation of the embryos occurs relatively still earlier and consists in the dissociation of the egg into embryonic masses, which vary in number according to the species, and which later form larvae. In the light of artificial blastotomy it is possible, theoretically, to have the process of dissociation carried still further forward into the cleavage stages—even to the two-celled stage; but so far as the writer knows, the ocurrence of blastotomy as early as the two- or four-celled stage has never been observed as a natural phenomenon in development.

From among the several forms having polyembryonic development, one can select a series in which embryonic fission or budding is carried farther and farther toward the adult stage, and as Marchal has observed, one can pass insensibly from these cases of budding in the egg to the more frequent and well-known phenomenon in which the budding occurs after the individual has already come from the egg, as, for example, in the coelenterates, Orthonectida, Dicyemida, platyhelminthes and tunicates. We may ask then, below what point in the developmental cycle must one cease to speak of asexual reproduction as budding, and refer to it as polyembryony? Evidently a sharp line cannot be drawn between the two.

It is best to regard polyembryony as a precocious type of budding; and this, perhaps, only in the sense that it occurs early in the embryonic life, and without the implication that it has pushed forward in the life cycle or superseded a budding which in the ancestral froms occurred at a late period of development. This would seem to be the case at least in the Polyzoa, in which the embryonic budding is followed in the sessile larval stage by the typical budding to produce the colony.

In the armadillo the facts of development which we have presented in the descriptive part of this paper are fully in accord with the theory of budding. We have seen that the early phases of differentiation are similar to those of other mammals in which the ovum produces but one embryo. Prior to the appearance of the embryos, the ectoderm, the entoderm, and the exocoelomic mesoderm are differentiated, and later all three of these layers are concerned in the formation of the embryonic buds. The initial step in budding apparently occurs in the embryonic ectoderm, but the entodermal layer is soon involved in the process. It is therefore entirely correct to say that the seat of budding in the armadillo is to be found in the blastoderm, that is, the blastoderm in the budding organ. It may be possible to extend this same conception to accidental or sporadic cases of polyembryony occurring in the lower forms which lay yolk-laden eggs.

The most important point brought out in this study is the fact that polyembryonic development in the armadillo can be interpreted as a type of budding; and, while to show that polyembryony is a budding process does not solve the question as to the determining cause of the division of the blastoderm, yet it is a distinct step toward the solution of that important problem.

It is perhaps premature to attempt an explanation of the ultimate cause of polyembryony. We first need a comprehensive study of each of the forms in which it occurs. Such investigations, followed by well-directed experiments, may yield results that will reveal at least some of the factors which control polyembryonic development. At present only a few suggestions need be made; and, first, we may briefly consider the ideas that have been expressed by some of those who have worked on the subject.

Harmer ('93), in his excellent paper on embryonic fission in Bryozoa, points out several interesting comparisons that can be drawn between the process of multiple-embryo formation in Crisia and budding in many other organisms. He calls attention to the fact that, in at least some of these forms, embryonic fission is connected with the deviation from the normal type of

segmentation of the egg. Furthermore, he points out that the early blastomeres of the egg of Crisia are separated from each other by follicle-tissue, and that they are surrounded by a rich nutritive material, which is obtained through the protoplasmic strands connecting the several units of the colony with the ovicell. He believes that the production of numerous larvae from the primary embryo in Crisia is a process comparable to artificial blastotomy in Echinus eggs, as shown by the experiments of Driesch ('92). His general conclusion is clearly stated in the closing paragraph of his paper. He says:

> The cases already quoted may be taken as showing that some of the abnormalities in the development of Crisia may be due to the nutritive conditions in which the development takes place. Just as the presence of food-yolk within the egg modifies the character of the segmentation and the formation of the layers, so the presence of copious stores of nutrient material in the maternal tissues outside the egg may also effect the early developmental processes. Thus the large number of relatively large larvae which develop from the minute egg of a Crisia could not be produced if the egg were not supplied with nutriment from outside itself. While some of the irregularity in the segmentation of the egg may be due to this cause, the extreme independence of the blastomeres at an early stage may be connected with the acquirement by the embryo of a habit of forming buds in the embryonic condition.[10]

Marchal ('04) has expressed somewhat similar views, as may be gathered from the following quotation.[11]

> As to the determining cause of the division of the germ, Marchal thinks that it is from the sudden surrounding with more dilute liquids in the interior of the nourishing mass and in a concomitant modification of the osmotic exchanges in the interior of the cellules. One sees, in fact, with Encyrtus that polyembryony reaches its greatest intensity at the moment when the larvae of the Hyponomeuta commences to feed (in the early days of April), and for the Polygnotus at the period when the young larva of the Hessian fly engorges itself with sap. Now, the production of the rapid changes bringing about osmotic pressure constitutes precisely the procedure employed to bring about the separation of the blastomeres and their evolution into several distinct individuals, as has been shown by the experiments already mentioned of Loeb and Bataillon.

[10] Loc. cit., p. 236.

[11] From Howard's ('06, p. 816) clear translation of Bugnion's ('06) review of Marchel's ('04) paper.

Moreover, in connection with his study on Polygnotus, Marchal observed that the polygerm is moved back and forth in the digestive tract as a result of contractions of the wall of the host. He believes that this movement is analogous to the shaking of Echinus eggs, and has a similar influence upon the division of the germ.

In the preliminary paper (Patterson '12) similar views were given, but expressed in a somewhat different way. It was stated that in all of the well known cases of polyembryony the cleavage of the egg is of the 'indeterminate' type, so that it was impossible to trace out a 'cell-lineage' for any particular embryo. It was also stated that the primary embryo or polygerm led a sort of parasitic existence, and that as a consequence it was surrounded an abundance of nutritive substances.

The cleavage of the mammalian egg is generally regarded as belonging to the indeterminate type, and, although the cleavage stages of the armadillo have yet to be studied, still we have no reasons for believing that they will be found to differ from those of other mammals; and if we may judge from the conditions of the earliest stages of the blastocyst that have been examined, there is no evidence to show that the early blastomeres have been separated by foreign nutritive substances. The development of the embryonic vesicle until the germ layers are differentiated can be compared to that of certain other mammals. However, it is a significant fact that at the close of the period of germ layer formation the embryotrophic phase of placentation, which is particularly striking in the armadillo, becomes well established. It may be that the nutritive substances produced by the action of the embryonic nuclei upon the maternal tissue furnish the stimulus which excites the blastoderm to bud off the embryonic tubes, just as the engorged sap of the Hessian fly is suggested by Marchal to be the determining cause of the division of the germ of Encyrtus. If this point be well taken, it is evidently not necessary to assume that the time of stimulation to polyembryonic development dates back to the early cleavage stages.

In this connection it might be well to call attention to another suggestion that has been made. It is generally believed that in

coelenterates, ascidians, and Polyzoa the germ cells antedate the formation of the buds in which they are found; and Montgomery ('06) has gone so far as to suggest that perhaps in all cases the products of asexual reproduction contain germ cells. He suggests that "If this were so, it might then be the case that the incapacity of any part of the body of an animal to reproduce asexually or even to regenerate, would be due to the absence of germ cells in it." It may be possible to explain in this way the appearance of rudimentary embryos that have been observed in both species of armadillo, or even, indeed, to account for the so-called asexual larvae in Litomastix. Such abortive attempts to produce normal organisms may be the result of a failure on the part of a bud to receive germ cells.

2. Origin of polyembryony in the Dasypodidae

One of the interesting problems which presents itself in connection with this study pertains to the question of the origin of specific polyembryony among the Dasypodidae. The question becomes all the more interesting, from the standpoint of the physiology of reproduction, in view of the fact that the uterus of the armadillo is of the simplex type. The same type of uterus in other mammals is adapted to the function of receiving and nourishing a single ovum, although occasionally it may receive and nourish two or more ova at the same time, in which event a multiple birth results ('fraternal twins,' 'triplets,' etc.). In the armadillo the uterus also receives a single ovum at a time, but instead of but one embryo arising from the egg, polyembryonic development comes on and increases the number of offspring to several individuals. Here we have a clear case of adaptation, in which the productivity of the ovum of a uniparous mammal is increased several fold.

Two quite distinct problems are presented. One of these is the most fundamental question of the whole problem of polyembryony, namely: What are the causal factors underlying the formation of two or more individuals from a single egg? The other problem concerns the phylogeny of specific polyembryony in

the group of armadillos. It must be stated frankly that both of these questions are wholly unanswered. But a few suggestions are given in the hope that they may point the way to their solution. In the remaining part of this section we shall discuss the question of phylogeny as the other problem was discussed in the preceding section.

So far as I am aware, the only armadillos in which specific polyembryony is definitely known to occur are the North and South American varieties of T. novemcincta, and T. hybrida, which inhabit the southern part of the South American continent. Judging from the description of Fernandez ('09) on comparatively late stages of T. hybrida, the two species agree very closely in many details of development; but there are evidently important differences, one of which relates to the number of offspring in a litter. In T. novemcincta a litter consists typically of four individuals, while the number in T. hybrida varies from seven to twelve, with a strong tendencey to produce eight or nine.

In T. novemcincta, out of 219 pregnant females that have been studied carefully, 176 had embryonic vesicles far enough advanced to permit a determination of the number of embryos, and of these all but four, or about 98 per cent, possessed four embryos each. Of the four exceptions, three had five-embryo sets and one a two-embryo set.

There is some doubt in the latter case, since the embryos were born in the laboratory and therefore an opportunity to study them in utero was not offered. But all of the five-embryo sets were carefully studied and the relations of the different parts of the blastocyst were determined. In each case two members of the litter (either on the right or on the left side) showed the normal paired condition, while the other three presented an asymmetrical arrangement, one of the primary placental discs being about twice the usual size and bearing the umbilical cords of two embryos.

This arrangement of the members of a five-embryo set is significant, in that it suggests that the two embryos arose in the normal fashion from one of the primary buds, and that the three embryos of the opposite side have come from the other primary

bud, either directly, through the formation of three secondary buds from it, or through a further division of one of the two secondary buds, after they had been formed in the normal way.

It is to be regretted that the rare case of a two-embryo set, referred to above, was not studied before birth occurred, as it would doubtless have been found that the embryos occupied the right and left sides of the chorionic vesicle, thus indicating that each primary bud had been directly transformed into a single individual. In that event, we should have had very strong evidence in support of our contention that specific polyembryony in the Dasypodidae began by the formation of a set of twins, perhaps at first as sporadic cases of gemelliparous development such as probably occurs in the production of duplicate twins in the human species.

However that may be, I have recently descovered certain evidence in the early development of the Texas armadillo which strongly supports this view concerning the origin of specific polyembryony in the Dasypodidae. It was pointed out in an earlier section that when the secondary buds first appear, Nos. II and IV apparently arise from the tips of the primary buds, as though they were merely prolongations of these buds; while each of the other secondary buds evidently arises slightly to one side of tip of a primary bud. That is to say, that Embryo I always arises to the left of its paired mate No. II, and likewise Embryo III to the left of IV (fig. 2). This may be expressed in another way by saying that buds I and III are outgrowths from the primary buds, and that consequently they follow chronologically the development of buds II and IV. The evidence upon which this interpretation is based is to be seen in several of the young blastocysts.

It has been pointed out elsewhere that the first sign of a secondary bud appears on the right side of the left-hand primary bud in blastocyst No. 247 (fig. 1). The other embryonic bud is the result of a prolongation of the extreme tip of the primary diverticulum. In figures 2, 3, 4, and 28 is seen further evidence of this same difference in the size of the two members of a pair of embryos, and it is also evident in the sections (figs. 67, 77).

Furthermore, it was observed in at least three living specimens, so that there can be no doubt but that during the early history of the embryonic buds, Nos. II and IV are more highly developed than their mates I and III. And what else can this mean than that phylogenetically the former pair should precede the latter.

This obvious difference in size does not last indefinitely, and indeed is no longer discernable after the embryonic rudiments have become slipper-shaped (fig. 5).

That primary buds may also be precursors of secondary buds in Mulita is, I believe, to be inferred from the work of Fernandez ('09) on that form. Unfortunately, he has but a few scattered stages at his command, and therefore was not able to give us a full history of the development of the embryonic rudiments. His specimen 46, which is the one next to his youngest stage (corresponding to Specimen 233), already has embryonic rudiments fully as well developed as those in Specimen No. 226 (plate 11). Nevertheless, in speaking of the relation which exists between the ectoderm of the common amnion and that of the embryonic diverticula in this specimen, he makes the following significant statement, "Der Cervix uteri zu, d.h. über dem Anfang der Medullarplatte, ist das Amnion keines Tieres geschlossen, as steht vielmehr durch eine sehr weite Öffnung mit dem Amnion eines oder mehrerer Nachbartiere in vollkommener Kommunikation."[12] Again in the same paragraph he says, "Das Amnion eines Einzelembryo kann sich auch direkt in diese Blase öffnen, ohne vorher mit den Amnia andrer Embryonen in Verbindung getreten zu sein."

