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# ASCIDIANS FROM THE COASTS OF CANADA.

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(*Read 8th April*, 1911.)

## The Ascidians of the Marine Biological Stations of Canada.

The writer has spent a portion of each summer for the last three years at the Marine Stations, in 1908 and 1909 at Departure Bay, British Columbia, and in 1910 at St. Andrews, New Brunswick. As the collections and observations were made during a very short time in each year, little knowledge has been acquired as to the annual and seasonal variations. However, sufficient has been learned to give some idea of the forms that are available for study at the two stations.

(A). The Atlantic Station.

The month of July, 1910 was spent at St. Andrews. Several rocky points were visited repeatedly at low tide and much material obtained. The most favourable places are rocky ledges on precipitous shores, where can be found plenty of flat stones. The lower surfaces of these stones are usually covered with Ascidians. The shores on either side of the station wharf and the shore of Sand Reef Point were the most productive of those that were visited. All the species that were found at low tide, occurred in the dredgings as well, but the majority of them could be obtained more easily and in greater quantity at low tide.

The facilities at the station and the willing co-operation of the station staff made possible a large amount of dredging. The writer was especially indebted to Dr. Stafford, the curator of the station, for generous assistance in every way, both in dredging and in other collecting. Dredgings were made at various points (1) in the St. Croix River, (2) in Passamaquoddy Bay, (3) in the approaches to Passamaquoddy Bay (Letite Passages, Quoddy River, Indian River &c.), (4) near L'Etang and (5) out in the Bay of Fundy around and near the Island of Grand Manan. It was only in the last locality that any Ascidians were obtained from muddy or sandy bottoms. Nearly everywhere, they were obtainable on hard bottom, stones, shells and gravel.

Of Compound Ascidians, *Amaroucium glabrum*, *Tetradidemnum* [*Leptoclinum*] *albidum* and *Holozoa* [*Distaplia*] *clavata* were generally

distributed. Two colonies of *Aplidium spitzbergense* were obtained near Grand Manan. *Didemnopsis tenerum* occurred at Grand Manan and in the approaches, though sparingly.

Of the *Cionidae*, a single specimen of *Ciona intestinalis* was dredged near Grand Manan. Verrill has reported it as abundant in the Bay of Fundy.

Of Phallusiids [Ascidiids], *Ascidiopsis prunum* was obtained in quantity at low tide and considerable numbers were taken in the dredge. *Phallusioides obliqua* was common at Grand Manan and a few specimens were obtained in the approaches, but none near the station.

Of the *Chelyosomatidae*, a single specimen of *Chelyosoma macleayanum* was dredged in the approaches.

Caesirids [Molgulids] are very abundant. At low tide were found *Caesira papillosa*, *C. littoralis*, *C. canadensis* and *C. retortiformis*. In addition to these, the dredgings gave in the approaches, at L'Etang and near Grand Manan a fifth species, *C. pannosa*. At the last locality, a number of *Eugyra pilularis* were dredged in 10 fathoms, sand.

Of Styelids, *Dendrodoa carnea* was rather numerous at low tide, and occurred sparingly, but well distributed, with *Goniocarpa placenta*, in the dredgings. Two specimens of *Cnemidocarpa mollis* and numbers of *Pandocia fibrosa* were found near Grand Manan.

The Tethyids [Cynthiids] are well represented. *Boltenia ovifera*, *B. hirsuta* and *Tethyum pyriforme americanum* occurred in large numbers at low tide and were found generally distributed on hard bottom.

To sum up, the following are obtainable in fairly large numbers near the station:—

*Amaroucium glabrum*,
*Tetradidemnum albidum*,
*Holozoa clavata*,
*Ascidiopsis prunum*,
*Caesira papillosa*,
*C. littoralis*,
*C. canadensis*,
*C. retortiformis*,
*Dendrodoa carnea*,
*Boltenia ovifera*,
*B. hirsuta*.
*Tethyum pyriforme americanum*.

The following can be procured less easily (those found at Grand Manan only are not included):—

*Didemnopsis tenerum*,
*Phallusioides obliqua*,

*Chelyosoma macleayanum*,
*Caesira pannosa*,
*Goniocarpa placenta*.

In addition to the above species, the following have been found or are to be expected in this locality, but were not obtained in 1910:—

*Aplidium pallidum* Verrill,
*Lissoclinum aureum* Verrill,
*Botrylloides aureum* Sars,
*Pelonaia corrugata* F. & G.

The fauna is subarctic, consisting of species that are peculiarly subarctic, most of which are closely related to, if not identical with, European species, and other species that are found in both subarctic and arctic regions or that have their nearest allies in arctic regions.

(B). The Pacific Station.

From June to August of both 1908 and 1909 were spent at the Departure Bay Station, and in the latter year three days were spent at Ucluelet on the outer coast of Vancouver Island as the guest of Prof. Macoun.

At the station, the best collecting places at low tide are the precipitous shores of the small rocky islands in the bay. The roofs of small caverns, the under surfaces of projecting rocks and the under surfaces of flat stones that are to be found on many of the rocky ledges are the favourite spots. A considerable amount of dredging was done at various depths ranging to about 25 fathoms. The curator of the station, the Rev. G. W. Taylor, assisted me in every way and through his courtesy I enjoyed several dredging trips to Northumberland Straits on the other side of Nanaimo and one trip to Burrard Inlet near Vancouver. The best dredging places were the channels, where the bottom was stony, shelly or gravelly, although the sandy bottoms frequently yielded an abundance of a few species. Much of the bottom gives poor results, because of the absence of stones, &c. of a size that can be brought up by the dredge, although these same bottoms are doubtless well populated with Ascidians.

*Compound Ascidians.* A species of *Amaroucium* occurs in quantity at low tide and is occasionally dredged. A very dark *Tridemnum* with few or occasionally no (?) spicules was taken several times in the dredge.

*Cionidae. Ciona intestinalis* occurs rather frequently as shown by the dredgings, but not in quantity.

*Phallusiidae. Ascidiopsis columbiana* was growing in large numbers at low tide and was occasionally dredged. *A. paratropa*, a large handsome species, occurs sparingly in 10 fathoms or more and 3 specimens of *A. nanaimoensis* were found outside the bay and at Northumber-

land Straits. *Phallusia ceratodes* grows in large beds in the bay south of Brandon Rocks and also in a sponge bed near the entrance to the bay. Elsewhere only occasional specimens were obtained.

*Chelyosomatidae. Chelyosoma productum* is occasional at low tide and abundant in deeper water. *C. columbianum* was found in from 10 to 20 fathoms but was not abundant. *Corella inflata* was growing in numbers near low water mark and *C. rugosa* in deeper water.

*Caesiridae. Caesira apoploa* and *C. cooperi* were dredged in sand in about 10 fathoms in front of the station.

*Styelidae. Katatropa vancouverensis* and *Cnemidocarpa joannae* were abundant at low tide and the latter occurred frequently in deeper water. *Goniocarpa coccodes* was dredged in small numbers from stony and shelly bottoms and with it was found *Styela gibbsii*. The latter was very abundant in many places in from 5 to 15 fathoms, sand.

*Tethyidae. Boltenia villosa* was growing in quantity at low tide and was abundant in the dredgings. *Pyura haustor* was found in large masses in from 5 to 20 fathoms, sand and occasionally elsewhere. *Boltenia echinata*, *Tethyum aurantium* and *T. igaboja* were obtained only very rarely and in from 10 to 25 fathoms, stones and shells.

The list for this station is as follows:—

(1) in quantity.

*Amaroucium* sp. A,
*Ascidiopsis columbiana*,
*Phallusia ceratodes*,
*Chelyosoma productum*,
*Corella inflata*,
*C. rugosa*,
*Katatropa vancouverensis*,
*Cnemidocarpa joannae*,
*Styela gibbsii*,
*Boltenia villosa*,
*Pyura haustor*.

(2) occasional.

*Tridemnum* sp. A,
*Ciona intestinalis*,
*Ascidiopsis nanaimoensis*,
*A. paratropa*,
*Chelyosoma columbianum*,
*Caesira apoploa*,
*C. cooperi*,
*Goniocarpa coccodes*,
*Boltenia echinata*,
*Tethyum aurantium*,
*T. igaboja*.

The completion of the railway from Nanaimo to Alberni will make it possible to reach the outer coast of the Island in a short time by means of the railway and the Alberni Canal. This is of importance, as the fauna of the outer coast appears to differ markedly from that of the inner coast. The following account is based on collections made at Ucluelet on Barkley Sound near the mouth of the Alberni Canal by Prof. Macoun and his assistants in 1909 and by myself at the same point at the end of July and the beginning of August of the same year.

The rocks at low tide on the exposed coast are rich in Ascidians, especially the compound forms. The following were found:—

(1) in quantity.
*Amaroucium* sp. A,
*Synoicum* (?) sp. A,
*Trididemnum* sp. B,
*Sycozoa* [*Colella*] sp.,
*Holozoa* [*Distaplia*] sp. A,
*Polycitor* (*Eudistoma*) sp. A,
*Clavelina sp.*,
*Perophora annectens*,
*Katatropa yakutatensis*,
*Styela montereyensis*.

(2) occasional.
*Corella rugosa*,
*Chelyosoma productum*,
*Caesira pacifica*,
*Katatropa vancouverensis*,
*Cnemidocarpa joannae*,
*Boltenia villosa*,
*Pyura haustor*,

Dredgings made in a few fathoms (5 to 10) yielded the following:—

(1) in numbers.
*Amaroucium* sp. A,
*Synoicum* (?) sp. A,
*Trididemnum* sp. B,
*Clavelina* sp.,
*Ascidiopsis columbiana*,
*A. paratropa*,
*Corella rugosa*,
*Chelyosoma productum*,
*Caesira apoploa*,
*Cnemidocarpa joannae*,
*Boltenia villosa*.

(2) occasional.
*Amaroucium* sp. B,
*Synoicum* (?) sp. B,
*Leptoclinum* [*Diplosoma*] (?) sp.,
*Holozoa* [*Distaplia*] sp. B,
*Polycitor* (*Eudistoma*) sp. B,
*Katatropa uclueletensis*,
*Styela gibbsii*,
*Pyura haustor*,
*Tethyum aurantium*,
*T. igaboja*.

Dall has remarked that the fauna of the inner channels of the British Columbian archipelago is of a distinctly more northern character than that of the open coast. This is well shown in the Ascidians. The list from Departure Bay includes arctic forms that are not represented at Ucluelet and among the Ucluelet species are a number of southern forms that do not occur at Departure Bay. It may be noticed that the arctic species of Departure Bay are not as plentiful and are not found in as shallow water as the corresponding species of the Atlantic Coast at St. Andrews.

(C). Some general features of interest.

Material for studying the early development of many of the Ascidians can be obtained very easily, as in many cases the eggs are retained in the parent and only the free-swimming larvae escape. This is the condition of affairs in practically all of the compound Ascidians, (those found at the stations). In the majority of the simple forms the eggs are not retained, apparently because the oviduct opens into the atrium very near the base of the atrial siphon and the strong current present at that point carries the eggs out. In some genera and species the oviduct opens

at some distance from the atrial siphon, where the current is not as great and as a result the eggs are usually retained. This retention of the eggs may be quite constant in a genus, may be characteristic of certain species only of a genus, or may occur only in occasional individuals of a species. In some cases the eggs are, though retained, laid in lots, so that practically only one stage can be obtained from one individual and all the individuals of one locality may have their eggs at the same stage of development. The following is a list of the simple forms of the stations that retain their eggs:—

Genera—*Dendrodoa*, eggs produced continuously.
*Katatropa* " " "
Species,—*Caesira cooperi*, eggs produced continuously.
*C. canadensis*, " " "
*C. littoralis*, " " "
Occasional individuals of
*Ascidiopsis prunum*, eggs produced in lots.
*Corella inflata*, " " "
*Boltenia hirsuta*, (?)