If we pass from these two statements to an examination of his figures 1 and 2 (plate 17), which are photographs of vesicles showing well-formed embryos, we shall find further evidence of this same nature. The specimen shown in figure 1 is from a vesicle containing 11 embryos, six of which appear in the photograph. On account of the advanced stage of development, the evidence that two embryos have come from a common diverticulum must be sought in the relation of the amniotic canals to the common

[12] Loc. cit., p. 314.

amniotic vesicle. Curiously enough it is found in two or three instances that the canals of two embryos unite and enter the vesicle as a single tube, and in one case at least three canals so unite. His figure 2 presents a still more striking case. It contains 9 embryos, and the embryo lying at the top of the figure, and slightly to the right of the center, sends its canal directly to the vesicle, while the canals from the four embryos on the right enter the vesicle very close together, two of them by a common tube. Likewise, the canals from the four embryos lying on the left enter the vesicle at a common point. Indeed, they apparently unite just before reaching the vesicle.

In view of the fact that we have shown that the union of two canals in T. novemcincta is a certain indication of their common origin from a primary bud, I believe we are justified in drawing a similar conclusion from the conditions to which we have just called attention in T. hybrida. And I venture to predict that when Fernandez shall have secured intermediate stages, he will be able to confirm this conclusion.[13]

It does not follow, of course, that in Mulita only two primary buds will be found to appear, for while in T. novencincta the polyembryonic process in the ovum is extremely stable, as expressed by the constancy with which litters of quadruplets appear, in T. hybrida, on the contrary, variability characterizes this process. Hence, there are no good reasons why in this species, regions on the ectodermal vesicle which correspond to those unoccupied by the two primary buds in the vesicle of novemcincta might not give rise to new primary diverticula. If this be found to be the the case it would in no wise nullify our conclusion regarding the origin of polyembryony among the armadillos; that is that it began in the ancestors by the formation of twins. Whether all of the species which might show such a primitive condition are

[13] After the above was written my attention was called to a report, in the Journal of the Royal Microscopical Society, June 1913, page 279, of Fernandez's communication at the Ninth International Congress of Zoologists. From the brief statement given it seems clear that Fernandez has observed exactly the same method of embryo formation in Mulita that I have described for T. novemcincta, that is, the embryonic primordia arise as diverticula. He states that the diverticula "*become the primordia of embryos, either directly or after division.*" (Italics my own).

now extinct is, of course, not known; but it can be stated that some of the living species, other than T. novemcincta and T. hybrida, are known to give birth sometimes to a single young, and at other times to two individuals.

3. Polyembryony and duplicate twins and double monsters

Another interesting problem with which polyembryony is concerned is that of the origin of duplicate twins in the human species. There is a vast literature bearing on duplicate twins and various types of double monsters, and several different theories have been advanced to account for their production. The reader is referred to Wilder's ('04) extensive paper in which the more important references are cited, and in which the leading theories are discussed.

It is well at first to distinguish between the different kinds of twins, 'duplicate twins' and 'fraternal twins', understanding by the former those cases in which the two members of a pair are supposed to have come from a single egg, and by the latter those supposed to be the product of two distinct eggs. This distinction is by no means an artificial one, but is based upon a considerable amount of data. It is supported not only by the general physical appearance of the members of a pair, but also by the intra-uterine relationships of the two members. In fraternal twins the two individuals do not resemble each other any more closely than do the several individuals of a litter belonging to a normally multiparous animal, and the intra-uterine relationship of two chorions indicate that their origin is from two distinct eggs. In duplicate twins the individuals are enclosed within a single chorion, and their close resemblance to each other is often so striking that it has gained for them the name of 'identical twins.' Furthermore, duplicate twins are invariably of the same sex, while fraternal twins may be of the same or of different sex.

Wilder points out that this fundamental principle upon which the distinction between duplicate and fraternal twins is based, also holds in multiple births involving more than two individuals, and that it can be extended to include cases of duplicate twins and similar combinations in other forms.

As to the origin of duplicate twins, Wilder advocates the blastotomy theory, believing that it is the result of a total separation of the first two blastomeres of the single egg. In case the blastomeres fail to separate completely, symmetrical double monsters (dislopagi) result. In this connection he says, "The double monsters of which we have authentic record are sufficiently numerous and diverse to represent every stage from that of the otherwise normal individual with a doubling of certain of the median parts, to that of two complete duplicate twins with a slight connection between them."[14] Finally, be believes that unequal duplicate monsters (autosite and parasite) are the result of a secondary fusion (due to the great contiguity) of two embryos which were at first duplicate twins.

The contention that duplicate twins and double monsters arise from a single egg is undoubtedly sound, but the conclusion that their origin is the result of a complete or a partial separation of the first two blastomeres, is, I believe, open to question. However, in the absence of any study in the early stages of these sporadic cases of polyembryony, and in view of the results from experiments on artificial blastotomy, this conclusion seemed both natural and logical. In the study of the embryology of the armadillo we have a most excellent opportunity to put the blastotomy theory to test; for here is a mammal with a simplex type of uterus, and one which habitually reproduces by polyembryony. I am therefore bold enough to suggest that the conclusions which I have drawn concerning the origin of the embryos in this mammal may also apply to cases of duplicate twins and double monsters in the human species. And I am encouraged to make this suggestion because of the recent discoveries in the human ovum (e. g. the Bryce and Teacher ovum, '08), in which the condition of the ectodermal vesicle is shown to be such as to require no great stretch of the imagination to picture how diverticula might arise from it, and thus initiate the development of two or more embryos. Nor is there any greater mental strain in accounting for the origin of composite monsters in this way than is required in the hypothet-

[14] Loc. cit., p. 462.

ical juggling of blastomeres to account for the various relationships and positions assumed between the components of these monsters.

4. Polyembryony and sex

One of the obvious biological bearings that the study of polyembryonic development has revealed concerns heredity, including the heredity of sex. The illuminating studies of McClung ('03), Stevens ('06), Wilson ('05–'12), Morgan ('09) and many others, including the recent excellent paper on Ancyracanthus by Mulsow ('12), have shown that in a large number of animal forms the heredity of sex is in some way bound up with certain co-called sex chomosomes, and that, as a consequence, the sex of a given individual is irrevocably fixed at the time of fertilization, or in the case of an unfertilized or parthenogenetic egg, not later than the time the egg starts to develop.

It is in this connection that polyembryonic development furnishes a very strong confirmation of the modern cytological view of sex heredity. For in every authenic case of polyembryony among dioecious species all of the individuals arising from one egg are invariably of the same sex; that is they are either all males or all females, never mixed. The most logical conclusion that can be drawn from these facts is that the sex character is stamped upon the egg prior to the origin of the several individuals to which it gives rise. That the sex of the egg is determined as early as the time at which development begins, seems certain in the case of parasitic hymenoptera. It will be recalled that Silvestri ('06) has shown in Litomastix (and he thinks that is is almost certainly true in Ageniaspis) that the fertilized egg produces females only, while the unfertilized egg gives rise to males only, exactly as in the well known case of the bee. Here fertilization or the lack thereof determines the sex of the offspring, and, no matter how many individuals the egg may produce by polyembryonic development—and in Litomastix the astonishingly large number of about 3000 may develop—they are all of the same sex.[15]

[15] Except, of course, the so-called asexual larvae—the origin and development of each needs further study.

It might be argued that the identity of sex among the several individuals of a polyembryonic litter is the result of similarity of environment. But here again the facts of fertilization in the development of Litomastix completely disproves the idea that external influences are in any way causal factors in sex determination, at least in this parasitic insect. Furthermore, in the case of the development of the armadillo the four embryonic primordia early become separated from each other, each embryo becoming enclosed within its own amniotic envelope, and in a great measure acquiring its own individual environment. All of this takes place long before the sexual organs develop, indeed, long before the so-called 'hermaphroditic stage' of the embryo appears. If external factors play any role in sex determination, it is difficult to understand, under the conditions obtaining in the armadillo, why litters containing both male and female individuals are never produced.

The study of polyembryonic development in Litomastix also calls to mind another important fact, viz., that polyembryony has nothing to do with the actual determination of the sex of the egg. This conclusion becomes evident if one considers the different sexual conditions which exist in the several species exhibiting polyembryony. It occurs in the typical dioecious species, like the armadillo, in which the fertilized egg produces all females or all males, it occurs in the parasitic hymenoptera, in which the fertilized egg produces females, and the unfertilized egg males; and finally, it occurs in hermaphroditic forms, like the earth worm, and also (probably) certain cyclostomatous Bryozoa.[16]

Polyembryonic development may obtain, therefore, no matter whether the egg be fertilized or not, or whether it is destined to bring forth a progeny of unisexual or one of bisexual individuals. Let us repeat, then, that polyembryonic development is not to be considered as a causal factor in sex determination. The facts of polyembryonic development adds, rather strong corroborative evidence to that of cytology, namely, that the sex potentiality of the egg is fixed at a very early stage of development—doubtless in all ordinary cases, at the time of fertilization.

[16] Robertson ('03) states that Crisia geniculata and C. cornuta (or edwardsiana, according to her later identification, '10) are probably monoecious.

SUMMARY

The main facts brought out in the descriptive part of the paper may be summarized as follows:

1. The arrangement of the folds of the uterine mucosa is such that there is formed a distinct cross-shaped area of comparatively smooth mucosa at the tip of the fundus. The center of the cross is the attachment zone for the embryonic vesicle, and its right or left arm, depending on which ovary functions, forms the pathway along which the vesicle travels from the Fallopian tube to the point of attachment (fig. 21, pp. 561–563).

2. The armadillos breed during October and the early part of November. A large majority of the old females are pregnant before October 15, but the second year females may continue to breed for some time after this period. The young are born in March and April, but an occasional litter may appear in February. The period of gestation is estimated at one hundred and forty days (p. 564).

3. Except in very rare instances, young females born in March or April do not breed during the succeeding fall (p. 564).

4. A 'period of quiescence' of the blastocyst was discovered. This period lasts for at least three weeks, and is probably similar to the quiescent period of the blastocyst of the deer described by Bischoff (pp. 564–565).

5. The right and left ovaries function with equal frequency (p. 567).

6. In no case has more than one ovum been found in the uterus (p. 567).

7. The youngest specimen obtained was a typical mammalian blastocyst, consisting of an outer trophoblastic layer of polygonal cells, and an inner cell-mass of embryonic cells (fig. 6, pp. 571–572).

8. The entoderm differentiates before implantation occurs, and arises through a migration of entodermal mother-cells from among the embryonic ectodermal cells of the inner cell-mass. These entodermal cells, migrate, either directly or after having undergone division, to the under surface of the mass, where they

first form a fenestrated structure which later is transformed into a continuous layer (figs. 8–14, 36–42, pp. 572–585).

9. After the entoderm becomes a continuous layer, it splits from the ectoderm, and its free margin passes beyond the limits of the ectodermal mass until the area covered by the entoderm equals an arc of about 80 degrees on the circumference of the blastocyst. The margin of the entoderm now unites with the trophoblastic wall. The remaining 280 degrees of trophoblastic wall never becomes lined with entoderm (figs. 15, 47, 48; p. 585).

10. The blastocyst becomes attached at the embryonic pole to the uterine wall. (figs. 16, 17, 43–46, 49, 50; pp. 585–587).

11. After the entoderm is split from the embryonic ectoderm, the latter rounds up into a spherical mass, which upon parting company with the trophoblast, pushes into the cavity of the vesicle and becomes included in the entodermal layer (figs. 15–17, 43-46, 48–50; pp. 587–590).

12. The ectodermal vesicle is formed by a vacuolization process, which results in disintegrating the core of the ectodermal sphere. When complete, the ectodermal vesicle consists of a single layered pole turned toward the Träger, and a uniformly thick pole, which faces the now inverted entoderm or yolk-sac (figs. 16, 17, 18, 43–46, 49–51; pp. 590–593).

13. During the process of inclusion of the ectodermal mass, there is created an extraembryonic cavity, which lies between the Träger and the endothelial-like pole of the ectodermal vesicle (figs. 17, 18, 49–51; pp. 592–593).

14. The extraembryonic mesoderm arises through cell proliferations from the ectodermal vesicle. These proliferations occur around the entire vesicle, at the angle formed by the vesicle and the entoderm, where the latter turns out to join the trophoblast. The mesoderm cells are given off in groups, which are quickly transformed into vesicular-like structures that fuse together to form a continuous lining or mesothelium for the extraembryonic cavity (figs. 19, 20, 55–60; pp. 593–595).

15. On the right and left sides of the ectodermal vesicle, the primary diverticula or buds appear from thickened areas that

have arisen through the shifting of cells from the thick pole of the vesicle (figs. 1, 21, 22, 59–61; pp. 598–603).

16. Soon after its origin, each primary diverticulum gives rise to two secondary diverticula or buds. One of these buds apparently is but an extension from the tip of the primary diverticulum, while the other takes its origin from the left-lateral portion of the tip of the diverticulum. The embryonic buds extend toward the Träger down along the inner side of the entodermal portion of the blastocyst wall as tube-like processes, which involve not only the thickened lateral plates of ectoderm, but also portions of the thin endothelial-like wall of the vesicle (figs. 23–29, 62–74; pp. 603–610).

17. The part of the ectodermal vesicle which remains after the embryonic tubes are given off becomes the common amniotic vesicle. It retains for some time connections with the embryonic tubes by means of the amniotic canals, which are differentiated from the proximal parts of the original diverticula. The characteristic paired condition of litters of T. novemcincta is the result of the method by which two secondary buds arise from each of the primary diverticula. The common amniotic vesicle eventually degenerates and disapppears (figs. 5, 80–84; pp. 611–613).

18. Each embryo differentiates within the secondary diverticulum, deriving its ectoderm from a portion of the lateral plate which was carried down into the diverticulum, and its entoderm in loco from the primitive entodermal sac or yolk-sac. The embryonic mesoderm arises from a typical primitive streak region in each embryonic primordium (figs. 75–84; pp. 614–617).

19. The region of the so-called Rauber's layer forms the seat of attachment of the blastocyst. This region is soon transformed into a syncytium, from which the embryonic nuclei pass over into the mucosa, destroying it by their phagocytic or histolytic action (figs. 31–33; pp. 617–619).

20. The more superficially situated nuclei of the embryonic syncytium organize the Träger epithelium, which, together with the overlying mesothelial layer, forms the lower portion of the chorionic wall. The Träger proper, which forms a thickened annular zone about the base of the attached vesicle, gives rise

to the villi of the definitive placental discs. Villi also appear on the Träger epithelium but they remain more or less rudimentary (figs. 34, 35, 62–65; pp. 619–625).

Marine Biological Laboratory
Woods Hole, Massachusetts
August 10, 1913

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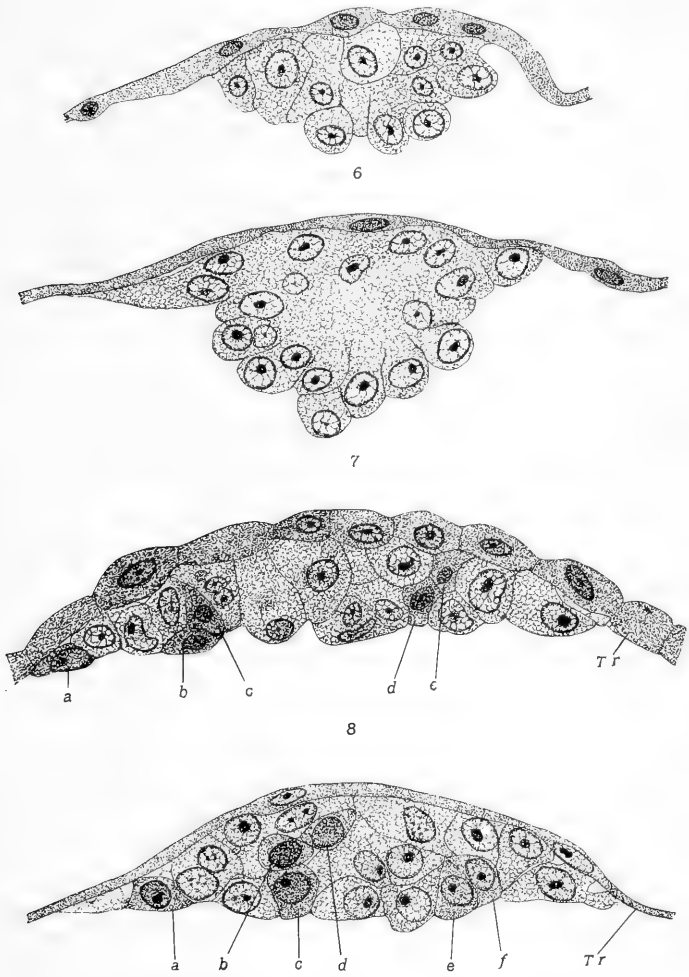
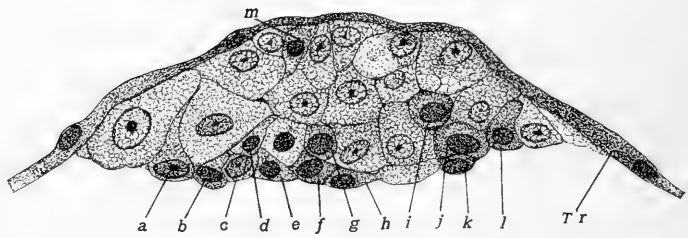


Fig. 6 Median section of the inner cell-mass of specimen No. 287. × 913.
Fig. 7 Median section of the inner cell-mass of specimen No. 310. × 913.
Fig. 8 Median section of the inner cell-mass of specimen No. 335. The letters *a* to *e* indicate the entodermal mother cells. × 728.
Fig. 9 Median section of inner cell-mass of blastocyst No. 244. × 728.



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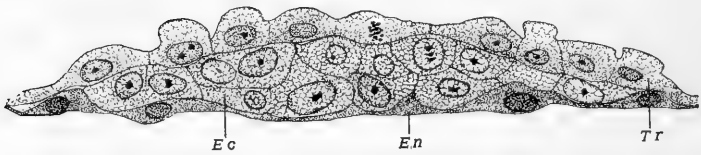

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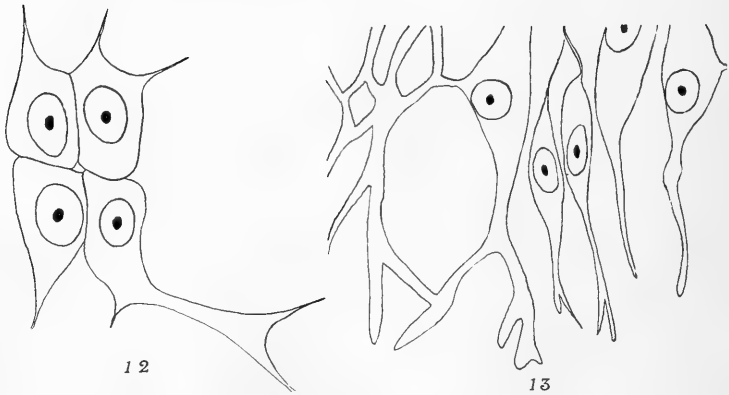

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Fig. 10 Median section of the inner cell-mass of blastocyst No. 296. This specimen shows with special clearness the formation of the entoderm. × 728.

Fig. 11 A section taken slightly to the side of the center of the inner cell-mass of blastocyst no. 311. × 596.

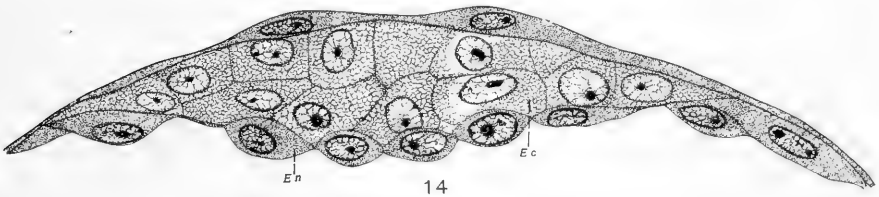
Fig. 12 Outline of the marginal entodermal cells from blastocyst No. 300.

Fig. 13 Marginal entodermal cells from blastocyst No. 320 (see plate 1).

Fig. 14 Median section of the inner cell-mass of blastocyst No. 249. × 1190.

Fig. 15 Median section of the embryonic spot of blastocyst No. 340. × 1190.

Fig. 16 A section passing through the center of the ectodermal mass of specimen No. 316. The sections are not quite perpendicular to the surface of the mucosa, and consequently the point of attachment does not show in this section (see plate 2). × 218.



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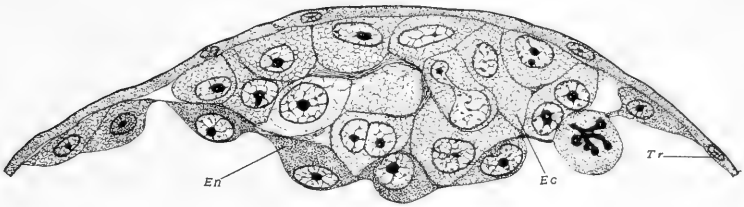

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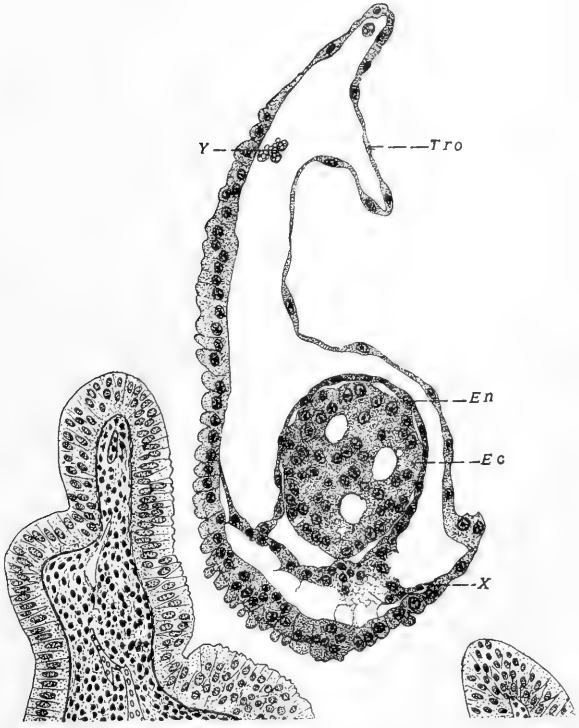

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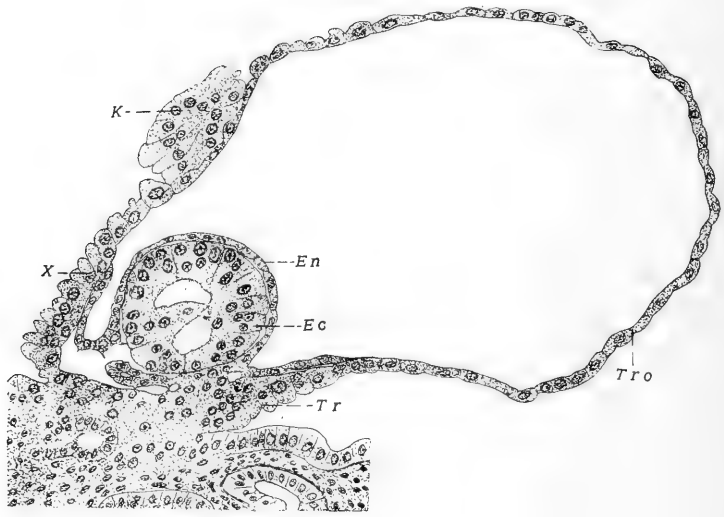

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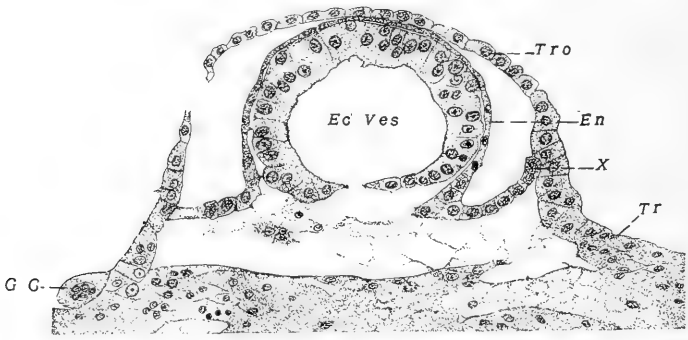

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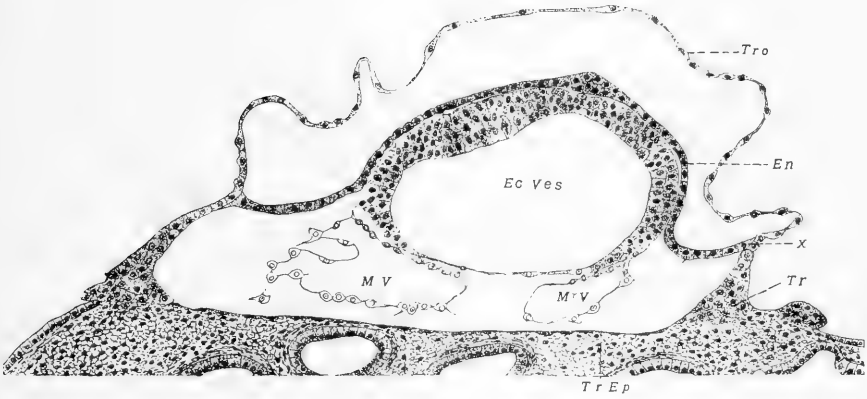
Fig. 17 Median section of the ectodermal mass of specimen No. 332. This specimen is just a little older than the preceding, and shows with great clearness the manner in which the ectodermal mass is included within the entoderm. At *k* is a knot-like growth from the trophoblast. Note that the ectodermal mass is undergoing vacuolization to form a vesicle. × 245.

Fig. 18 Median section of specimen No. 233. This shows the condition just at the close of the formation of the ectodermal vesicle (see text for description). × 162.