Young individuals, for studying the post-larval development, can be obtained in the case of the commoner species by carefully examining the free surface in individuals of those species which have a roughened test. Individuals, that were anaesthetized with cocaine, killed in the extended condition and well fixed, have furnished me with an abundance of stages of some of the commoner species. A series of sections made of an adult *Dendrodoa carnea*, yielded in addition, (1) an almost complete series of stages of the same species from the fertilized egg up to the free-swimming larvae, (2) a 'young adult' of the same species, (3) two 'young adults' of *Ascidiopsis prunum* and (4) a 'young adult' of some species of *Caesira*!

As is well known, the Ascidians harbour many commensals. *Protozoa* are to be found in the pharynx and atrial cavity in many of the simple forms of both coasts, the majority being attached to the oral tentacles. Various kinds of Copepods and Amphipods are to be found in the same cavities. Pea-crabs occur in the atrial cavity in most specimens of *Tethyum igaboja*, *Ascidiopsis paratropa* and *Phallusia ceratodes* of the West Coast. A hydroid* is abundant at Departure Bay, coating the prebranchial zone of certain species of Ascidians and small colonies were occasionally found on the wall of the atrial cavity. Nearly every individual of *Phallusia ceratodes* contained this form and it was also found in *Ascidiopsis paratropa*, *Ciona intestinalis*, and *Tethyum aurantium*.

*Mr. C. McLean Fraser has recently described this form (Bull. Lab. Nat. Hist. Univ. Iowa, vol. VI, No. 1) as the type of a new genus, belonging to the family *Turridae*. He has given it the name *Crypta huntsmani*.

Parasitic *Protozoa* occur in the glandular folds of the stomach in most species and in the 'liver' of Caesirids [Molgulids] and Tethyids [Cynthiids]. An Isopod was found in the endostylar vessel of *Styela gibbsii* and *Pyura haustor*.

Although Ascidians are not used for food on this continent, there are a number of species that might be so used. In most of the forms the musculature is so small in amount that when the test has been removed the great bulk of the animal consists of sea-water. In the Styelids and Tethyids, however, the musculature is well developed and frequently quite thick. Two species of *Tethyum*, very similar to or the same as those of our coasts, are, according to Oka, eaten by the Japanese. The inhabitants of Peru and Chili use as food two species of *Pyura* that occur on their coasts and species of the genus *Microcosmus* are exposed for sale in the markets of southern Europe (Grube).

## THE HOLOSOMATOUS SPECIES OF THE WEST COAST.

The complete account of these species was sent in July, 1910, for publication in the Report of the Biological Stations of Canada. In the following account it is intended to give provisional diagnoses of the new genera and species, as well as some notes on the other species. The full extent of the variation noted in the various species is not always given in this account. Further study has shown that certain changes should be made in the original account and these have been incorporated in this article.

The writer is indebted to the Rev. Mr. Taylor for a large amount of material from Departure Bay, Hope Island, Banks Island, Goose Island, Lowe Inlet, China Hat, Stephen Island, Port Simpson, Prince Rupert, Rose Spit and Hecate Straits. Prof. Macoun communicated to the writer the collection of the Geological Survey, which contained a few specimens collected by Dr. Dawson in 1885, and a large amount of material from Departure Bay and Ucluelet, collected by Prof. Macoun and his assistants in 1908 and 1909.

Many of the Ascidian genera are inconveniently large and heterogeneous, e.g. *Tethyum* [*Cynthia*, *Halocynthia seu Pyura*], *Styela*, *Caesira* [*Molgula*], and *Phallusia* [*Ascidia*]. It would be a distinct advance to have these genera divided into smaller natural groups, so that the relationships of the species would be shown. I have attempted a division of *Tethyum*, *Styela* and *Phallusia* as far as the material at my disposal would permit. Some of these groups are quite small and it is questionable whether they should have the rank of genera. If they are given generic rank temporarily, it will call attention more forcibly to the characters which seem to be of importance in separating these groups. Many

of these characters are entirely neglected in descriptions of new species. The final determination of their rank may be left until our knowledge of all the species is such as to make revisions of the various families possible.

Family—*Perophoridae.*

*Perophora annectens* Ritter. Proc. Cal. Ac. Sc., ser. 2, vol. IV, p. 37.

In numerous colonies from Ucluelet the zooids differ from Ritter's description only in being yellowish-orange instead of yellowish-green and in having a maximum of 24 stigmata in a row instead of 18. These differences seem unimportant. The individuals in all cases formed typical social colonies and in no case were imbedded in a common test.

Family—*Agnesiidae.*

The genus *Agnesia* Mchlsn. should not be placed in the family *Corellidae* (*Corellinae*) or *Chelyosomatidae* (*Chelyosomatinae*), as has been done by Michaelsen, Seeliger and Hartmeyer. The position of the intestinal canal on the left side of the pharynx is of major importance and shows that its closest allies are the *Cionidae* and *Phallusiidae.* It differs sufficiently from either of these groups to warrant its being placed in a separate family.

*Agnesia septentrionalis* sp. n.

Shape oval, laterally flattened. Dimensions of largest specimen, 15×11×8 mm. Oral aperture terminal, atrial at anterodorsal angle. Surface entirely sand-covered, sand adhering to filamentous processes of the test. Apertures indistinctly 7- and 6-lobed respectively.

Dorsal and ventral bands of transverse muscular fibres in addition to the usual siphonal musculature.

About 30 simple tentacles, varying in size, scattered over inner surface of oral siphon. Dorsal tubercle apparently behind peripharyngeal groove, its aperture transverse and slightly bent. Six very large dorsal languets, with long 'roots.' No longitudinal bars. Transverse vessels carry a number of large sickle-shaped processes. Stigmata forming short infundibula, as many as three turns in each spiral; two rows of infundibula between successive transverse vessels.

Stomach large, smooth-walled. Intestine with the usual forwardly directed loop on the left side of the pharynx.

Ovary a rounded mass in the intestinal loop. Testicular lobes scattered over the intestinal loop near the ovary. Gonoducts accompany rectum.

Collected near Stephen Island in 1906 by Rev. Mr. Taylor.

This form differs from A. *glaciata* Michaelsen (Zoologica, Bd. 12, Ht. 31, p. 6), the only other species described, in details concerning the surface of the test, pharyngeal wall, &c.

Family—*Cionidae.*

*Ciona intestinalis* (L.)

Specimens were taken frequently in and near Departure Bay, but never in quantity. They seem to differ from European specimens only in the small number (5) of muscular bands on each side of the body. To judge from the published figures, the number is variable in European specimens (6 or 7).

Family—*Phallusiidae.*

Genus—*Ascidiopsis* Verrill (*sens. nov.*). (=*Ascidia seu Phallusia auct. partim*).

The type species is *Ascidia callosa* Stimpson (=*A. prunum* Müller). Verrill instituted this genus for the type because of the plicated condition of the pharyngeal wall. This is a character common among Phallusiids and one that cannot be used as a distinctive feature. The diagnosis may be changed so as to include those forms with the pharynx extending beyond œsophageal aperture but not beyond the posterior side of the stomach, longitudinal bars bearing papillæ and intermediate papillæ, and renal vesicles occurring over intestinal loop and in the adjacent parts of the body-wall. Also the ganglion is close to the dorsal tubercle. This genus is intermediate between *Ascidiella* (as restricted by Hartmeyer) and the typical *Phallusiae* (e.g. *P. mentula*).

*A. nanaimoensis* sp. n.

Oblong, laterally flattened; attached by left side. Up to 27 mm. in length. Oral aperture terminal, 7- (or 5-) lobed; atrial, distant from oral from one-third to one-half the length of the body, 6- lobed. Surface nearly smooth (minute papillæ over part of surface). Musculature practically confined to right side of body.

From 45 to 105 tentacles. Prebranchial zone with indistinct papillæ. Aperture of dorsal tubercle crescent-shaped, opening between horns directed forwards. Ganglion is the width of peripharyngeal groove behind tubercle. Dorsal lamina prominently ribbed, its margin with coarse teeth corresponding to the ribs. Longitudinal bars, from 41 to 50 on the left side and from 48 to 66 on the right. Plications fewer than the bars. From 3 to 6 stigmata in each mesh.

Stomach with 17 shallow folds; intestine without typhlosole. Ovary in intestinal loop and on right side of first part of intestine. Testicular lobes in intestinal loop posteriorly, on both sides of first part of intestine and on right side of stomach. Gonoducts pass along posterior side of last bend of intestine.

Three specimens obtained at points near Departure Bay.

This species somewhat resembles in appearance *Ascidia adhærens*

Ritter from Alaska, but differs from it in the number of stigmata in each mesh, in the number of plications between successive bars and probably also in the number of bars.

*A. columbiana* sp. n.

Oblong, laterally flattened, attached by the entire left side. Up to 4.5 cm. in length, 3.5 cm. in width and 2 cm. in thickness. Apertures placed as in last species. Surface more or less roughened, with numerous short papillæ, which differ greatly in size in several varieties of this species which occur. Those near the apertures are always very distinct and longer than the others. Musculature as in last species.

From 20 to 45 tentacles. Prebranchial zone smooth. Aperture of dorsal tubercle horseshoe-shaped, the horns frequently bent inwards or outwards. Ganglion directly behind tubercle. Dorsal lamina strongly ribbed, its margin with teeth corresponding to the ribs and occasionally from 1 to 5 indistinct intermediate teeth. From 19 to 24 bars on the left side and from 21 to 26 on the right. From 1 to 2 plications between successive bars. From 4 to 20 stigmata in each mesh.

Stomach with from 12 to 22 shallow folds; intestine usually with typhlosole. Anus at level of anterior end of intestinal loop or somewhat behind it. Ovary chiefly on right side of first part of intestine. Testicular lobes on left side of loop and on both sides of stomach. Oviduct passes across lower (left) side of last bend of intestine and then along posterodorsal border of rectum.

Numerous specimens from Departure Bay, Ucluelet and Port Simpson.

Differs from *Ascidiella incrustans* Herdman from Puget Sound in the plain prebranchial zone and the toothed condition of the dorsal lamina. *Ascidia adhærens* Ritter is without the papillated surface and the peculiar course of the oviduct. Its closest ally is *A. prunum* (Müller) from the North Atlantic, which differs from it chiefly in the absence of the papillæ of the surface, and in the smaller number of bars (rt. 18 to 20, lt. 15 to 19).

*A. paratropa* sp. n.

Short cylindrical, attached by small area at posterior end. Up to 11 cm. in length and about 4.5 cm. in diameter. Oral aperture terminal, 7-lobed, turned toward the right side; atrial aperture, 6-lobed, at the end of a short siphon which extends from the anterodorsal angle to a point in front of the level of the oral aperture. Surface with large irregular tubercles. Musculature, extensive on the right side, consisting chiefly of longitudinal fibres; on the left side, longitudinal fibres from the siphons extend nearly to intestinal loop.

From 15 to 30 rather short tentacles. Prebranchial zone smooth. Aperture of dorsal tubercle as in last species; horns may be slightly coiled. Ganglion about width of peripharyngeal groove behind tubercle. Dorsal lamina ribbed on left side, its margin irregularly toothed, the largest corresponding to the ribs. On right side of œsophageal aperture and extending posteriorly is another lamina also toothed. From 28 to 34 bars on the left side and from 32 to 42 on the right. From 1 to 1.5 plications between successive bars. From 4 to 12 stigmata in each mesh.

Stomach with from 40 to 46 shallow folds; intestine with typhlosole; intestinal loop directed forwards but not bent toward dorsal side. Ovary in intestinal loop, on both sides of loop anteriorly and on right side of stomach. Testicular lobes in the loop and on both sides of posterior part of loop, as well as on both sides of stomach. Gonoducts pass along posterior side of last bend of intestine.