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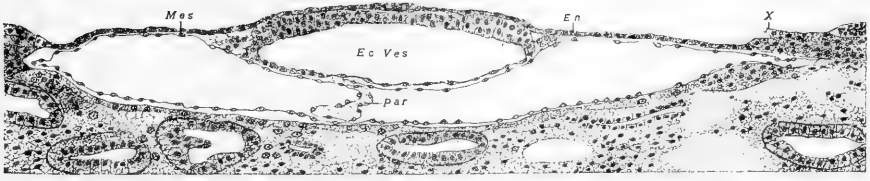

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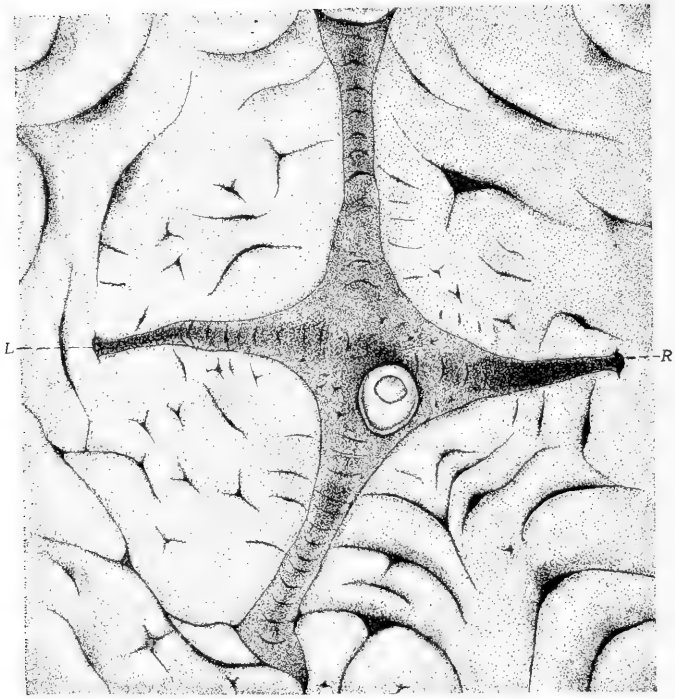
Fig. 19 Median section in a right-left plane of specimen No. 234. The development of the mesoderm has made considerable progress, and the smaller of the two vesicles shows at '*x*' the point where the entoderm joins the chorionic ectoderm (trophoblast). × 142.

Fig. 20 A section similar to the preceding, from specimen No. 256. The vesicle has collapsed and thus appears very much flattened in the figure. The chorionic ectoderm has sloughed off and disappeared. The mesodermal vesicles have expanded until they now fill up the entire extraembryonic cavity. × 113.



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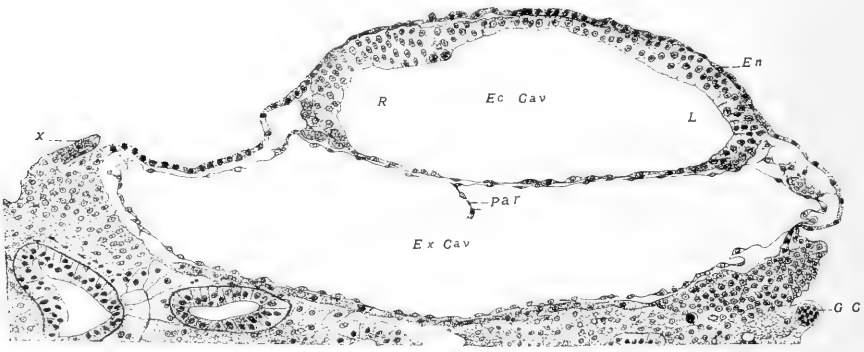

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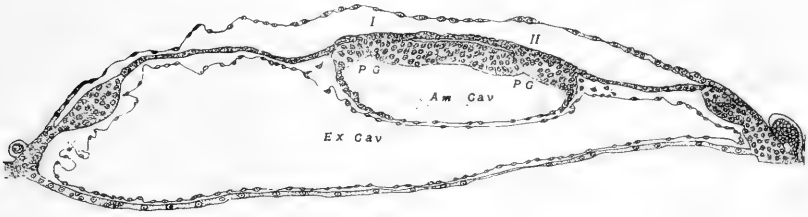
Fig. 21 Drawing of the inner surface of the fundus end of the uterus, from specimen No. 247. It shows the cross-shaped area, in the center of which is an attached vesicle. The vesicle has come from the right ovary and is attached on the right side of the placental area. × 14.5.



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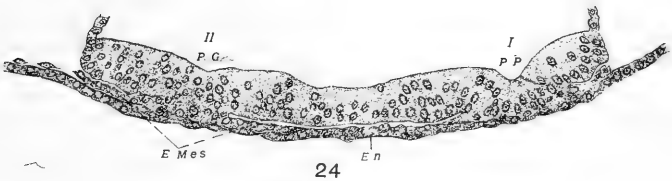

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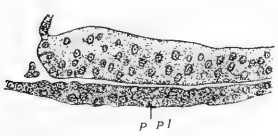

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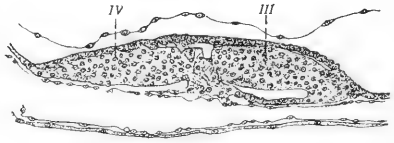

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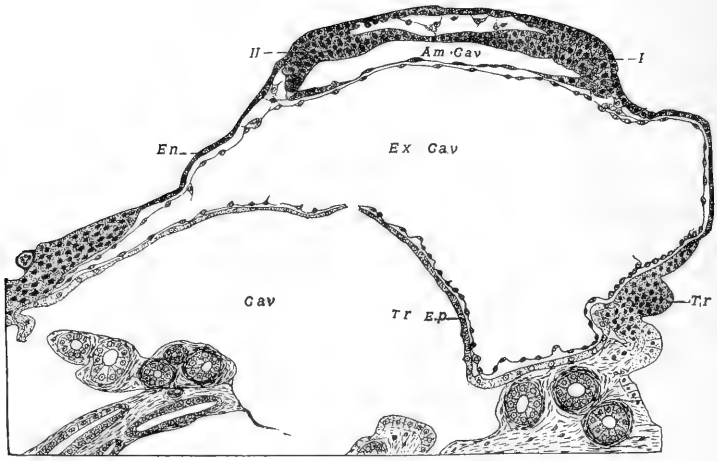
Fig. 22 A median section through plane *a* to *b*, figure 1. The right and left bays (*R* and *L*) of the primary buds are well shown in the figure. A remnant of the partition which previously separated the two mesodermal vesicles is seen at *par*. × 130.

Fig. 23 The section through *e* to *f* of figure 2. It shows the transverse section across Embryos I and II. This specimen shows a rather rare condition for a blastocyst so far advanced in development, in that the chorionic ectoderm has not as yet disappeared. However, it shows many signs of degenerating. × 71.

Fig. 24 Enlarged view of the embryonic portion of the ectodermal vesicle of the preceding figure. The figure is inverted in order to bring the dorsal sides of the embryonic primordia uppermost. The primitive streak region of each embryo is proliferating mesodermal cells (*E. Mes.*). In the case of Embryo I the section cuts across a pit-like depression (*P.P.*) of the primitive groove. × 153.

Fig. 25 A portion of the section passing through the anterior end of Embryo II. It shows the entoderm beginning to thicken preparatory to a proliferation of mesodermal cells. This region of the entoderm corresponds to the protochordal plate of Hubrecht. × 153.

Fig. 26 A section across the tips of the secondary buds of Embryos III and IV. × 153.



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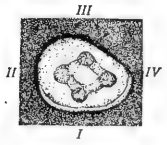

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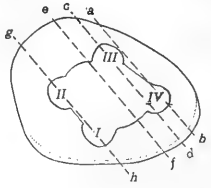
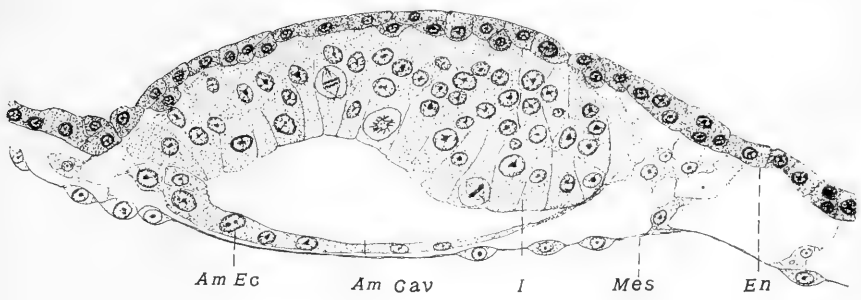


29

Fig. 27 A section through *g* to *h* of figure 29. The buds of Embryos I and II are shown in section. The enlargement of the cavity (marked *Cav.*) lying below and to the left of the extraembryonic cavity is an artifact, caused by the lifting up of the Träger epithelium from the mucosa. × 79.

Fig. 28 Sketch of the blastocyst No. 175, showing the four embryonic rudiments. × 14.5.

Fig. 29 Diagram of the same specimen. The broken parallel lines show the planes of the sections illustrated in plate 10. × 29.



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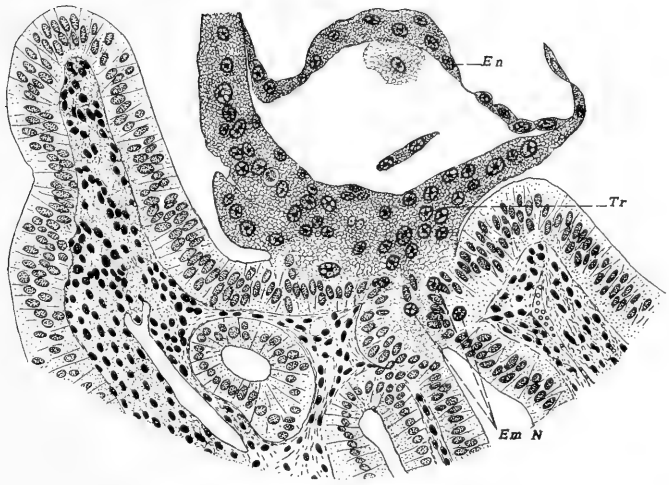
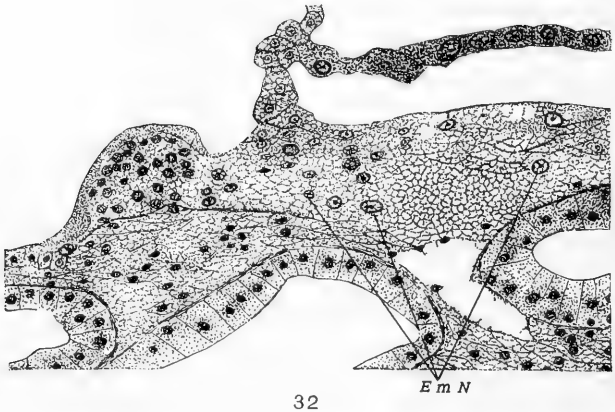


31

Fig. 30 Detailed drawing of a transverse section across bud I of specimen No. 257. × 312.

Fig. 31 Section through the Träger region of blastocyst No. 316. × 271.



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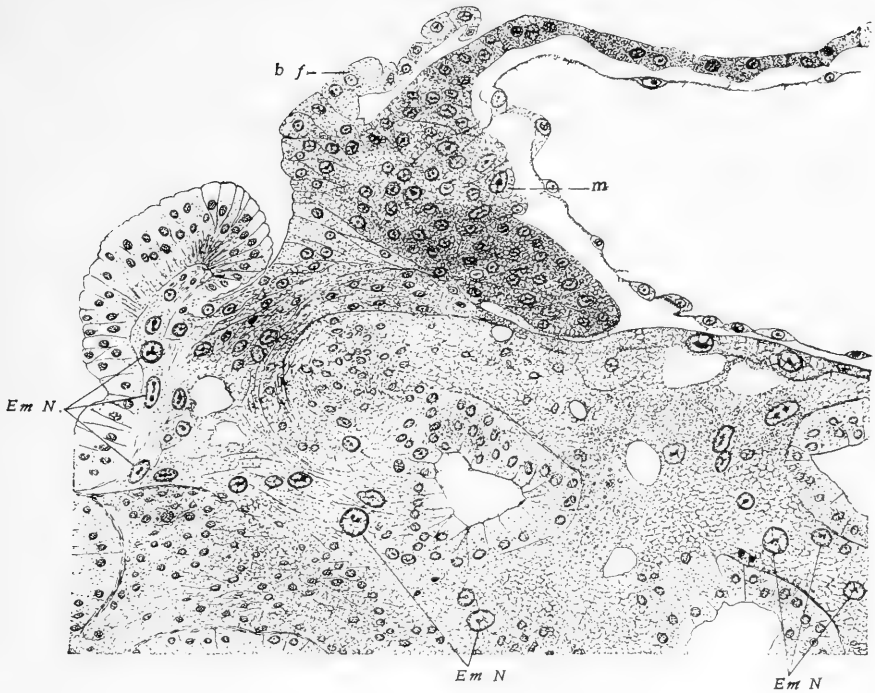
33

Fig. 32 A portion of the left side of the section shown in figure 51. × 306.

Fig. 33 A similar region from the section shown in figure 58. *D.C.*, cells in mitosis; *G.C.*, giant cell, which is frequently found at the base of the blastocyst At *m* is the beginning of a mass of Träger cells which later extends up to receive the entoderm. × 306.

Fig. 34 A portion of the left side of the section shown in figure 60. The mass at *m* now carries the entoderm, while the outer layer of Träger cells is seen as the basal fragment (*b.f.*). Note that the vacuoles are beginning to appear in the syncytium. × 319.

Fig. 35 A portion of the right side of a section from specimen No. 175, plate 8. The Träger epithelium (*Tr.Ep.*) is quite well organized, and root-like growths of Träger tissue are seen extending into the mucosa at *Em.* × 485.



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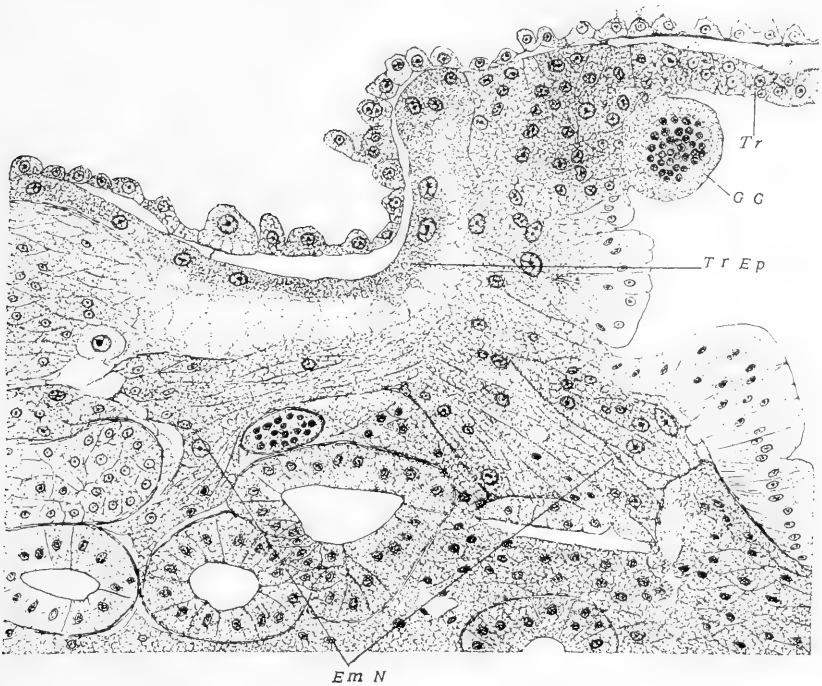


35

DESCRIPTION OF PLATES

All of the photographic figures shown in these plates were made directly from the negative without retouching. The photographs were made by the use of the Bausch and Lomb combination micro-photographic and drawing apparatus. Various combinations of Zeiss objectives and oculars were used in the work. In practically every case the magnification is given after the description of the figure.

ABBREVIATIONS

C.A.V., common amniotic vesicle
Ec., ectoderm
En., entoderm
E.Mes., embryonic or primitive streak mesoderm
Em.N., embryonic nuclei
Ec.Ves., ectodermal vesicle
Ec.Cav., ectodermal cavity
G.C., giant cell
Mes., mesothelium
M.V., mesodermal vesicle
P.G., primitive groove
P.P., primitive pit
Tr., Träger
Tr.Ep., Träger epithelium
Tro., trophoblast
I, ventral embryo
II, right-lateral embryo
III, dorsal embryo
IV, left-lateral embryo

PLATE 1

EXPLANATION OF FIGURES

36 Median section of blastocyst No. 310. This shows the knob-like inner cell-mass lying against the thin trophoblast. × 186.

37 Median section of blastocyst No. 296. The deeply staining entodermal cells are seen in the act of migrating to the lower or inner side of the cell-mass. × 186.

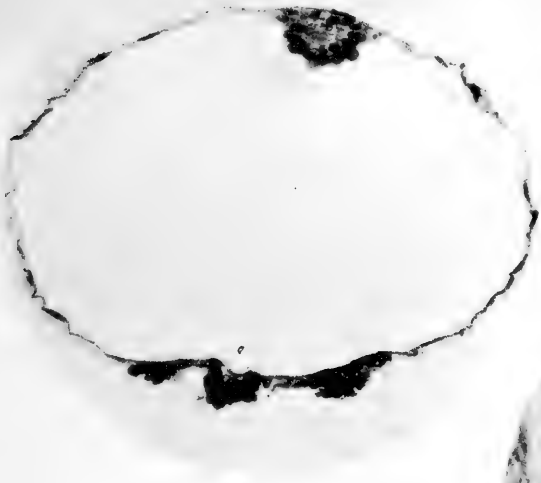
38 Median section of blastocyst No. 335. Note that the trophoblast has not yet become thinned out or attenuated. × 186.

39 Enlarged view of the inner cell-mass of the blastocyst shown in the next figure. It clearly shows the pseudopod-like processes extending out from the marginal entodermal cells. × 225.

40 Upper view of blastocyst No. 320. From an unstained glycerin jelly preparation. × 90.

41 Median section of blastocyst No. 249. The section does not lie flat on the side, and since the microscope was focused on the trophoblastic cells, the inner cell-mass is slightly out of focus. Note how sharply Rauber's layer stands out from the mass. × 417.

42 Enlarged view of the inner surface of the entoderm of blastocyst No. 300.



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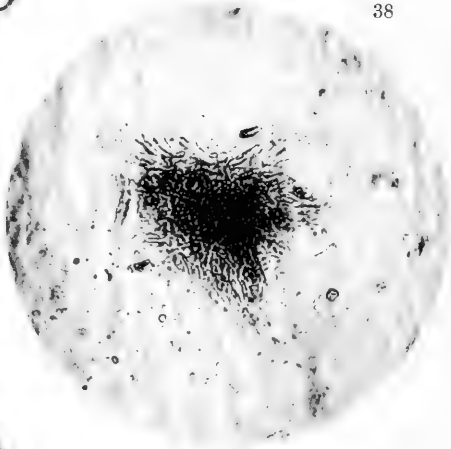

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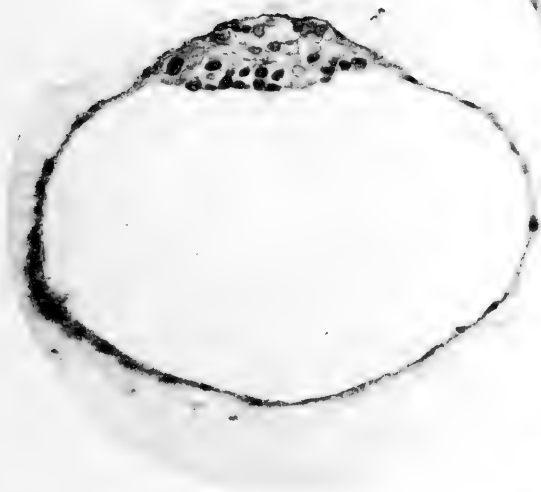

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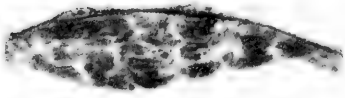

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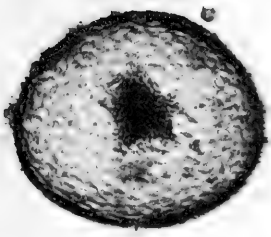

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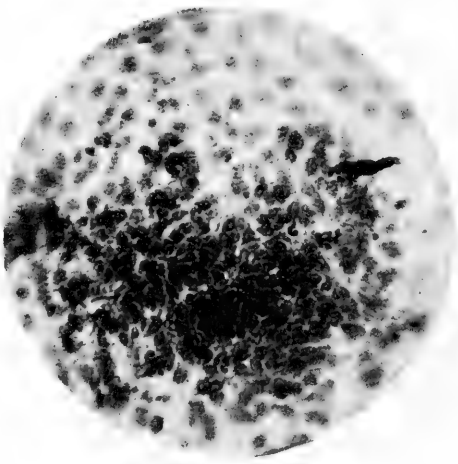
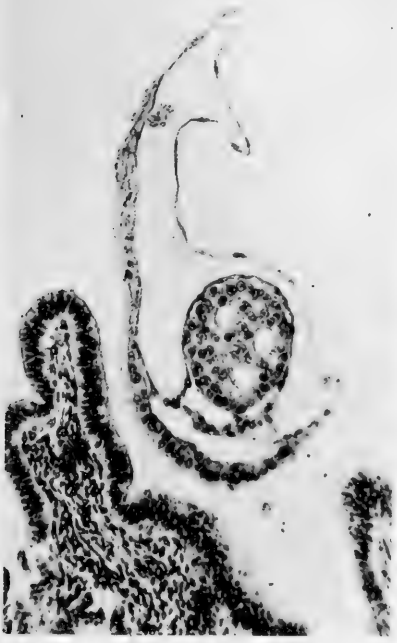


42

PLATE 2

EXPLANATION OF FIGURES

43 to 46 A series of four sections from specimen No. 316 (see text for descriptions). × 148.



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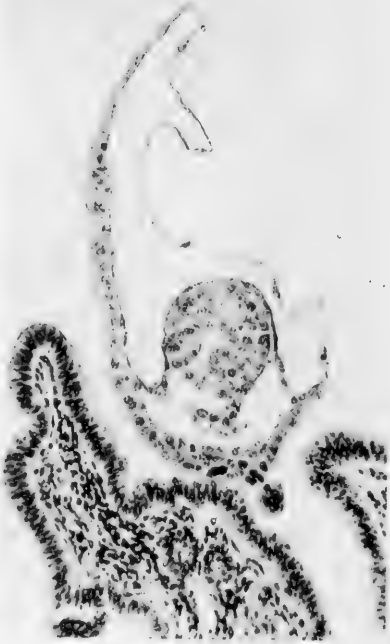

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PLATE 3

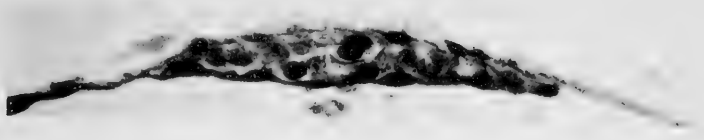
EXPLANATION OF FIGURES

47 Portion of a section from blastocyst No. 311. It shows the well-organized layer of entoderm, which takes a deeper stain than does the overlying ectoderm. × 417.

48 Similar section from specimen No. 340. This shows the first step in the rounding up of the ectoderm to form a ball-like mass. × 416.

49 Median section of blastocyst No. 332. The ectodermal mass has become included within the entoderm. × 149.

50 This section is taken four sections to the right of the preceding, and shows with especial clearness the vacuolization of the ectodermal mass to form a vesicle. × 149.



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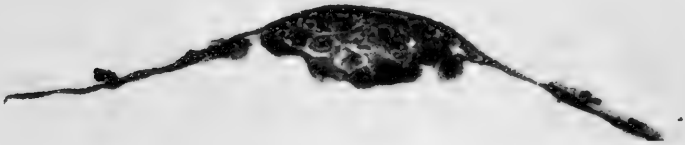

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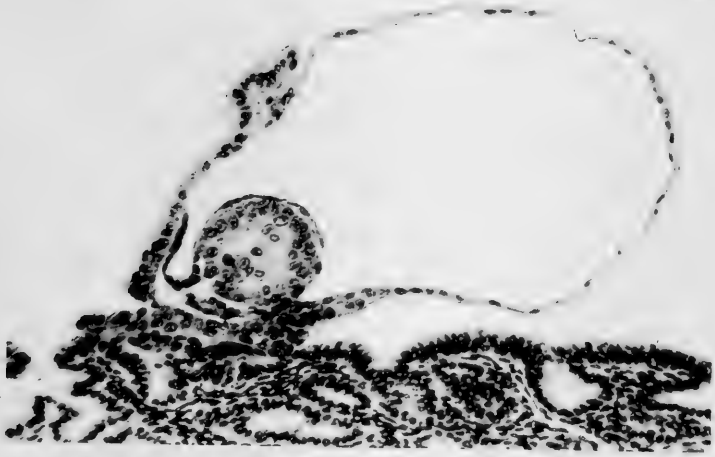

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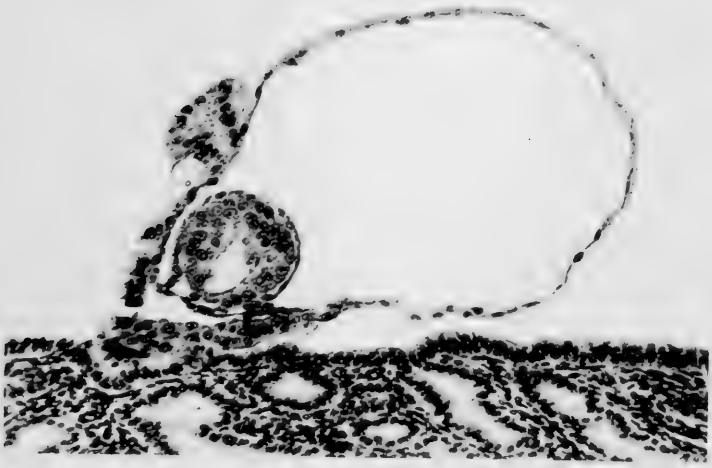

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PLATE 4

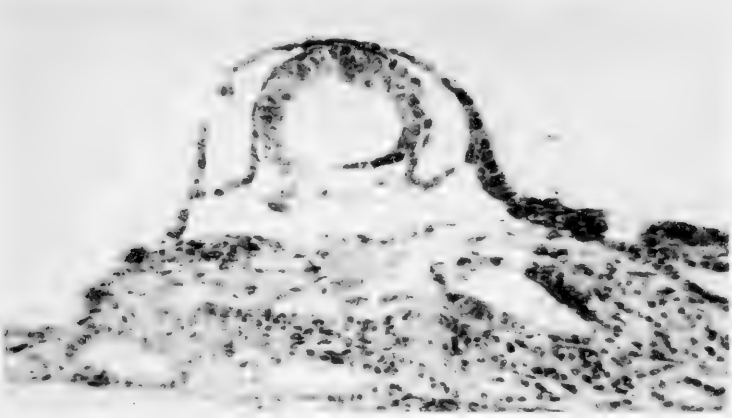
EXPLANATION OF FIGURES

51 Median section of specimen No. 233. The ectodermal mass has become transformed into a vesicle, the lower wall of which has a small pore. × 167.