About 30 specimens from Departure Bay, Ucluelet, Banks Island and Goose Island, in from 5 to 20 fathoms.

No Phallusiid that has been described seems to have the peculiar shape and tubercles of this species. *Ascidiella griffini* Herdman from Puget Sound may be near it, but, as described, it differs in shape, character of surface, number of tentacles (60-70) and the presence of prebranchial papillæ.

Genus—*Phallusia* Savigny (*sens. restr.*)

Syn. *Ascidia seu Phallusia auct. part.*

This genus may be restricted to those forms that can be grouped around the type species (*Ascidia mentula* Müller ?) and which have the pharynx extending behind the posterior border of the stomach, longitudinal bars with papillæ, dorsal lamina extending behind œsophageal aperture, ganglion a considerable distance behind dorsal tubercle and renal vesicles restricted to the intestinal wall (or absent ?).

*P. ceratodes* sp. n.

About twice as long as broad, laterally flattened, attached by greater part of left side; up to 7 cm. in length, 3.2 cm. in width, and 1.5 cm. in thickness. Apertures sessile or on short siphons; oral 6- or 7- lobed, terminal; atrial 5- or 6-lobed, placed about half the length of the body back along the dorsal edge. Surface irregularly wrinkled and minutely roughened. Musculature practically confined to right side.

From 50 to 150 tentacles. Prebranchial zone smooth. Aperture of dorsal tubercle horseshoe-shaped, horns incoiled, with one turn in each coil; opening between horns directed forwards. Ganglion from 3 to 7 mm. behind tubercle. Dorsal lamina ribbed on left side, its margin finely toothed; from 2 to 5 teeth between successive teeth corresponding

to the ribs. Bars, 35 to 54 on right and 32 to 51 on left side. Papillæ at junctions, but not between. One plication or less between successive bars. From 3 to 9 stigmata in each mesh.

Intestinal canal occupies from ⅓ to ½ of left side, leaving from ⅓ to ¼ of length of pharynx uncovered at both anterior and posterior ends. Stomach with about 17 folds. Intestinal loop bent forwards and somewhat upwards. Ovary in intestinal loop and on its right side. Oviduct follows posterior margin of last bend of intestine and postero-dorsal margin of rectum. Testicular lobes in a thick layer on right side of stomach and scattered over both sides of intestinal loop. *Vas deferens* on right side of first part of oviduct and in groove on the left side between second part of oviduct and rectum. As a result of this, when one looks at the lower side of the body (test removed) only a short terminal part of the *vas deferens* is seen.

In and near Departure Bay, in from 10 to 30 fathoms, locally abundant.

Its closest ally appears to be *P. longistriata* Hartmeyer from Japan, from which it differs in having the margin of the dorsal lamina toothed and in the situation of the gonads.

Family—*Chelyosomatidae.*

As is shown farther on, the intestinal loop in the genus *Chelyosoma* is always on the right side of the pharynx. Whether it is on the right or left side of an arbitrary median plane, is of little moment. With this genus brought into line, we have the utmost constancy in the position of the intestinal loop with reference to the pharynx in each of the genera of Ascidians. The only exceptions are those individuals that show an *inversio viscerum*. It seems right, therefore, that the genus *Agnesia* should not be placed in this family.

*Corella willmeriana* Herdman.

One specimen from Elk Bay was collected by Dr. Dawson, in 1885.

The surface is smooth. The atrial aperture is not on a distinct siphon. There are 24 bars on right side of pharynx and 22 (?) on left side. Spirals of infundibula are for the most part broken up into short stigmata.

*C. rugosa* sp. n.

Syn. *C. willmeriana* Ritter, Ann. N.Y. Ac., vol. 12, p. 604.

Oblong, laterally compressed or more or less cylindrical. Attached by posterior end or by right or left side. Apertures on same level at anterior end; atrial often at the end of a short siphon. Surface irregularly wrinkled and rough with fine irregular processes of the test. Up to 4 cm. long, 2 cm. wide and 1.5 cm. thick. Musculature consists of the usual siphonal fibres and of longitudinal fibres extending from the siphons for a short distance back over the body.

From 50 to 80 tentacles. Aperture of dorsal tubercle varying from a transverse slit to a horseshoe-shaped opening. From 14 to 20 languets. From 20 to 22 bars on each side, with a few rudimentary ones near dorsal and ventral margins of pharyngeal wall. Stigmatic infundibula deep and nearly square; the spiral of each with 5 or 6 turns, often slightly divided into shorter stigmata, the divisions occurring at the angles of the square.

Intestinal loop of the usual form, placed across posterior end of pharynx. Rectum long, reaching nearly to base of atrial siphon. Gonads on both sides of intestinal loop, the testicular lobes, but not the ovary, usually extending to beginning of rectum.

Numerous specimens from Departure Bay, Burrard Inlet, Ucluelet, Banks Island and Hecate Straits.

This species differs from the last in the more anterior position of the atrial aperture, the roughened test and the smaller number of longitudinal bars.

*C. inflata* sp. n.

This is very similar to the preceding species. No intermediates have been found. It was obtained only at Departure Bay, occurring there in quantity at low tide and in very shallow water (8 fathoms or less). The most characteristic feature is the great enlargement of the atrium (the median part of the peripharyngeal cavity just beneath the atrial siphon). As a result of this, the shape is more nearly cubical than in the last species and the rectum is very much shorter (less than half the length of the body). There is a smaller number of tentacles (40 to 60) and also of longitudinal bars (16 to 18). Many of the latter (especially dorsally) are represented only by T-shaped processes, the pharynx not reaching, even in large individuals as complete a stage of development as that of the last species. The testicular lobes do not extend as far as the beginning of the rectum. The apertures are at the same level and the surface of the test is roughened with small irregular processes.

Genus—*Chelyosoma.*

There have been divergent accounts of the position of the intestinal canal in members of this genus. The most recent statements are that the loop is sometimes on the right side of the body and sometimes on the left side. As its position in other genera is quite constant, this seemed rather remarkable. An examination of a large number of specimens belonging to two species which occur on the West Coast and of a single specimen of the type species from the East Coast has shown that, in all, the loop is on the lower attached side of the body, which in this case corresponds for the most part with the right side of the pharynx, as the

endostyle has been displaced toward the left side. Although the loop is sometimes more to the right, sometimes more to the left of a plane passing through the apertures perpendicularly to the disk, it is always on the morphological right side of the pharynx, just as in *Corella*. It is directed forward, however, instead of transversely.

*C. productum* Stimpson.

Numerous specimens from Departure Bay and Ucluelet and one from Hecate Straits.

Characteristic of this species are the symmetry of the disk, the large size of mature individuals and the absence of muscle bands across many of the lines between the plates of the disk.

*C. columbianum* sp. n.

Usually flattened and depressed, attached by a broad area on side opposite the disk. Margin of disk sharp, not raised above level of disk. Disk broad behind. Apertures nearer right margin of disk than left. There are typically 2 central, 12 marginal and 2 left intermediate plates, but there is a fairly wide range of variation. Up to 19 mm. in length and 14 mm. in breadth. In addition to the siphonal and marginal muscles, there are short strands crossing all the lines that are some distance from the margin.

From 50 to 100 tentacles. From 12 to 22 languets. Aperture of dorsal tubercle a transverse slit. Funnel asymmetrical, the duct connecting with left side of tubercle. From 33 to 42 bars on each side of pharynx. Stigmata more or less coiled, with as many as 2 ½ turns in a coil, irregularly disposed.

Gastric folds for the most part longitudinal. Intestine narrow. Loop narrow, some distance to right of posterior half of endostyle.

About 40 specimens from Departure Bay and Burrard Inlet in from 10 to 20 fathoms, stony and shelly.

Easily distiguished from the last by the presence of the series of muscle fibres connecting the central plates. It reaches maturity at a much smaller size. It differs from *C. sibogae* Sluiter of the East Indies and Japan, in the irregularity of the musculature, in the coiling of the stigmata and in the aperture of the dorsal tubercle being transverse instead of longitudinal.

Family—*Cæsiridae* [*Molgulidae*].

*Cæsira apoploa* sp. n.

Nearly spherical. Usually free in the sand. Siphons equal, in length about half the diameter of the body. Surface covered with sand grains, with the exception of that of the siphons and a variable part of the surface near them. Long simple radicoid filaments over the sand-

covered surface. Up to about 15 mm. in diameter. In addition to the usual siphonal musculature, there are two circular bands (deficient ventrally) of short fibres on either side of the median plane.

From 18 to 37 tentacles, the largest bi-pinnate. Aperture of dorsal tubercle horseshoe-shaped; horns slightly inturned; opening between horns directed backwards and slightly towards right side. Dorsal lamina narrow, its margin smooth. Seven folds each side (in one specimen a rudimentary eighth on right side dorsally). As many as 4 bars on a fold, on the ventral side only. Stigmata forming the usual infundibula, each stigma forming from ¼ to ¾ of a circle. An occasional small accessory infundibulum between folds.

Intestinal loop very narrow, bent into a semicircle, the concavity of which is entirely filled by the left gonad. Anus with thickened smooth margin. Gonads oblong, massive, with central ovary. Testicular lobes massed along upper and lower sides of the ovary. Oviduct short, projecting upward from posterodorsal angle. Several (in one case 6) *vasa deferentia* of medium length projecting inwards along the middle of each ovary. Renal organ about equal in length to right gonad and of the usual sausage shape.

About 30 specimens from Departure Bay, Ucluelet, Alert Bay and Hecate Straits.

This form appears to be more nearly related to the following species than to any other.

*C. hecateia* sp. n.

Rounded oblong, laterally flattened. Apertures rather close together on dorsal edge near anterior end. In the contracted condition, they are at the bottom of a shallow furrow. Apertural lobes pointed. Surface (except a narrow zone around each siphon) closely covered with sand and fragments of shells. Up to 32 mm. in length, 20 mm. in depth and 15 mm. in thickness. Musculature as in preceding species, but the circular bands of fibres are more numerous (2 to 4 on each side) and irregular.

Probably about 35 or 40 tentacles, the largest bushily branched, bipinnate or slightly tripinnate. Aperture of dorsal tubercle horseshoe-shaped, directed toward right side. Dorsal lamina rather broad; its margin incised, presenting about 7 large teeth or lobes, which are most distinct posteriorly. It extends behind œsophageal aperture. Seven folds each side. Up to 6 or occasionally 7 bars on each fold, on the ventral side only. Stigmata as in last species, but the accessory infundibula are more numerous.

Intestinal loop narrow, horizontal, nearly straight. Gonads much

as in last species. Eight *vasa deferentia* were counted in the right gonad of one individual.

Several specimens were collected in Hecate Straits by Rev. Mr. Taylor.

*C. pugetiensis* (Herdman) from Puget Sound differs from this species in the extent of its musculature, in having fewer longitudinal bars (3 to 4), and in the direction of the dorsal tubercle (backwards).

*C. occulta* (Kupffer) of Europe is still nearer this species, but the short descriptions that have been given of it permit of only a few points of difference being given, viz. the shape of the body and the positions of the apertures. These seem unimportant, but it would be best to keep them separate until they can be more closely compared.

*C.* sp.

A small specimen was obtained by Dr. Dawson in 1885 between Cortez and Hernand Islands. It appears to differ from the two preceding species in the extent of the musculature (the circular or transverse fibres covering practically the whole body) and in the condition of the dorsal tubercle (a longitudinal slit).

*C. pacifica* sp. n.

Nearly spherical, 15 mm. long, 13 mm. deep and 10 mm. thick Attached by lower surface and part of right side. Siphons contracted, atrial the longer. Surface overgrown with seaweed &c., the lower half, at least, with radicoid filaments. In addition to siphonal musculature, there are irregularly scattered fine, short fibres over the surface of the body.

About 40 tentacles, the largest slightly tripinnate. Aperture of dorsal tubercle horseshoe-shaped, directed toward right side, the horns approximated. Dorsal lamina short and narrow, its margin smooth; it extends only a short distance behind œsophageal aperture. Seven folds each side. Bars on both sides of each fold, as many as 11 on a fold. Stigmata form the usual infundibula, each stigma extending from ¼ to ½ of a circle. No accessory infundibula.

Intestinal loop narrow, bent into a semicircle. Renal organ and gonads as in *C. apoploa*, but only 2 *vasa deferentia* could be seen (right side).

A single specimen was obtained at Ucluelet at low tide, attached to rock.

It differs from the preceding species in having bars on both sides of each fold, and from *C. pannosa* of the East Coast in the direction of the dorsal tubercle. There are other differences in both cases.

*C. cooperi* sp. n.

Nearly spherical or flattened against the object of attachment. Apertures with small pointed lobes. Siphons very short. Surface, including that of siphons, entirely covered with closely placed sand grains, which adhere to the usual filaments. Up to 15 mm. in length. In addition to the usual siphonal musculature, there is an almost uniform layer, continuous with the circular fibres of the siphons. It is thin over the intestine, gonads, &c.

About 75 (?) tentacles. Aperture of dorsal tubercle crescent-shaped, turned toward the left. Dorsal lamina narrow, its margin smooth; it does not extend beyond œsophageal aperture. Six folds on each side, their posterior ends fringed. Up to 14 bars on a fold, occurring on both sides of each fold. Stigmata forming the usual infundibula, with 10 or more turns in each spiral. Each stigma represents ¼ of a circle, so that more or less regular transverse rows are formed, such as are characteristic of the genus *Ctenicella* as defined by Hartmeyer.

Intestinal loop narrow, bent into a semicircle. Margin of anus smooth. Gonads much elongated. The left is in the concavity of the intestinal loop and anteriorly bent over the tip of the loop. It is thus closely applied to the intestine for a considerable distance. The right gonad is much longer than the renal organ, to which it is closely applied. The latter is of the usual shape. Each gonad consists of an axial ovary, with the testicular lobes scattered along its upper and lower margins. The *vas deferens* runs along the inner side of the ovary and projects upward with the oviduct from the posterior end of the gonad.

Several specimens were obtained in 5 to 15 fathoms, sand and gravel, in Departure Bay.

This species is doubtfully distinct from *C. regularis* (Ritter) from California. From the data available at present, there are the following differences,—a smaller number of tentacles (10), the absence of siphons (?), the aperture of dorsal tubercle a longitudinal slit, which is not curved, and the larger diameter of the stomach in *C. regularis*.

*Rhizomolgula globularis* (Pallas).

Syn. *Ascidia globularis* Pallas, Nov. Act. Ac. Petr., vol. 2, p. 241.
? *Rhizomolgula gigantea* Redikorzew, Mem. Ac. St. Peters., ser. 8, vol. 18, no. 11.

Laterally compressed, somewhat elongated parallel to a line joining apertures. Largest specimen is 19 mm. long, 17 mm. deep, and 12 mm. thick. Apertures about 7 mm. apart, not on distinct siphons. Surface sparsely covered with sand grains. On the side of the body opposite the apertures there are usually 2 short 'roots,' each with numerous long branches. The usual siphonal musculature; on each side near the

'roots' a few bands of longitudinal fibres over the surfaces of the glands; a median band of short transverse fibres encircling the body in the median plane; and a short band of similar fibres extending downwards on each side about half the length of the body. That of the left side connects with the atrial siphon and that of the right side with the oral.

From 17 to 20 tentacles, the largest tripinnate. Aperture of dorsal tubercle horseshoe-shaped, directed forwards and slightly toward the left. Dorsal lamina narrow, its margin smooth; it does not extend beyond œsophageal aperture. Six folds on each side. Bars on both sides of each fold, as many as 7 on a fold. Stigmata form the usual infundibula and some small accessory infundibula between folds. The stigmata are short and not numerous nor closely placed.

About 12 shallow gastric folds. Intestinal loop broad anteriorly, straight. Inner margin of anus fused with pharyngeal wall, outer margin smooth or with a single tooth. Gonad fills the intestinal loop and covers its inner side, consisting of a central ovary (its duct following rectum) and an upper and lower mass of testicular lobes. The *vasa deferentia* are numerous (9 in one specimen), their free portions short, placed in an irregular row above the middle of the gonad on its inner side. Renal organ below stomach. Heart along right side of renal organ. Glands small, disk-shaped. Ectodermal processes of mantle few or absent.

Several specimens were collected by the Rev. I. O. Stringer at Herschel Island, Arctic Ocean and communicated to me by Prof. Wright.

Pallas' *A. globularis* is undoubtedly a *Rhizomolgula*. The identification of these specimens with his species rests upon external characters alone. He has given very characteristic figures. Redikorzew's *R. gigantea* appears to be the same species.

Family—*Styelidæ*.

Subfamily—*Polyzoinæ*.

*Metandrocarpa dermatina* sp. n.

Colonies thin, encrusting, dark red or purplish in colour. Individuals irregularly disposed, about 2 mm. distant from each other. They are flattened parallel to the surface of the colony or elongated perpendicularly to it, depending upon the thickness of the colony. They are from 4 to 5 mm. in length. The colonies are up to 10 cm. in length. The apertures are transverse slits.

About 24 tentacles. Aperture of dorsal tubercle a transverse slit. Dorsal lamina narrow, its margin smooth. Five bars on each side, the two uppermost approximated. Small transverse vessels cross stigmata. Stigmata narrow, about 50 in a row.

About 15 gastric folds. Apparently about 12 atrial tentacles. Up to 10 or 11 tentacles grouped at anterior end beneath pharynx. A row of testes on each side posteriorly and ventrally. In the right row there are from 6 to 12 and in the left from 3 to 5. They are imbedded in the test.

Several colonies were obtained on the beach at Hope Island, by Rev. Mr. Taylor in 1906.

This form is doubtfully distinct from *M. dura* Ritter from Santa Barbara, California. It differs from the descriptions of the latter, given by Ritter and Michaelsen, in having a smaller number of oral tentacles and a larger number of gastric folds. The differences in the reproductive organs are probably referable to the greater maturity of the colonies from Hope Island.

*M. taylori* sp. n.

This is a social species, the individuals being connected by stolons alone. The largest individuals are $7 \times 5 \times 4.5$ mm., in shape more or less hemispherical. Apertures are transverse slits. The surface is smooth or slightly wrinkled. The test is thin.

The structure of the pharynx is the same as has been described for the last species.

Thirteen or 14 gastric folds. Atrial tentacles minute. In one individual there were counted 11 ovaries, 9 testes on the right side and 11 testes on the left.

This form is so nearly identical in anatomical details with the preceding species, that one considers the possibility of their being different forms of the same species, just as Ritter has considered that *Perophora annectens* may form either social or compound colonies. With our present knowledge we must consider this form distinct from *M. dermatina*, the differences being,—'social' instead of compound colonies, larger individuals and colonies white instead of dark purple.

Subfamily—*Styelinae.*

Genus, *Katatropa* nov.

Syn.—*Styela auct. part.*

Siphons with spinules.

Four folds on each side, the second from above smaller than the first or the third. Aperture of dorsal tubercle horseshoe-shaped, directed toward left.

Normally 2 gonads on each side, placed obliquely; the anterior ends which bear the ducts, being directed downward toward endostyle. Ovary tortuous, rather short; testicular lobes grouped along either side of ovary, little (if at all) branched; their long axes are perpendicular to plane of

body-wall and not bound together, but each projects freely into pharyngeal cavity. Eggs retained.

Siphonal vela narrow, adnate to siphonal wall, the atrial with scattered short filiform tentacles on its lower (inner) surface. Alimentary canal more or less Z-shaped. Anus with lobed margin.

Type species—*K. vancouverensis.*

This genus comprises a small group of species all from the West Coast of North America. In the current classification they would be placed in the genus *Styela.*

*K. vancouverensis* sp. n.

Short cylindrical, length being about twice the diameter. Attached by posterior end and part of ventral surface, therefore ascending from the attached surface. Surface minutely roughened, with indistinct tubercles on siphons. Up to 25 mm. in length and 9 mm. in diameter.

From 10 to 22 tentacles. Formula for longitudinal bars,—example, Right side. 2 (10) 1 (6) 1 (9) 1 (6) 1.

Intermediate (internal) transverse vessels. From 9 to 13 long narrow stigmata in each mesh.

From 12 to 18 gastric folds. From 8 to 12 anal lobes. Testicular lobes chiefly along the ventral side of the posterior part of each ovary.

Numerous specimens attached to rocks at low tide mark, Departure Bay and Ucluelet.

*K. ucluletensis* sp. n.

Cylindrical, attached by posterior end, which may have radicoid processes.

From 30 to 36 tentacles, for the most part two sizes which alternate with each other.

Eighteen gastric folds. About 16 anal lobes. Testicular lobes along both sides of posterior part of each ovary.

In other respects this species is the same as the last. They are doubtfully distinct, but as yet I have seen no intermediates.

Two specimens were obtained in a few fathoms at Ucluelet.

*K. yakutatensis* (Ritter).

Syn. *Styela yakutatensis* Ritter, Proc. Wash. Ac. Sc., vol. III, p. 239.

This is a stalked species, with smooth surface, and the oral aperture bent ventrally.

It occurs in numbers near low tide mark, attached to rocks, at Ucluelet.

*K. greeleyi* (Ritter) of Bering Sea is another stalked species of this genus. It differs from this one in having a shorter body, a longer stalk, longer testicular lobes and spinules which are acicular. In the three

British Columbian species the spinules are short, channeled above, with truncated toothed extremities.

Genus—*Styela* (*sens. restr.*).

Dorsal tubercle directed forwards or to left.

Gonads very long, ending just beneath atrial siphon, hence directed dorsally. Testicular lobes large, more or less branched. Eggs not retained. Otherwise as in last genus.

Type species—*S. canopus* (Savigny).

This genus is widely distributed in the tropical and temperate zones. *S. canopoides* Heller, *S. variabilis* Ald. & Hanc. and *S. partita* (Stimpson) belong to the genus as thus restricted and probably many other species as well, which are too insufficiently described for one to be certain of their position.

*S. gibbsii* (Stimpson).

Numerous specimens from Departure Bay, Ucluelet and Banks Island, taken in from 5 to 30 fathoms sand, gravel or shells.

*S. montereyensis* (Dall).

The stalked form of this genus.

Numerous specimens taken at low tide, attached to rocks, at Ucluelet and one specimen from Hope Island (Mr. Taylor).

Genus—*Goniocarpa* nov.

Syn. *Styela auct. part.*

Dorsal tubercle directed forward or to left.

One gonad on each side, bent more or less in the form of a right angle. The vertical limb of the gonad ends in the genital ducts, just beneath the atrial siphon. Ovary tortuous; testicular lobes grouped around horizontal limb of ovary, each one lobulated, the lobes bound together into a biscuit-shaped mass. Eggs not retained. Otherwise as in *Katatropa.*

Type species—*G. loveni* (Sars), as described by Hartmeyer (Fauna Arctica, Bd. III, 1903).

The species of this genus would currently be placed in *Styela.* It is apparently a northern group and includes *G. rustica* (L.), *G. armata* (Lac.-Duth. & Del.), *G. granulata* (Alder), *G. coriacea* (Ald. & Hanc.), *G. northumbrica* (Ald. & Hanc.) and *G. placenta* (Packard).

*G. coccodes* sp. n.