52 This section lies ten sections to the right of the preceding. × 167.

53 Section from blastocyst No. 329. This specimen was unfortunately crushed in transportation from the field to the laboratory, but the general relations of the different parts can be made out. × 167.

54 Section from specimen No. 289. The relation of the different parts is particularly clear in this section. Note that the large extraembryonic cavity, which lies between the ectodermal vesicle and the mucosa, is entirely free from cells. × 85.



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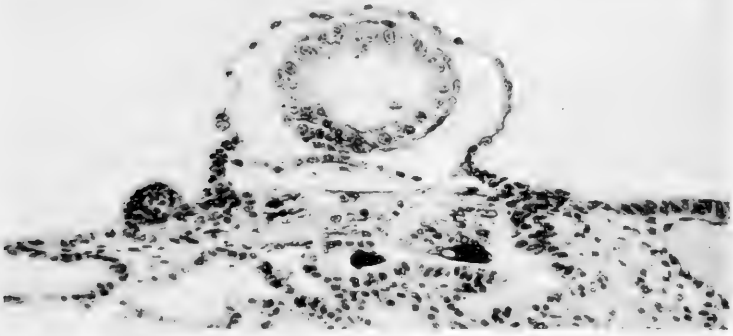

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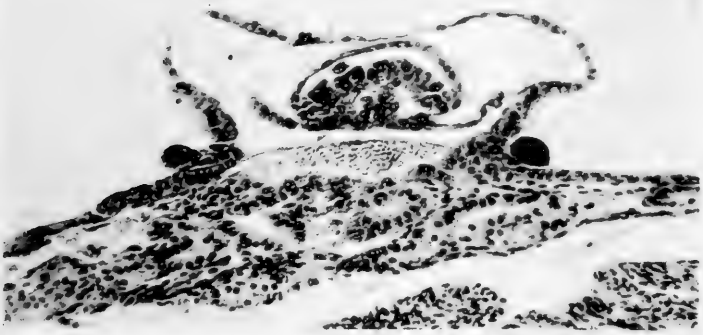

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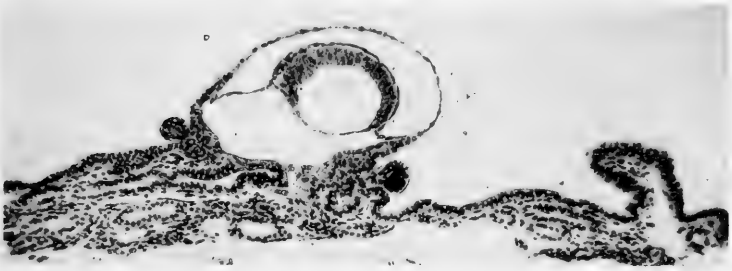

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PLATE 5

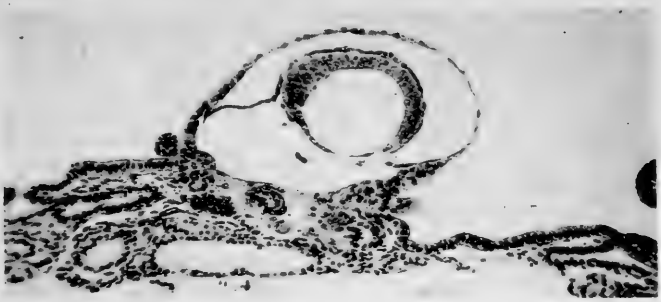
EXPLANATION OF FIGURES

55 Median section of specimen No. 289. The two deeply-staining cells lying at about the center of the extraembryonic cavity are degenerating entodermal cells the single cell situated toward the right side of this cavity is of ectodermal origin, and indicates the beginning of a cell proliferation which will give rise to the extra-embryonic mesoderm. × 106.

56 Median section from specimen No. 298. It shows an early stage in the development of the mesoderm, which appears in the form of small vesicles. × 85.

57 A section lying four sections to the left of the preceding, and showing well-formed but small mesoderm vesicles. × 106.

58 Median section, taken in a right-left plane, of No. 234. This shows two relatively large mesodermal vesicles, which have been formed by a fusion of smaller vesicles, such as appear in the preceding figure. × 106.



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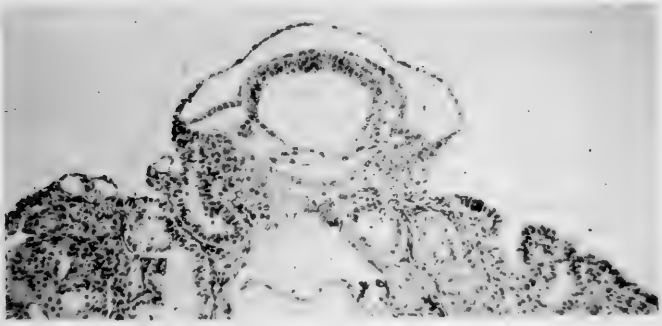

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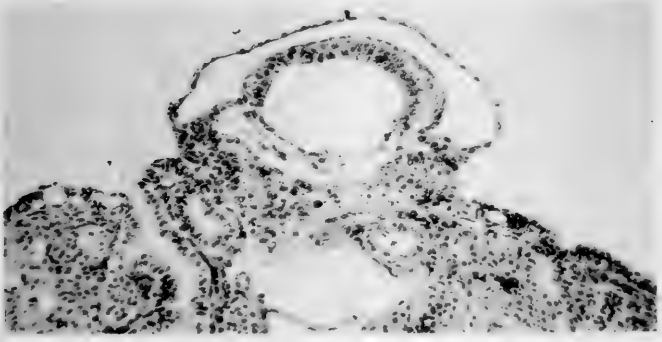

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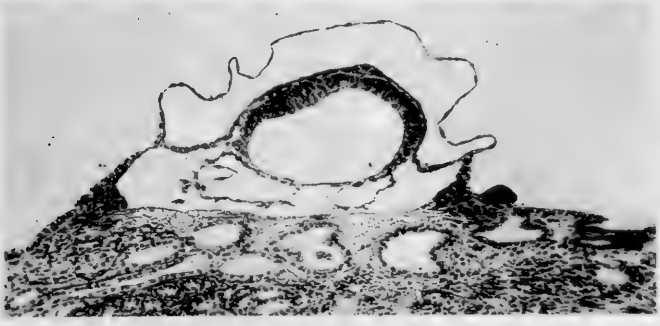

58

HELIOTYPE CO., BOSTON.

PLATE 6

EXPLANATION OF FIGURES

59 Median section, taken in a right-left plane, of specimen No. 256. The two mesodermal vesicles have expanded until they now fill up the entire large extra-embryonic cavity; but their adjacent walls still remain, forming a double-walled partition between the cavities of the vesicles. × 95.

60 Median section of specimen No. 247. It passes through plane *a* to *b* of figure 1. × 90.

61 A section taken through plane *c* to *d* of figure 1. × 90.

59

60

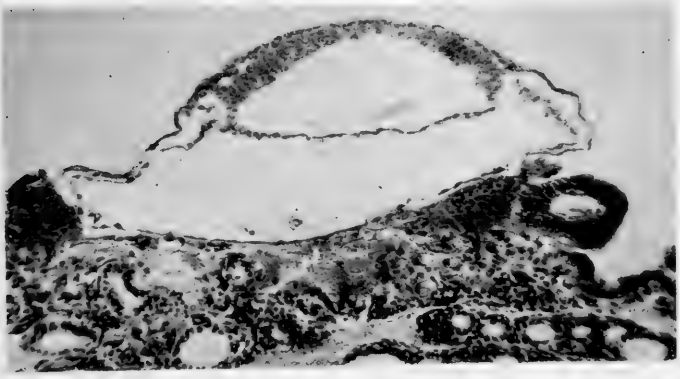

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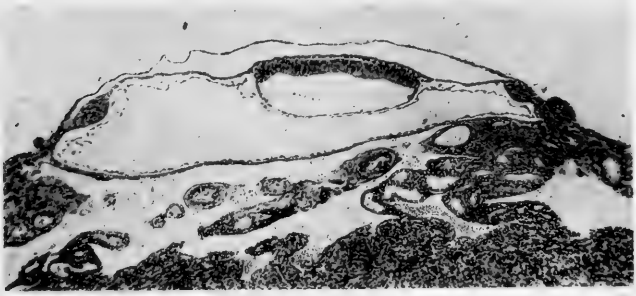
PLATE 7

EXPLANATION OF FIGURES

62 to 65 A series of four sections from specimen No. 290; all × 46.
62 Taken through plane *a* to *b*, figure 2.
63 Taken through plane *c* to *d*, figure 2.
64 Taken through plane *e* to *f*, figure 2.
65 Taken through plane *g* to *h*, figure 2.

62

63



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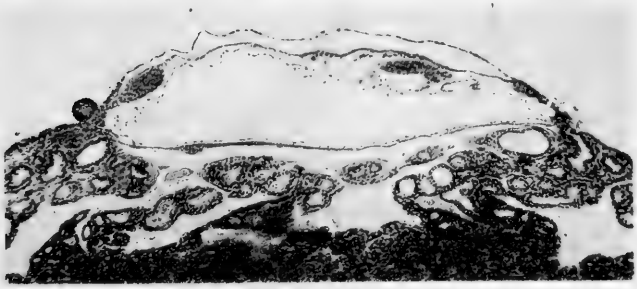

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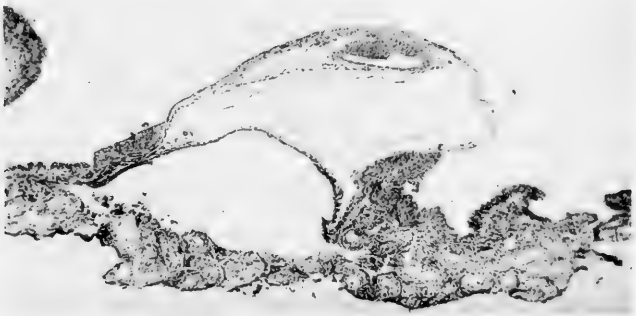
PLATE 8

EXPLANATION OF FIGURES

66 to 69 A series of four sections from specimen No. 175; all × 56.
66 Taken through plane *a* to *b*, figure 29.
67 Taken through plane *c* to *d*, figure 29.
68 Taken through plane *e* to *f*, figure 29.
69 Taken through plane *g* to *h*, figure 29.



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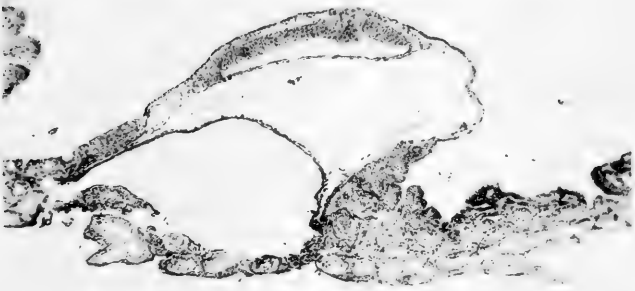

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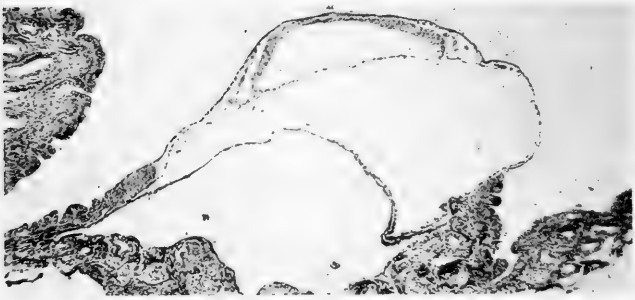

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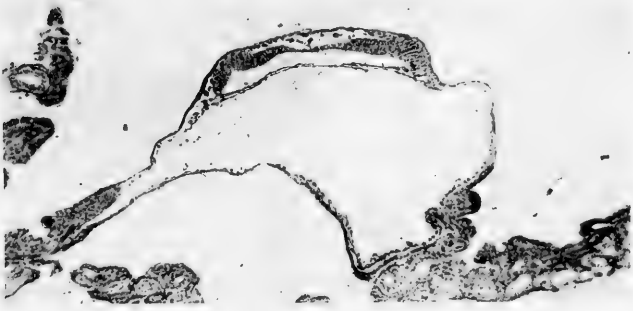

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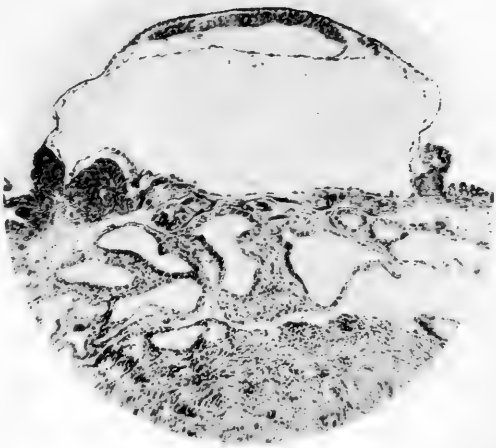
PLATE 9

EXPLANATION OF FIGURES

70 to 74 A series of five sections from specimen No. 257; all × 63.
70 Taken through plane *a* to *b*, figure 3.
71 Taken through plane *c* to *d*, figure 3.
72 Taken through plane *e* to *f*, figure 3.
73 Taken through plane *g* to *h*, figure 3.
74 Taken through plane *i* to *j*, figure 3.