Exceedingly variable in shape, from scale-like to elongated oval. Surface pebbly, owing to the presence of rounded tubercles, from 1/16 to 1/8 mm. in diameter. Siphons short. Up to 2 cm. in length.

From 25 to 35 tentacles. Formula for longitudinal bars,—example—

Right side— 4(19) 4 (11) 4 (18) 6 (9) 4.

From 1 to 3 internal transverse vessels crossing each stigmatic row. From 3 to 7 long narrow stigmata. From 20 to 26 gastric folds. About 12 anal lobes. Testicular lobes chiefly ventral to horizontal limb of ovary.

A number of specimens from Departure Bay, Burrard Inlet, Lowe Inlet, China Hat and Prince Rupert, in from 10 to 30 fathoms, stony or shelly.

Most nearly related to *G. placenta* of the East Coast and *G. coriacea* of England, from which it seems to differ in certain details. Further study may show the necessity of uniting them into one species.

*Pelonaia corrugata* F. &. G.

A few specimens were obtained by Rev. Mr. Taylor at Rose Spit in 1906 in a few fathoms, sand. They do not appear to differ in any respects from the descriptions of European and Arctic specimens.

This form does not deserve to be placed in a separate subfamily, the only respect in which it differs markedly from its nearest relatives (e.g. *Styela*, *Goniocarpa* &c.), being the absence of folds in the pharyngeal wall. This condition may be approximated in other forms when the pharynx is expanded (e.g. *Styela gibbsii*). The current statement that the intestinal canal is behind the pharynx is only partially correct. It is distinctly on the left side of the pharynx and only slightly farther back than it is in *Styela gibbsii.*

Genus—*Cnemidocarpa* nov.

Syn. *Styela auct. part.*

Spinules rudimentary or absent.

Gonads variable in number, 3 or more on each side, elongated, tortuous, radiating more or less from atrial siphon. Ducts at upper ends. Each gonad consists of an ovary on the inner side and a layer of testicular lobes on the outer side. The *vas deferens* runs along the inner side of the ovary.

Siphonal vela broad, applied to walls of siphons and reaching nearly to the margins of the apertures. A single row of tapering atrial tentacles at base of atrial velum.

Having examined only two members of this group, I am unable to give more characters. The members of this genus are currently included in *Styela*. It includes *Polycarpa finmarkiensis* Kiaer, *Styela elsa* Hartmeyer, *Glandula mollis* Stimpson, *Styela vestita* Alder and probably a large number of other species, but it is difficult to be certain in most cases because of the incomplete descriptions.

*C. joannae* (Herdman).

Syn.—*Cynthia coriacea* Stimpson, Proc. Ac. Phil., ann. 1864, p. 160.
*Styela joannæ* Herdman, Tr. Liv. Biol. Soc., vol. XII, p. 264.
*Styela stimpsoni* Ritter, Ann.N. Y. Ac., vol. XII, p. 602.

Numerous specimens from Departure Bay, Ucluelet, China Hat and Banks Island, attached to rocks &c., from low tide mark to at least 20 fathoms.

From the abundance of the material in my possession, all, as far as examined, agreeing with Ritter's description, I judge that Herdman's and Ritter's species are the same and that Herdman was mistaken in describing the dorsal lamina as being a 'plain membrane.' Stimpson's name was preoccupied by Alder & Hancock in 1848.

Family—*Tethyidæ.*

[*Halocynthiidæ seu Pyuridæ, auct., non Tethyidæ* Hartmeyer, 1909]

In my opinion, the valid type of the genus *Tethyum* Bohadsch is the *Ascidia papillosum* of Linné. *Cynthia* and *Halocynthia* will then be absolutely synonymous with *Tethyum* and are to be replaced by it. *Halocynthiidæ* and *Pyuridæ* are to be replaced by *Tethyidæ.*

Genus, *Boltenia* (*sens. nov.*)

Syn. *Boltenia auct. part.*+*Halocynthia auct. part.*

Body elongated parallel to a line joining apertures. Surface covered with simple or branched spines. Short, channeled, siphonal spinules.

Aperture of dorsal tubercle bent, opening between horns directed toward right side. Dorsal groove with languets. At least 6 folds on each side, the second and sixth, counting from above, being the smallest. Stigmata transverse, arranged in longitudinal rows, which are traversed from end to end by the longitudinal bars.

One gonad on each side, the left in the intestinal loop. The ducts are at the posterior end of each. Each consists of an axial ovary and peripheral testicular lobes.

Type species—*B. ovifera* (L.)

This is a very sharply defined group and includes only a few of the stalked forms that have been referred to this genus. It appears to be confined to the Arctic and Subarctic regions. In addition to the species mentioned in this article, it includes *B. thompsoni* Hartmeyer of Bering Sea. Some of these species have been placed in the old genus *Boltenia* and some in the genus *Halocynthia* or *Pyura.*

*B. echinata* (L.)

Syn. *Cynthia echinata plur. auct., non Boltenia echinata* Ritter, 1907.

A few specimens were obtained in 10 to 20 fathoms, stony or shelly, at Departure Bay. Hartmeyer has recently (S.-B. Ges. naturf. Freunde

Berlin, ann. 1910, p. 231) come to the conclusion that the series of forms which have been referred to the *Ascidia echinata* of Linné, cannot be divided into two distinct species. These Pacific specimens agree well with the descriptions that have been given of Arctic specimens.

*B. villosa* (Stimpson).

Syn. *Cynthia villosa* Stimpson, Proc. Ac. Phil., ann. 1864, p. 160.
*Cynthia castaneiformis* v. Drasche, Denk. Ak. Wien, Bd. 48, p. 373.
*Boltenia echinata* Ritter, Univ. Cal. Publ. Zool., vol. IV, p. 14.

Numerous specimens from Departure Bay, Ucluelet, Goose Island and Prince Rupert, from between tides to 30 fathoms, attached to rocks, sea-weed &c.

In a series of specimens taken at one locality such a range of variation is shown, that it seems impossible to consider the species listed above in the synonymy as distinct.

Genus, *Pyura* (*sens. restr.*)

Syn. *Cynthia, Halocynthia, Pyura auct. part.*

Surface rough with irregular warts, corrugations &c. Test usually more or less encrusted with sand. Siphons usually rather long. Siphonal spinules acicular (always ?).

Aperture of dorsal tubercle bent, directed forwards. Dorsal groove with languets. Six folds on each side. In very young specimens the second and sixth folds are much smaller than the others. Stigmata longitudinal.

One gonad on each side, the left in the intestinal loop. Each is divided into (usually) two rows of hermaphroditic masses, the genital ducts passing back between these rows and ending near the anus.

Type species, *P. chilensis* Molina.

Michaelsen has described what purports to be Molina's species (Mt. Nat. Mus. Hamburg, Bd. XXI, p. 15). It would be included in the group of species with the above characters and hence becomes the type. Other species are *P. dura* (Heller), *P. jacatrensis* (Sluiter), *P. riiseana* (Traustedt), *P. karasboja* (Oka) &c. I have been able to examine only the one species of this group and consequently the diagnosis given above is more or less tentative. Further study will show the correct limits of this group. The most important characters seem to be the irregularity of the surface, the number of folds and the division of the gonads.

*P. haustor* (Stimpson).

Syn. *Cynthia haustor* Stimpson, Proc. Ac. Phil. ann. 1864, p. 159.

Numerous specimens from Departure Bay, Ucluelet, Hope Island and Banks Island, from between tides to 30 fathoms, usually in sand.

Genus, *Tethyum* (*sens. nov.*)

Syn. *Cynthia*, *Halocynthia*, *Pyura auct. part.*, *non Tethyum* Hartmeyer, 1909.

Oral aperture terminal, atrial on dorsal side. Siphonal spinules acicular. Surface with simple or branched spines.

Aperture of dorsal tubercle curved, usually forming two cone-shaped coils; opening between horns directed forwards and to left. Dorsal groove with languets. Number of folds variable, increasing with age, at least 6, the second not smaller than the first and third. Stigmata longitudinal.

Two to many gonads on each side, those of the left side placed across the inner side of the intestinal loop (which is transverse to the long axis of the body). The two genital ducts open at the anterior end of each gonad. The gonads of each side are fused together posteriorly. The testicular lobes are grouped around the posterior ends of the ovaries.

Type species—*T. papillosum* Gunner.

Hartmeyer (Zool. Ann., Bd. III, 1908) has indicated *Ascidia rustica* L. and *A. quadridentata* L. as the types of *Tethyum* Bohadsch. He seems, however, not to have considered Art. 30 of the International Rules, in which we find the following:

"(*e*) The following species are excluded from consideration in selecting the types of genera.

(*a*) Species which were not included under the generic name at the time of its original publication."

It is possible that he may interpret this to mean only those species that have been named binominally. In that case he would neglect the four species of Bohadsch. Following Sherborn, he has accepted the species of Gunner as validly named. Gunner (Trond. Selsk. Skrift., III) names three species which are identical with three of the species described by Bohadsch. Hartmeyer states that Gunner's article appeared in the same year as the 12th edition of Linné's *Systema Naturae* (1767), and considers that Linné's work has the priority. He has evidently not seen the original article by Gunner, which (according to Sherborn and Hopkinson) appeared in 1765, but only the German translation (Dront. Gess. Schrift., III). That it antedates Linnæus is shown by a reference of the latter under *A. intestinalis*, viz. "Act. nidros. 3. p. 81, t.3. .3, 4. Tethyum." This refers to Gunner's description and figures of *Tethyum sociabile*.

As Hartmeyer has not indicated a type from among the species originally included in the genus—either practically (those of Bohadsch) or binominally (those of Gunner)—a type remains to be indicated. Of the species of Bohadsch, the one which we can identify to-day with the

greatest degree of certainty is *Tethyum coriaceum*, the T. *papillosum* of Gunner and the *Ascidia papillosum* of Linné. This may be taken as the type of *Tethyum*.

Heller has indicated the same species as the type of *Cynthia* Savigny. *Halocynthia* Verrill and *Lais* Gistel were instituted to replace *Tethyum*. All three are therefore absolutely synonymous with *Tethyum*.

As defined above, this genus comprises a group of species, which differ from all other Tethyids in the position of their gonads. It includes *T. pyriforme* (Rathke), *T. aurantium* (Pallas), *T. roretzii* (Drasche), *T. hilgendorfii* (Traustedt), *T. igaboja* (Oka)and probably a number of others which have not yet been sufficiently described for one to be sure as to their position.

*T. aurantium* (Pallas).

Syn.—*Ascidia aurantium* Pallas, Nov. Act. Ac. Petr., vol. II, p. 240.
*Cynthia pyriformis* Dall, Amer. J. Conch., vol. VII, p. 157.
" " *alt. auct.* (Pacific).
" *superba et deani* Ritter, Ann. N.Y. Ac., vol. XII, p. 590.

A very few specimens from various points—Departure Bay, Ucluelet, Banks Island and between Cortez and Hernand Islands, in from 10 to 30 fathoms.

*T. pyriforme* from North Europe and the Arctic Ocean has, according to Hartmeyer (1903), 4 gonads on the left side and from 4 to 6 on the right.

All the Pacific specimens, that I have been able to examine, have 3 gonads on each side. They seem to be for that reason, quite distinct from *T. pyriforme*. From Traustedt's account (Vid. Meddel. Kbhvn., 1885, p. 34), I conclude that his Corean specimens had 3 gonads on each side. That would make the Asiatic and West American forms identical, Pallas' name, being the first one given, is the valid one for this group.

*T. igaboja* (Oka).

Syn.—*Halocynthia igaboja* Oka, Ann. Zool. Jap., vol. VI, Pt. 1, p. 45.
? " *okai* Ritter, Univ. Cal. Publ. Zool., vol.IV, p. 11.