70



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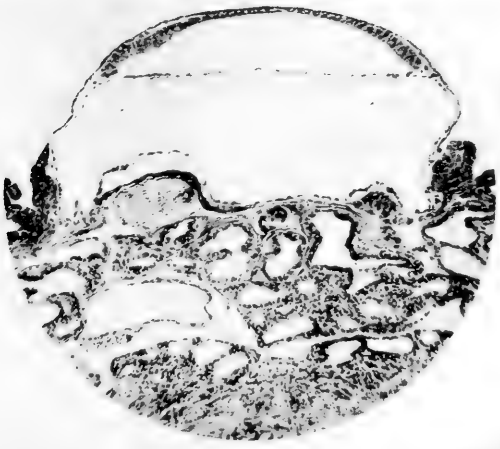

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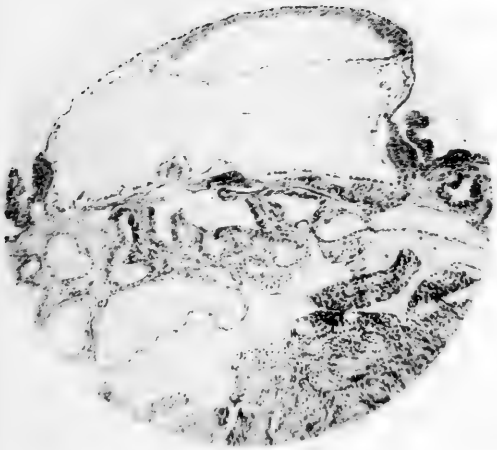

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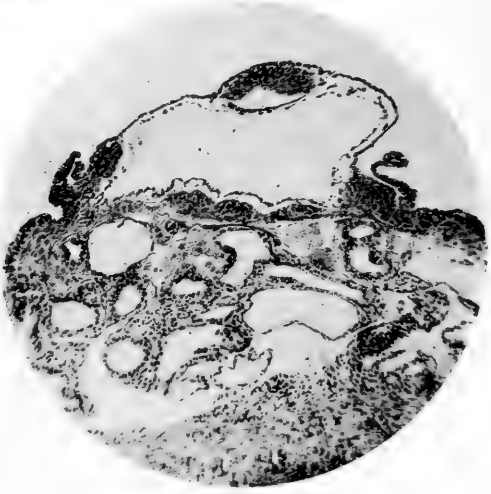

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PLATE 10

EXPLANATION OF FIGURES

75 to 77 A series of three sections from specimen No. 170; all × 75.

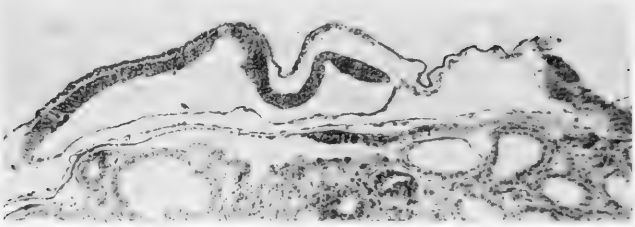
75 Taken through plane *e* to *f*, figure 4.

76 Taken through plane *c* to *d*, figure 4.

77 Taken through plane *a* to *b*, figure 4.

78 Transverse section through the middle of one of the embryos from specimen No. 276 (fig. 5).

79 Litter of four embryos attached to the placentae of the chorion, which has been cut open on the ventral side. The paired arrangement of the embryos is very clearly shown. × ⅓.



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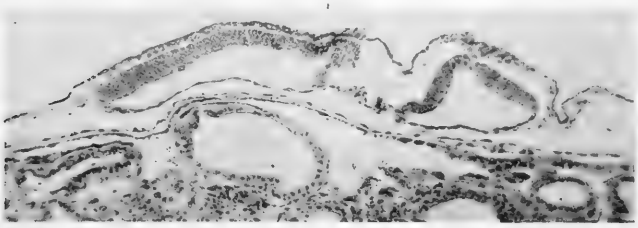

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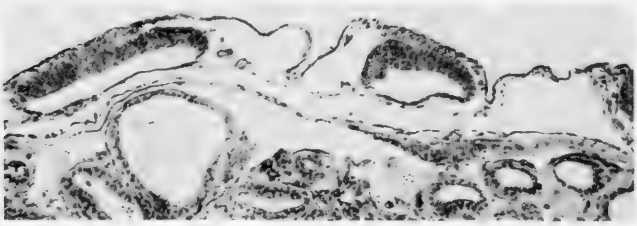

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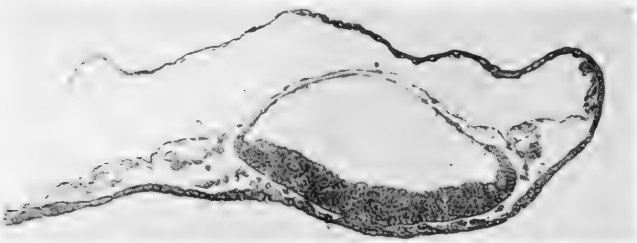

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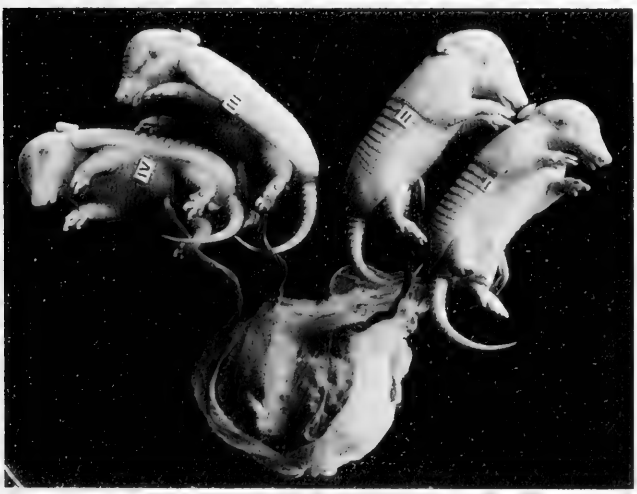

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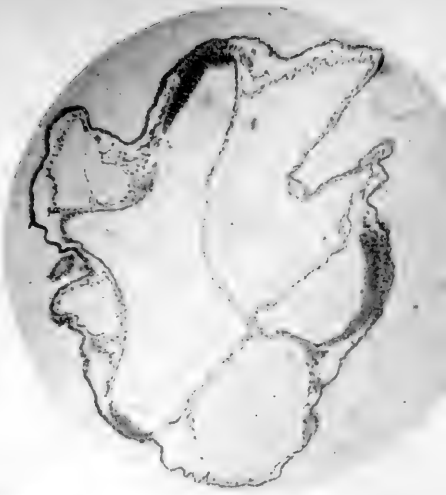
PLATE 11

EXPLANATION OF FIGURES

80 to 84 A series of five sections from specimen No. 226. The sections are cut almost transversely to the long axes of the embryos, that is, parallel to the surface of the mucosa (see text for descriptions). Figures 80 to 81, × 46; figures 82 to 83, × 42; and figure 84, × 37.

80



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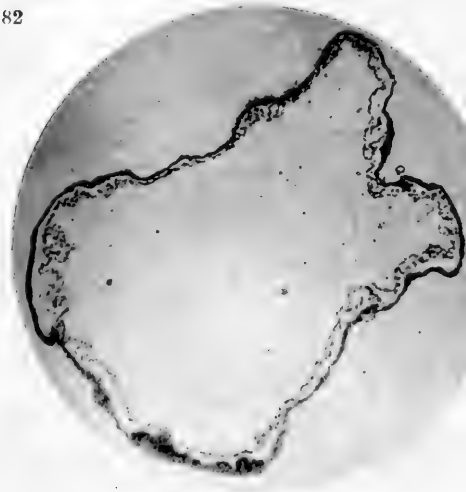

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A STUDY OF THE SUBMENTAL FILAMENTS CONSIDERED AS PROBABLE ELECTRIC ORGANS IN THE GYMNOTID EEL, STEATOGENYS ELEGANS (STEINDACHNER)

ANNA LOWREY

Biological Laboratory of the University of Colorado

FOUR FIGURES

INTRODUCTION

Specimens of Steatogenys elegans (Steindachner) are very rare, since this fish is found only in the tropical rivers of South America. The seven specimens used in this study were obtained by the Gimbel Expedition to British Guiana in 1910. They were caught in the Demarara river by a native woman.

The submental filament consists of a series of saucer-shaped discs, placed parallel to each other, surrounded by a cylindrical sheath of connective tissue, and with a large nerve running between these discs and the sheath. From the minute structure of the discs, and from the relation of the nerve to them, it seems that these discs are electroplaxes, and that each submental filament is an electric organ.

TECHNIQUE

The fishes were killed in formaldehyde and 85 per cent alcohol. The submental filaments were embedded in 56° C. paraffin, and serial sections, six micro-millimeters thick, were made. Both transverse and sagittal series were examined, the two filaments for comparison being from the same fish. All sections were stained by Heidenhain's iron hematoxylin method and counter stained with licht grün.

DESCRIPTION OF THE FISH

Steatogenys elegans (Steindachner) was described in 1880 from specimens collected in the Barra de Rio Negro, Brazil. More recently it has been taken in the Rio Jurua, Brazil;[1] in the Demarara river, British Guiana;[2] and in the Amazon at Manaos, Brazil.[3]

Like other members of the family of Gymnotidae this fish is found only in fresh waters. It is a small flesh-colored, eel-shaped fish marked with six or more large purplish, wedge-shaped blotches, and distinguished from other gymnotids by two peculiar submental filaments. An average individual is about 125 mm. in length, and 13 mm. in the region of greatest depth. The head is small and chubby, and the small eyes are placed nearer the tip of the snout than the gill-openings. Like all gymnotids of the subfamily Sternopyginae, Steatogenys elegans possesses but pectoral and anal fins. The caudal region terminates in a long, rat-like tail composed of elongated, cylindrical vertebrae. In the submental region there are two fleshy filaments.

THE SUBMENTAL FILAMENTS

These fleshy filaments were discovered by Steindachner who figures them in his original description, and calls them fatty filaments, but offers no explanation of their function, but it has been suggested[4] that these peculiar structures were probably electric organs.

Each filament is about 9 mm. long and 5 mm. broad and terminates in a minute, median cup-shaped depression, located two or three mm. posterior to the anterior margin of the lower jaw. Posterior to this depression the filaments diverge, curving outward and upward to a point just in front of the base of the pectoral fin. Here the filament is anchored and receives a large nerve and blood vessel. Each filament, completely covered by the dermis, lies within a groove.

[1]Boulenger, Trans. Zool. Soc., vol. 14, p. 428, 1898.

[2]Eigenmann, Fresh water fishes of British Guiana, Mem. Carn. Mus., vol. 5, p. 422 et seq., 1912.

[3]Ellis, The gymnotid eels, Mem. Carn. Mus., in press.

[4]Ellis, l. c.

After the removal of the dermis, the filaments appear as small white cylinders which are swung at the base of each operculum, and are attached only at their ends.

The filament consists of an outer cylindrical sheath of connective tissue, arranged in layers. Inside this cylinder is a series of about forty parallel discs placed vertically to the outer sheath of connective tissue which supports the discs, and from this layer small strands of connective tissue support the nerve fibers and blood vessels.

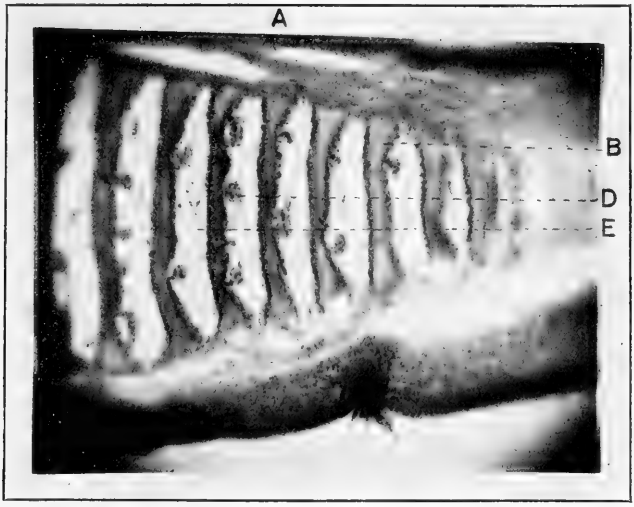

Fig. 1 Sagittal section of submental filament. Photograph. *A*, connective tissue; *B*, electroplax; *D*, papillae on electric surface; *E*, electrolemma.

The main nerve, supplying each filament, enters the organ at a point near the basal angle of the operculum, and traverses the filament in a sinuous path along the ventral side between the connective tissue and the plates. This nerve divides, sending branch nerves to each disc. These branch nerves ascend the posterior surfaces of the discs and cross to the anterior surface of the adjacent discs. Entering the organ with, and paralleling the main nerve, is the main blood vessel which also sends branches to each plate.

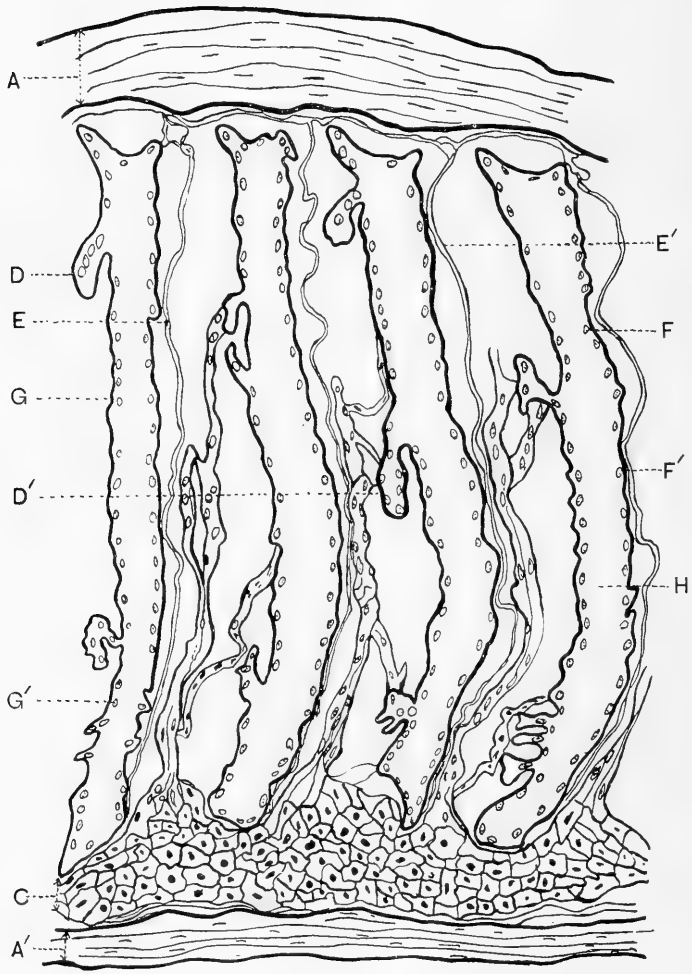

Fig. 2 Diagram of a sagittal section of four electroplaxes. *A*, *A′*, connective sheath; *C*, main nerve; *D*, *D′*, papillae on electric surface; *E*, *E′*, electrolemma; *F*, *F′*, nuclei on the nutritive surface; *G*, *G′*, nuclei on electric surface; *G*, middle layer.

Each disc is more or less bi-concave, with its edge broadened and flattened into a flange. In the ventral margin of each is a semi-circular excavation under which the main nerve passes. Average plates are about 430 micro-millimeters long and 34 micromillimeters thick, the anterior curvature the greater.

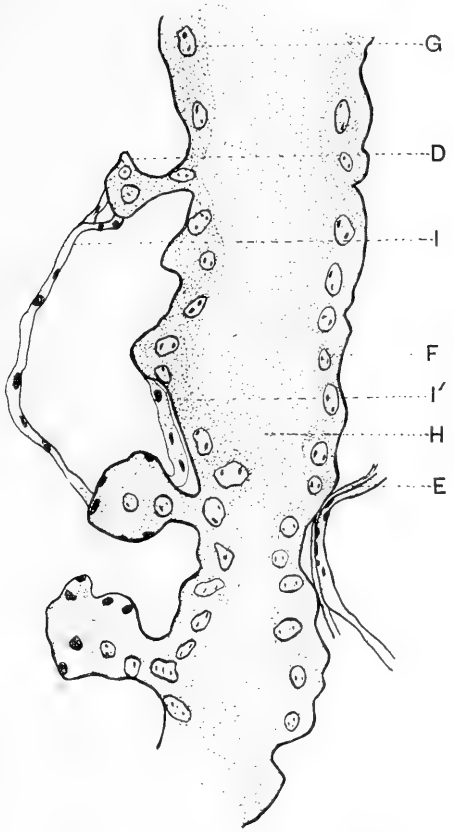

Fig. 3 Portion of an electroplax. Sagittal section; camera lucida. *D*, papillae on electric surface; *E*, electrolemma; *F*, nucleus in nutritive layer; *G*, nucleus in electric surface; *H*, middle layer; *I*, nerve fiber.

In sagittal section of the filament, the plates appear as bars, much the shape of capital I, crossing the space enclosed by the sheath of connective tissue. From this view the structure of the plates can be seen most satisfactorily. Each plate is made up of a central broad syncytium with a row of regularly placed nuclei along each surface. On the anterior surface of each plate there are two or three, sometimes as many as six, fungiform papillae which contain a number of nuclei. Most of these papillae receive a small blood vessel and nerve fiber from the posterior sur-

face of the adjacent plate. The larger of these papillae are lobed, always receiving several branches of the main nerve and blood vessel. The posterior surface of each plate is rather regularly and finely convoluted, the convolutions being occasionally raised into small papillae.

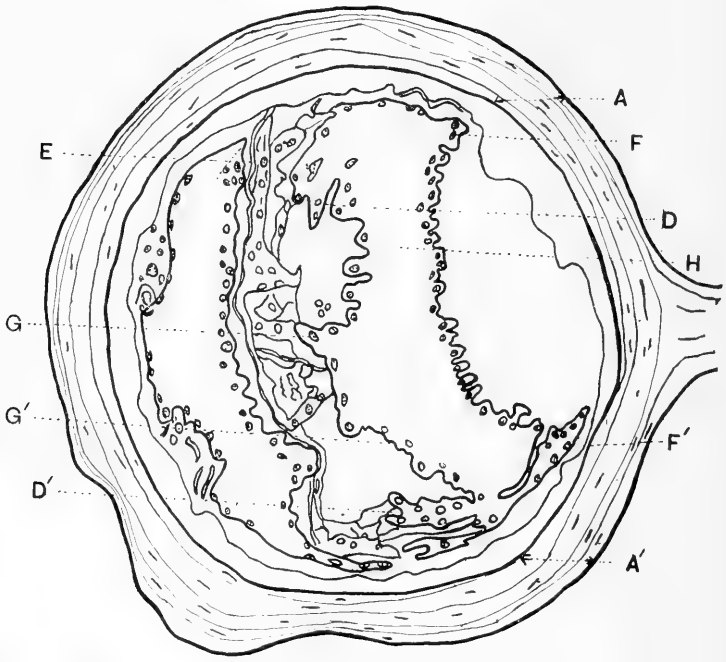

Fig. 4 Diagram of an oblique cross-section of an entire filament. *A*, *A′*, connective sheath; *D*, *D′*, papillae on electric surface; *E*, electrolemma; *F*, *F′*, nuclei on nutritive surface; *G*, *G′*, nuclei in electric surface; *H*, middle layer.

Between each pair of plates is a thin webbing of supporting connective tissue which forms a rather distinct partition in the dorsal ventral regions, but which is arched forward and usually attached to the middle of the posterior surface of the anterior plate. This supporting tissue carries the branches of the main nerve and blood vessel.

COMPARISON WITH KNOWN ELECTRIC TISSUE

Electric organs are found in certain rays of the Atlantic Ocean and southern seas, and in the African Siluroid, Malopterurus electricus. The organs of the Rajas show unmodified electric tissue while those of the Electrophorus electricus possesses a specialized electric tissue.

In Raja ocellata[5] the electric tissues occupy two unmodified regions of the tail muscle, forming a symmetrical, spindle-shaped region on each side. The electric organ is divided into a series of minute spindle-shaped compartments whose dividing walls are connective tissue, which surround a jelly-like mass of connective tissue supporting the electroplaxes.

The electroplax of Raja ocellata is a large, disc-shaped syncytium occupying the entire width of each compartment, not extending the whole length of the compartment, but leaving anterior and posterior spaces filled with connective tissue. The size of these spaces varies with the species. Each electroplax consists of three layers, the electric and nutritive which are continuous around the edge; the middle or striated which forms a core and is the thickest layer. The blood vessels are usually introduced from the nutritive side, and branch in the electric connective tissue. The nerve supply enters the anterior corner or edge of each compartment, lose their medullary sheath, and the fibers begin to divide and subdivide as they approach the electric surface on which they terminate. The electric surface is flat, and the nutritive surface is evaginated into many papillae which contain a portion of the striated layer. The nuclei are found in all three layers, forming a regular close arrangement in the electric layer; sparingly scattered through the striated portion; and irregularly arranged and somewhat smaller in the nutritive layer. Surrounding each nucleus is a mass of granular cytoplasm which connected with the other masses form a separate layer. The syncytium is surrounded by a delicate cell membrane, the electrolemma.

The electric tissue of Electrophorus electricus[6] consists of a number of electroplaxes placed vertically and facing forward to

[5]Dahlgren and Kepner, Principles of animal histology, pp. 105–122, 1908.
[6]Dahlgren and Kepner, l. c.

form several masses of tissue on the sides of the rear of the body. The electroplaxes have a myotome arrangement, with the electric surface facing posteriorly and with the nutritive surface facing anteriorly. In front and behind each electroplax is a layer of connective tissue. The electric surface is evaginated into large, short papillae while the nutritive surface is drawn into short, thick closely-set papillae. Each papilla system is said to be as thick as the middle layer. As in Raja ocellata each electroplax consists of three layers, but in this fish the electric and nutritive layers alone carry the nuclei, and the middle layer forms a non-striated core. The nuclei are near the edge and nearly always in the papillae. The nerve fibers approach the electroplax in the posterior jelly-like connective tissue as medullated fibers which branch and send non-medullated fibers to the ends of the electric papillae.

From the description of the two types of electric tissue, the unspecialized and specialized, electric tissue may be said to possess the following characteristics. It is made up of a series of disc-like electroplaxes or plates, each of which is composed of three distinct layers, the outer ones continuous around the edge and containing regularly arranged nuclei. One or both of the outer layers of an electroplax may be evaginated into papillae. The electric surface always receives the nerve endings. The middle layer is the core to each electroplax and may have the striations omitted. Each electroplax is a syncytium, well supplied with small blood vessels and nerves; is preceded and followed by a space filled with jelly-like connective tissue; and is separated from the others by a delicate cell membrane, the electrolemma. The whole series of plates is surrounded by connective tissue.

In comparing the structure of the submental filaments of Steatogenys elegans with known electric tissue, the following similarities are found: The entire series of disc-like plates described in the filaments of Steatogenys elegans, is surrounded by a general sheath of connective tissue. Each plate of the series consists of three layers, two of which are distinctly nucleated, and one of which receives the nerve endings; each is preceded and followed by a space filled with connective tissue, and is separated

from the others of the series by a delicate cell membrane; and each is well supplied with nerves and blood vessels.

The plates in each filament are arranged as myotomes and placed vertically to the surrounding connective tissue. The individual plates possess papillae on both anterior and posterior surfaces, the surface receiving the nerves possessing the larger. The nuclei are arranged regularly and usually in the papillae of both anterior and posterior surfaces. The middle layer possesses neither striations nor nuclei. These last characters are very similar to those peculiar to Electrophorus electricus.

Since the tissue of the submental filaments of Steatogenys elegans possesses the general characters of the rudimentary electric tissues, as in Raja ocellata, and also of the specialized electric tissue, as Electrophorus electricus, there seems to be little doubt but that this particular tissue is electric tissue. Final proof of this must come from Golgi preparations made from fresh material. The function of this probable electric organ is yet unknown.

ELECTRIC TISSUE OF OTHER GYMNOTIDAE

Since other gymnotid material was at hand, it seemed desirable to look for electric tissue in the myotome region, part of which is occupied by electric tissue in the electric eel, Electrophorus electricus.

By examining cross sections of the entire fish and paraffin sections of tissue from the region corresponding to that occupied by the pseudo-electric tissue of Electrophorus electricus, from five species, Sternopygus macrurus, Gymnotus carapo, Adontosternarchus sachsi, Eigenmannia virescens, and Sternarchus hasemanni, it was found that in or between the first and second muscular units of the ventral portion of the great lateral muscles, there was a slight degeneration of parts of the muscles. The larger units had been reduced to two minute oval muscles embedded in either strands of cartilage, or strands of cartilage and fat, and occupied parts of two triangular spaces, one on each side of the median septum just above the unit which controls the anal fin. No plates, special nerve fibers, or nerve endings were seen.

The cross section of Steatogenys elegans which was taken from the myotome region of the body, showed no modifications of the muscle units.

This study was made in the Biological Laboratory of the University of Colorado during the year 1912–1913. The writer is indebted to Dr. Max M. Ellis for assistance and suggestions.

SUMMARY

1. The submental filaments of Steatogenys elegans are not adipose tissue.

2. These filaments possess all the characters of electric organs.

3. The minute anatomy of the filaments agrees with that of the electroplaxes from known electric tissue.

4. No electric tissue was found in the myotome region of the several species of gymnotids examined.

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SUBJECT AND AUTHOR INDEX

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Sept. 2, 1915.