A number of specimens from Departure Bay, Ucluelet, Lowe Inlet and Prince Rupert, in from 10 to 30 fathoms shelly or gravelly.

These specimens are in accord with Oka's description and differ from Ritter's only in regard to the inrolling of the horns of the dorsal tubercle. The gonads are quite variable, there being from 2 to 16 on the right side and from 5 to 14 on the left.

## NOTES ON THE SPECIES OF THE ATLANTIC COAST.

With the exception of the compound forms, which have been recently thoroughly treated by Dr. Van Name (Proc. Bost. Soc. N. H., vol. 34, No. 11, 1910), the species of the East Coast have for the most part been only imperfectly described. It will be necessary therefore to give an account of the anatomy of many of the species. It has been very difficult in many cases to refer, with much certainty, my specimens to the species that have been described from this coast, owing to the imperfect descriptions of the older authors. As many of the specimens have been obtained from or near the localities which gave the types of the species, the identifications should have a greater probability of being correct. Dr. Van Name, who is at present engaged in work on the simple Ascidians of this coast, has been most kind in giving me help in the identification of my specimens with Verrill's species. He has corrected some errors into which I had fallen and confirmed some of my surmises.

*Aplidium spitzbergense* Hartmeyer, Fauna Arctica, Bd. 3, Lf. 2, p. 341.

A single capitate colony was obtained in Long Island Bay, Grand Manan, in about 8 fathoms. This species has been previously reported only from Spitzbergen. The agreement with Hartmeyer's description seems, however, to be perfect.

The colony is 15 mm. by 10 mm., with a thick stalk 8 mm. long. The test contains very numerous sand-grains.

The zooids are about 2.5 mm. long. Oral aperture 6-lobed. Atrial aperture round, at the end of a short tubular siphon, placed opposite the interval between the first and second stigmatic rows. A long atrial languet is present a short distance in front of the siphon. Four stigmatic rows. Four gastric folds. Abdomen and postabdomen together are slightly longer than the pharynx. Ovary small and no embryos present.

Another colony, not capitate, 20 mm. long, 9 mm. wide and 6 mm. thick, seems to be referable to the same species. There are much longer and narrower zooids with the ovaries well developed, embryos in the peripharyngeal cavity, and the postabdomen nearly equal in length to the thorax and abdomen together. The colour of this colony, when living, was decidedly greenish.

This second colony was obtained off Long Island, Grand Manan, in about 35 fathoms shelly and muddy.

Of the characters which distinguish *Aplidium* from *Amaroucium* the only one possessed by this second colony is the small number of stigmatic rows. It might be best to place it in the genus *Amaroucium*, near *A. diaphanum* (v. Drasche).

*Amaroucium glabrum* Verrill.

Numerous colonies, apparently belonging to this species, were obtained at nearly all points at low tide and in the dredgings.

*Tetradidemnum albidum* (Verrill).

Both the white and salmon-coloured varieties of this species were found generally distributed at low tide and where dredgings were made.

*Didemnopsis tenerum* (Verrill).

Syn. *Lissoclinum tenerum* Verr.

Several colonies were dredged in the approaches to Passamaquoddy Bay and one off Swallow-tail Light, Grand Manan.

*Holozoa clavata* (Sars)?

Soft, light yellow, encrusting colonies of *Holozoa* were obtained at low water mark and practically throughout in the dredgings, though never in large numbers. Dr. Van Name has referred all the colonies from along this coast, that were examined by him, to Sars' species. None of the colonies in my collection show even an approximation to the clavate condition.

*Ciona intestinalis* (L.)

As only a single small specimen was obtained (off Grand Manan), no detailed study of it was made. It doubtless is identical with the European species.

*Ascidiopsis prunum* (Müller).

Syn.—*Ascidia callosa* Stimpson, Proc. Bost. Soc. N.H., vol. IV, p. 228.

*Ascidiopsis complanata* Verrill, Amer. J. Sc., ser. III, vol. 3, p. 289.

Characteristic of this species is the small number of longitudinal bars (from 15 to 19 on the left side and from 18 to 20 on the right), the presence of intermediate papillæ and the crossing (slightly) of the last bend of the intestine by the genital ducts. Eggs and larvae were found in the peripharyngeal cavities of some of the breeding individuals. The large individuals seemed to be uniform in having undeveloped reproductive organs.

Found in large masses at low tide mark. It is generally distributed as shown by the dredgings. At Grand Manan it seems to be largely replaced by the next species.

Genus *Phallusioides* nov.

This genus is formed for the reception of *Ascidia* (*seu Phallusia*) *obliqua*, which differs from *Phallusia* in that the pharynx and dorsal lamina do not extend beyond the œsophageal aperture, in this respect

resembling *Ascidiella*. From the latter it differs in having papillæ on the bars and in not having renal vesicles. It is thus intermediate between *Phallusia* and *Ascidiella*. The ganglion is close to the dorsal tubercle and there are no intermediate papillæ. In the absence of renal vesicles, it resembles some *Phallusiæ*. As if to offset this lack of vesicles, there is a very great development of what appears to be the pyloric gland. This forms a thick layer of coarse branches, covering all parts of the intestinal canal.

*P. obliqua* (Alder).

Syn. *Ascidia mollis* Verrill, Amer.J. Sc., ser. III, vol. 7, p. 409.

This can be distinguished from the preceding species by the thinner test (which is more collapsible), the more numerous (about 50) longitudinal bars, and the course of the genital ducts (not crossing last bend of intestine), as well as by the differing generic characters.

Large numbers were dredged at various points and depths near Grand Manan and occasional specimens were obtained in the approaches to Passamaquoddy Bay.

*Chelyosoma macleayanum* B. & S.

Syn. *Ascidia geometrica* Stimpson, Proc. Bost. Soc. N.H., vol. IV, p. 229.

A single specimen was obtained in the approaches. It is rather unusual in the asymmetry of the plates of the disk. Those of the left side are larger than those of the right and two additional plates are interposed between the middle and posterior marginal plates. In both these respects, it approaches *C. columbianum* of the West Coast.

*Caesira papillosa* (Verrill).

Syn. *Molgula papillosa* Verrill, Am. J. Sc., ser. 3, vol. 1, p. 57.

Surface with numerous radicoid filaments, those on the siphons being quite short. Siphons quite variable in length, frequently as long as the diameter of the body, nearly equal.

From 15 to 25 bipinnate tentacles. Aperture of dorsal tubercle horseshoe-shaped; opening between horns directed backwards. Dorsal lamina of medium width, not extending beyond œsophageal aperture; its margin is coarsely toothed. Six folds on each side of pharynx. Posterior end of each fold coarsely toothed along its free border. Bars on both sides of each fold, as many as 8 on a fold, the dorsal bars weak.

Intestine forming a double loop. Outer lip of anus with about a dozen rounded lobes. Gonads elongated, the right horizontal, the left oblique and filling the secondary loop of the intestine. Oviduct of medium length, projecting upward from posterior end of gonad and ending at base of atrial siphon. Each ovary with an upper and lower row of

pouches. From the outer side it has the appearance of a double row of rounded lobes. Testicular lobes scattered along upper and lower margins of each ovary; usually on the right side the lobes are above anteriorly and below posteriorly, whereas on the left they are more variable, the majority being below. From 1 to 4 *vasa deferentia* on each side (usually 2) opening not far from the centre of the inner side of the ovary; the free part of each *vas deferens* is extremely short and can be seen only with difficulty.

Specimens obtained at the roots of eel-grass have very short siphons and seemed to fit Verrill's description of *Molgula manhattensis* better than that of *M. papillosa*. In internal anatomy they agree with specimens obtained beneath stones at low tide and in the dredgings, which correspond with the description of the latter species. Some of these specimens have siphons as long as those figured by Verrill for *Eugyra pilularis*. Specimens of *M. manhattensis* from Connecticut and Rhode Island, kindly sent me by Dr. Van Name, are distinctly different from all northern individuals. They have, as Dr. Van Name stated to the writer in a letter, a narrow dorsal lamina with smooth margin. Other differences are—a smaller, more rounded dorsal tubercle; the testicular lobes are not scattered but massed, being confined to the lower side of the ovary and the inner side of its anterior tip (on the left side, seen from without, the testicular mass appears to curl around the anterior end of the ovary, as figured in 1847 by Van Beneden for his *Ascidia ampulloides*, a related species); and the free portions of the *vasa deferentia* are much longer than in *C. papillosa*.

The nearest allies of the latter are *Molgula simplex* Ald. & Hanc. and *M. siphonata* Alder of the coasts of England. In both of these the testes are in the form of one or two large masses, confined to the inner side of the ovary. It is interesting to note that the English forms are short- and long-siphoned respectively, corresponding with the extreme individuals of the series of specimens of *C. papillosa* taken at St. Andrews.

This appears to be the *Caesira* that is most abundant and most generally distributed near St. Andrews.

*C. canadensis* sp. n.

This is the North American representative of the group to which Lacaze-Duthiers gave the name *Ctenicella*.

Body nearly spherical or flattened against the object of attachment. Attached usually by the right side. Up to about 1 cm. in diameter. Apertures fringed, each oral lobe with 3 teeth, each atrial with from 6 to 8. Exposed surface always more or less dirty. Along the margin of the attached area are numerous irregular radicoid filaments. If the

animal is sand-covered, these are present over the entire surface, including that of siphons. If not sand-covered, the free surface has numerous minute adhesive tubercles.

From 15 to 25 tentacles, pinnate or slightly bipinnate. Aperture of dorsal tubercle varying from a simple slit to the shape of an imperfect S, which Hartmeyer suggests is characteristic of the genus *Ctenicella*. Dorsal lamina with tapering distant teeth. Seven folds on each side of pharynx. Bars on both sides of each fold, as many as 4 (or occasionally 5) on a fold. Stigmata in infundibula (divided once), each stigma usually representing ¼ of a circle and simulating the longitudinal stigmata of other groups.

Intestinal loop narrow, more or less bent. Anus with smooth margin. Gonads some distance above intestinal loop and renal organ respectively. Ovary short, bent with the concavity ventral; oviduct passing from its anteroventral angle; testicular lobes along the upper side of the posterior end of the ovary or in a semicircle around its posterior end; the single *vas deferens* projects from the centre of the inner side of the ovary.

The species to which this form is most nearly related, and the respects in which it differs from them are as follows:—

*Molgula complanata* Ald. & Hanc.—7 folds on left side instead of 6, smaller number of bars and larger infundibula with the stigmata in transverse rows.

*Ctenicella lanceplaini* Lac.-Duth.—more teeth on each atrial lobe, deeper infundibula, more regular transverse rows of stigmata, a larger number of bars.

*C. morgatae* Lac.-Duth.—the smaller number of bars, the toothing of the posterior ends of the folds and the position of the testicular lobes.

At first I referred this species to Verrill's *Molgula littoralis*, but Dr. Van Name has informed me by letter that the latter (from his preliminary study of Verrill's specimens) has the long bent oviduct of the next species. He also states that he has not yet found any *Ctenicellæ* among his material. He suggests that it is something new to the region. There is the probability that it has been introduced from Europe since the time that Verrill collected in the Bay of Fundy region. Its derivation from *C. tenax* (Traust.), a nearly related Arctic form (occurring in Greenland) with usually only 6 folds, and its subsequent extension down the coast is another possibility. It is possible that further study will make it necessary to unite this species with the three from Europe into a single species.

Hartmeyer has retained Lacaze-Duthier's *Ctenicella* with an alteration of the diagnosis. His group does not seem to be a more natural

one than that of the latter author. Savigny's *Cynthia dione*, the type of the genus *Cæsira*, doubtless belongs to this same group. His description of the oral aperture as being 4-lobed and of the dorsal lamina as being smooth-margined was probably due to faulty observation. In that case *Ctenicella* will be synonymous with *Cæsira*.

*C. littoralis* (Verrill).

Syn.—*Molgula littoralis* Verrill, Amer. J. Sc., ser. 3, vol. I, p. 56.

Surface usually clean, at least in the neighbourhood of the apertures. Few radicoid filaments on surface. Siphons quite variable in length, usually rather short. Rows of papillæ on the outer surface of the siphons, corresponding with the apertural lobes. The papillæ are usually small and few in each row. Nearly globular in shape, somewhat laterally compressed. Siphons on dorsal edge, nearer anterior end.

From 20 to 30 bipinnate tentacles. Aperture of dorsal tubercle curved, the horns usually approximated so as to form a circle; opening between horns directed toward the right side. Dorsal lamina narrow, not continued behind œsophageal aperture, its margin smooth. Seven folds on each side, their posterior ends with smooth margins. Bars on both sides of each fold, as many as 10 on a fold. Stigmata in the usual infundibula (once branched), each stigma forming from ¼ to ½ a circle.

Intestinal loop rather narrow, bent with the concavity dorsal. Anus with smooth margin, Gonads in the usual positions close to intestine and renal organ. Ovary small, narrow; oviduct, which passes from its posteroventral angle, is long and bent so as to form a right angle, the terminal part passing up toward atrial siphon. Testicular lobes variously disposed, usually ranged along the upper and lower borders of ovary, sometimes forming a large mass covering the greater part of both inner and outer surfaces of the ovary; the free portion of the single *vas deferens* is of moderate length and projects from near the middle of the inner surface of the ovary.

A large number of specimens were obtained at low tide beneath rocks and in the dredgings from stony and shelly bottoms.

This form is very close to two European species, *Molgula citrina* Ald. & Hanc. and *M. echinosiphonica* Lac.-Duth. The former has fewer bars (6) and fewer tentacles (12 to 14). The latter has very conspicuous spines on the oral siphon whereas the atrial is smooth and the testicular lobes are placed at some distance from the ovary. It is doubtful whether these differences are important.

*C. pannosa* (Verrill).

Syn.—*Molgula pannosa* Verrill, Amer. J. Sc., ser. 3, vol. I, p. 55.

Surface, except that of siphons, with numerous fine, long filaments and entirely covered with shell-fragments, sand-grains &c. Siphons

short, rather close together near anterior end; when retracted they occupy depressions, which are surrounded by projecting ridges or collars. Apertures with the usual lobes; the oral lobes occasionally have more than the single tooth or process and the atrial appear to have regularly 4 or 5 teeth, just as in Lacaze-Duthier's genus *Ctenicella*. Body elongated, laterally compressed, up to 2½ cm. in length.

About 20 (?) bipinnate tentacles. Aperture of dorsal tubercle horseshoe-shaped; opening between horns directed backwards. Dorsal lamina narrow, its margin smooth. Seven folds on each side. Bars on both sides of each fold, as many as 12 on a fold. Stigmata rather short, each forming only about 1/8 of a circle at base of infundibulum. Infundibula branched dichotomously once or twice.

Intestinal loop narrow, horizontal. Each gonad a large oblong mass, with ovary central and testicular lobes chiefly above and below ovary. Oviduct directed upward from posterodorsal angle. There are as many as 7 *vasa deferentia* projecting from the inner side of the ovary in an irregular row.

This species was obtained at most points where dredgings were made in gravel, but never in quantity.

It resembles *C. pacifica* in the structure of the gonads and pharynx (7 folds, bars on both sides of folds, smooth dorsal lamina), but differs from it in having the surface covered with radicoid filaments and the dorsal tubercle directed backwards. From *C. oculata* (Forbes) of Europe it differs in having a smaller number of bars on the folds and the horns of the dorsal tubercle not rolled in.

*C. retortiformis* (Verrill).

Syn.—*Molgula retortifromis* Verrill, Amer. J. Sc., ser. 3, vol. I, p. 56.

This species occurs sparingly at low tide beneath rocks near the station and was dredged at various points in the approaches to Passamaquoddy Bay on stony and shelly bottoms.

It is by far the largest Cæsirid occurring at St. Andrews, the majority of the specimens being about 3 cm. in diameter.

Characteristic of this form are—its thick test, long atrial siphon (when extended) and the separation of the testes from the ovary. The latter has the usual position—above the intestinal loop on the left and above the renal organ on the right. The testes are below the renal organ on the right side and rather extensively distributed below the ovary, on the inner side of the intestinal loop on the left side. The oviduct of each side is long, ending just beneath the atrial velum. The *vasa deferentia* are very numerous. In one specimen 12 were counted on the right side and 25 on the left. They are scattered over the inner surface of the testicular mass. Their free portions are extremely short.

*Eugyra* (*Bostrichobranchus*) *pilularis* Verrill.

Syn.—*Bostrichobranchus manhattensis* Traustedt, Vid. Meddel., ann. 1884, p. 22.

No specimens were found in the vicinity of Eastport (where Verrill obtained it). But in 10 fathoms sand at Grand Manan numerous specimens were obtained which seem to be referable to Verrill's species. The tubes are strongly retracted in all the specimens, but the 'collar' at the bases of the siphons is very distinct.

This is very evidently Traustedt's species as well. The only differences apparent are explicable as due to a difference in size. Traustedt having specimens with a diameter twice as great as that of the largest in my collection. There is one exception. He describes the margin of the anus as smooth. In two specimens examined, the margin is reflected, but distinctly lobed (about 16 lobes). Evidently he has overlooked this reflected margin.

In *E. glutinans* and *E. adriatica* the entire margin of the anus is lobed and free. In this species the inner margin or lip is fused with the pharyngeal wall and the line of fusion seems to be represented by an irregular row of about 16 papillæ, placed just in front of anus on the outside of the pharyngeal wall.

There are 15 tentacles, the largest bipinnate. The dorsal lamina is broad and continued back behind œsophageal aperture and downwards toward endostyle.

Infundibula as in typical *Eugyræ*, consisting each of two stigmata spirally coiled and not broken up into short stigmata. They are not in regular rows. In a very small specimen not more than one row can be made out between successive longitudinal bars. In one 8 mm. in diameter there are two irregular rows and Traustedt has figured a larger number for a much larger specimen.

The oviduct passes along the left side of the rectum and opens only a short distance from the anus. The testes are along the upper and lower margins of the ovary. The *vasa deferentia* are numerous (9 in one specimen) and their free portions short, opening at various points along the middle of the inner side of the ovary.

The irregularity in the arrangement of the infundibula is not of enough importance to warrant the formation of a genus for this species as Traustedt has done, especially when there are as many points of agreement between it and typical *Eugyræ* as the following:—

Musculature reduced to siphonal region, with the exception of short fibres arranged in one or two rows encircling the body in the median plane.

Dorsal tubercle horseshoe-shaped, opening between horns directed toward left side and slightly forwards.

Dorsal lamina with smooth margin, extending behind œsophageal aperture.

Pharyngeal folds represented by single longitudinal bars, which are very thin and broad. Infundibula as described above.

Margin of anus lobed.

Only one gonad and that placed on the inner side of intestinal loop; oviduct accompanying rectum; testes peripheral, their duct or ducts not accompanying oviduct.

It might be well to retain *Bostrichobranchus* as a subgenus, if there prove to be species more closely related to *E. pilularis* than to the typical members of the genus (*E. glutinans*, *E. translucida* and *E. adriatica*).

*Goniocarpa placenta* (Packard).

Syn.—*Cynthia placenta* Packard, Mem. Bost. Soc. N.H., vol. I, p. 277, part.

? " *pulchella* Verrill, Amer. J. Sc., ser. 3, vol. I, p. 99.

Easily recognized by the small, rounded, granular elevations that thickly cover the test. Near the apertures they are almost papilliform. I have not yet determined the differences (if any exist) between this form and the nearly related European and Pacific forms. It is as variable in shape as they.

Verrill states that Packard's specimens belonged to two different species and the one which he does not name but describes appears to be this species. Verrill himself probably confused this with *Dendrodoa carnea*, if the latter was as abundant and widely distributed in the Bay of Fundy when he made his collections as it is now. His *Cynthia pulchella* appears to have been a rounded form of one of these species, probably G. *placenta*.

*Cnemidocarpa mollis* (Stimpson).

Syn.—*Glandula mollis* Stimpson, Proc. Bost. Soc. N. H., vol. IV, p. 230.

" " Traustedt, Vid. Meddel., ann. 1880, p. 422.

" *arenicola* Verrill, Amer. J. Sc., ser. 3, vol. III, p. 288.

*Tethyum arenicolum* Hartmeyer, Zool. Anz., vol. 34, p. 147.

The two specimens obtained came from 10 fathoms sand not far from the locality from which Stimpson procured his specimens. They correspond with the descriptions given by the authors listed in the synonymy. I have placed this species in the genus *Cnemidocarpa*, as it agrees in the condition of the gonads and atrial tentacles with the type of the genus. It belongs to a group, consisting of forms with radicoid processes of the test to which sand-grains adhere, including *Styela vestita* Alder and *S. villosa* (Kupffer).

*Dendrodoa* (*Styelopsis*) *carnea* (Agassiz)?

Many specimens were obtained at low tide near the station and by the dredge at nearly every point, but never in large numbers. I have identified this with the *Ascidia carnea* of Agassiz with some hesitation. Hartmeyer, after examining specimens of this genus from Casco Bay, Maine, has considered Agassiz' species to be synonymous with *D. aggregata* (Rathke). I have not been able to find in my material any specimens that would correspond with Hartmeyer's description of the latter species. They are all more nearly related to *D.* (*Styelopsis*) *grossularia* (Beneden). They differ from it in having a very small number of longitudinal bars. This form shows, in fact, a much greater reduction in the number of bars than any other member of the genus. In nearly every case the formula is,

Right side. 0 (4) 0 (1) 0 (1) 0 (1) 0.
Left side. 0 (1) 0 (1) 0 (1) 0 (1) 0.

In one specimen the formula for the left side is

0 (2) 0 (1) 0 (1) 0 (1) 0.

In another specimen, which may belong to another species, the formula is—

Right side. 0 (4) 1 (3) 0 (2) 0 (1) 0.
Left side. 0 (2) 1 (2) 1 (2) 0 (2) 0.

This specimen differs from other individuals of the same size in having an immature gonad and no eggs in the brood-chamber.

The shape varies greatly—from scale-like to elongated oval, occasionally attached by a small base. Shallow-water specimens are a bright red. Those from deeper water are paler. As in *Cnemidocarpa joannæ*, the test in the living animal is transparent and the pigment is confined to the 'mantle.'

In a specimen 8 mm. in diameter there are 35 oral tentacles and 34 small tapering atrial tentacles. From 16 to 25 stigmata in each mesh.

The single gonad is similar to that of *D. grossularia*, the ovary forming the inner part and the testicular lobes a layer on the outer side. There are several *vasa deferentia*. The eggs are retained in a posterior brood-chamber into which the oviduct opens. There is a distinct pyloric cæcum. The anus is two-lipped, its margin indistinctly lobed.

*Pandocia fibrosa* (Stimpson).

Syn. *Glandula fibrosa* Stimpson, Proc. Bost. N.H., vol. IV, p. 230.

Specimens agreeing with Stimpson's description and obtained from the same locality as his specimens (the Hake Bay, off Grand Manan), prove to be closely related to the *Cynthia comata* of Alder. According to Hartmeyer, *Pandocia conchilega* Fleming the type of Fleming's genus *Pandocia*, is the same as *Cynthia comata*. Heller has specified *Glandula*

*fibrosa* as the type of *Glandula*. The latter genus is consequently synonymous with *Pandocia*.

Two hauls of the dredge made at a point off Long Island in about 35 fathoms, mud, brought up numerous specimens of this species.

The shape is spherical, and there is a thick coating of mud, which adheres to long fibrous processes of the test. These cover the entire surface, with the exception of a small area near the siphons. They are of three kinds, which intergrade—(1) simple threads, most numerous ventrally, (2) numerous threads arising from small tubercles of the test and (3) long processes with threads arising from each at different levels in a verticillate manner. Siphons verrucose and rusty.

From 45 to 55 tentacles. Dorsal tubercle horseshoe-shaped, opening between horns directed backwards and slightly towards the left. Dorsal lamina narrow. Four folds on each side. From 9 to 15 bars on each fold and from 1 to 4 bars in a space. Intermediate transverse vessels. From 5 to 8 long narrow stigmata in each mesh.

Diameter of stomach scarcely greater than that of intestine. About 24 gastric folds. Intestinal loop narrow, horizontal. Margin of anus with about 20 rounded lobes. Gonads hermaphroditic, about 2 mm. in length and 1 mm. in width. The end of each, that bears the ducts, is directed in most cases toward the atrial siphon, but occasionally downwards. From 10 to 15 gonads on each side, more numerous on the right. Endocarps numerous, many with enlarged opaque summits.

Siphonal vela narrow, free from wall. Atrial tentacles small, filiform, irregular in size, placed in an irregular row near attached edge of velum.

This species differs from *P. comata* (Alder) in having larger, verticillate processes of the test, more numerous gonads and a habitat in mud instead of sand.

*Boltenia ovifera* (L.).

This well-known species occurs at nearly every point and frequently in large numbers.

*B. hirsuta* (Agassiz).

Syn.—*Ascidia hirsuta* Agassiz, Proc. Am. Assn., vol. 2, p. 157.
*Cynthia (seu Halocynthia) echinata auct. americ.*

Hartmeyer (Fauna Arctica, vol. 3) has queried whether or not the North American form that has gone by the name of *Cynthia (seu Halocynthia) echinata* is identical with the Arctic and European form for which the same name has been used. The study of a number of specimens from St. Andrews has shown that we have on this coast a distinct form which differs from European, Arctic and Pacific specimens in hav-

ing rudimentary tentacles and very short gonads. The spines of the test are somewhat similar to those described by Hartmeyer for subarctic European specimens, but in this case we have large individuals with spines of this character.

*Ascidia hirsuta* Agassiz appears to be the only name that has been given primarily to Eastern North American specimens and is, therefore, the valid one for this species.

Specimens of large size were obtained at low tide near the station. It occurs generally distributed as shown by the dredgings.

*Tethyum pyriforme* (Rathke) subsp. *americanum* nov.

Syn.—*Cynthia* (*seu Halocynthia*) *pyriformis auct. americ.*

In contrast with the great constancy in the number of the gonads in the Mediterranean *T. papillosum* and in the Pacific *T. aurantium*, the number in *T. pyriforme* of Arctic and European seas is stated to vary from 4 to 6 on the right side and to be constantly 4 on the left. An examination of a series of individuals from St. Andrews, shows a variation of from 3 to 10 on the right side and from 5 to 14 on the left, the number on the left being usually greater than that on the right. It seems best to consider this as a subspecies of the Arctic *T. pyriforme*.

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# University of Toronto Studies

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# A CONTRIBUTION TO THE MORPHOLOGY AND BIOLOGY OF INSECT GALLS.

BY

A. COSENS, M.A.

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# A CONTRIBUTION TO THE MORPHOLOGY AND BIOLOGY OF INSECT GALLS.

BY A. COSENS, M.A.

The earliest explanations offered to account for the origin of galls are more or less fanciful, as must needs be, since nothing was known concerning the reciprocal relations of host and parasite involved in their life histories. Some of these theories dating from a less scientific age are crystallized in the popular names attached to certain classes of these structures. Thus the bristling masses of twigs, produced on several species of trees by the stimulation of plant or animal parasites, are known by some as "thunder bushes," a term that implies the expenditure of electrical energy in their origin. Also the more common name, "witches' brooms," indicates an equally imaginative explanation.

With the gradual advances of science much of the mystery surrounding galls has been dispelled, but they still present many interesting problems. Among these are two standing out so prominently that they seem to include all others. The first of these concerns the causes that are operative in producing the gall. It is now generally recognized that its origin is directly ascribable to the stimulation of a parasite, but the problem still remains concerning the nature of the stimulus and the principle governing the response. Concerning the nature of the stimulus, Fockeu and his school ascribe it to mechanical means, while Küster, Küstenmacher and others believe it to be referable to a chemical action. With regard to the response by the host, the conventional view endows the stimulated protoplasm with power to originate something foreign and without a prototype in the normal host. But a view is developed in this paper proposing an entirely different explanation, namely, that the supposedly new types of organs, tissues, etc., are due to the awakening of dormant characteristics in the protoplasm.

The second problem deals with the apparent philanthropy that characterizes the host plant in its care for the parasite. Concerning this, several explanations have been offered. The parasite may be simply taking advantage of structures thrown out by the plant in its own defence, or as Adler[1] figuratively expresses it, the besieger is making use of the water in the moat in pushing forward the attack on the fortifications. Perchance, even what Darwin regarded as impossible has taken place, and the plant is producing the gall entirely for the welfare of the parasite. In opposition to this the theory is proposed by some

investigators that in reality the host does derive benefit from the abnormal swelling since it has been the means of restricting the ravages of the parasite to a limited area.

I have confined the subject-matter of this research to the Zoocecidia including the Insecta and Arachnida. In the Insecta the following orders are dealt with—Hemiptera, Lepidoptera, Diptera and Hymenoptera. No attempt has been made to treat any one order exhaustively, but the work was restricted to the living gall material at hand. As a preliminary to the botanical work, several seasons were spent in rearing the producers in order that unquestioned identification could be assured of the various forms studied.

Concerning the anatomy of our American galls, the only work done previous to this is that by Cook,[22] who must be considered the pioneer in this branch of the subject. The anatomical studies pursued are considered important as forming a foundation for deductions.

The recent investigations of Weidel[45] on the early developmental stages of Neuroterus have added valuable data to the ontogenetic work. It is exceedingly necessary that some of the American species should be worked out in the same manner, but before this can be done with assurance of success, an American Adler must settle the questions concerning the alternate generations of our native forms.

Special attention has been paid to the Sawfly and Lepidopterous galls, since the anatomy of the former has hitherto not been dealt with at all and the ontogeny only very inadequately. The latter have also been neglected to nearly the same extent. The order Diptera and the family Cynipidæ have presented the most interesting biological phenomena, notably concerning the feeding habits and the nature of the gall-producing stimulus, two factors that are closely parallel if not indeed identical in these two sections of the gall producers.

With the exception of *Pachypsylla celtidis-mamma* Riley all the material was collected in the vicinity of Toronto. It was fixed in picro-sublimate or Flemming's weaker fluid and cut in series in paraffin or celloidin. A double stain of safranin and hæmatoxylin was invariably used.

The investigations described in this paper were carried on in the Botanical Laboratories of the University of Toronto under the supervision of Prof. J. H. Faull, who suggested the subject and to whom I wish to acknowledge my indebtedness for invaluable criticism and assistance throughout. To Dr. C. D. Howe I wish to express my obligations for direction in the physiological experiments. My thanks are also due to Mr. W. A. McCubbin, M.A., for valuable assistance in the photography and to Mr. J. H. White, M.A., for normal material.

## ORDER ACARINA.

The following species are described in this section:—

Order Acarina.

Fam. Eriophyidæ.

*Eriophyes Sp.* (*Fagus grandifolia* Ehrh.)
*Eriophyes querci.* (*Quercus macrocarpa* Michx.)
*Eriophyes Sp.* (*Acer negundo* L.)
*Eriophyes Sp.* (*Populus grandidentata* Michx.)
*Eriophyes Sp.* (*Populus tremuloides* Michx.)
*Eriophyes Sp.* (*Prunus nigra* Ait.)
*Eriophyes abnormis.* (*Tilia americana* L.)
*Eriophyes serotinæ* Beut. (*Prunus serotina* Ehrh.)

The numbers referred to are from "A Catalogue of the Phytoptid Galls of North America," by George H. Chadwick, 23rd Report of the State Entomologist, New York State Museum, 1907.

### *Eriophyes Sp.*

Chadwick's No. 65

Host *Fagus grandifolia* Ehrh.

This gall occurs on the under side of the leaves of the host plant. It is an erineum, white and frost-like at first, turning to a brown colour at a later stage.

The anatomical structure of the interior of the leaf remains perfectly normal, the effect of the stimulation being confined to the epidermis. This produces a large number of unicellular, capitate hairs, resembling miniature mushrooms, the resemblance to which is the more striking since the stalks of the hairs are bulbous at the base, as shown in Fig. 3. The hairs on the normal leaf are long, acicular and unicellular. As this gall is produced with no increase in the number of cells in the leaf, anatomically it stands as a simple type of "hypertrophy", the remaining forms all exhibit the more advanced phenomenon of cell proliferation.

### *Eriophyes querci* (Garman).

Chadwick's No. 112

Host *Quercus macrocarpa* Michx.

In this gall, which is of the "dimple" type, the depression is on the under and the elevation on the upper side of the leaf. The hollow is filled with a dense mass of brown pubescence. In rare cases the elevation is on the under side of the leaf, when the pubescence then covers it.

The leaf blade in this case has become of nearly twice the normal thickness. This has resulted from cell division which has occurred in the tissues of both the palisade and the spongy parenchyma, producing a compact mass of undifferentiated cells, entirely without intervening air spaces. This tissue is shown in Fig. 6.

The epidermis has responded to the stimulation by producing many long, acicular, unicellular hairs, which are narrowed at the base. The hairs are stellate on the normal leaf of the host, but are simple, unicellular and acicular on its reproductive axis. The same feature is true of *Quercus robur var. pedunculata*, and in all probability of the other oaks. This appears to be a clear case, not of the production of a new type of trichome, but of a reversion.

*Eriophyes Sp.*
Chadwick's No. 2
Host *Acer negundo* L.

A shallow dimple on the under side of the leaf, filled with a white pubescence.

In this gall as in the preceding species, the leaf blade has been very much thickened by proliferation in the mesophyll. The cells produced are circular in outline and of about the same size as those of the normal spongy parenchyma. The hairs produced in this case not infrequently consist of from 2 to 3 cells which are very much convoluted. They are well shown in Fig. 4. The hairs on the normal leaf of the host are straight or only very slightly curved, but the glandular, convoluted type of hair is found on the inflorescence. Both the normal and abnormal hairs are composed of the same number of cells.

*Eriophyes Sp.*
Chadwick's No. 84
Host *Populus grandidentata* Michx.

A deep dimple gall with the convex part on the upper surface of the leaf. The gall is light green above, with the contents of the depression dark red.

The cells produced by the palisade parenchyma can be distinguished in this case from those arising from the spongy parenchyma. The former are almost square in outline and placed in two very regular rows immediately below the upper epidermis, and parallel with it. This tissue can be seen in Fig. 2. The latter constitute a tissue made up of cells which are circular to elliptical in outline. These cells are much smaller than those produced by the palisade layer. The outgrowths from the lower epidermis described in various ways as trichomes, granules, etc., are in reality produced by a complicated folding, in which only the lower epidermis and the spongy parenchyma participate, as shown in Fig. 2.

A cross section of the gall shows a number of vascular strands, but in my opinion the gall-producing stimulus only enlarges the veins that are already present in the normal leaf; it does not originate a special vascular system for the gall.

*Eriophyes Sp.*
Chadwick's No. 88.

Chadwick considers this form, first described by Jarvis,[28] the same as his No. 87. But the latter is characterized by a depression on the under side of the leaf, the former by an elevation, so that the two are constantly distinct.

Host *Populus tremuloides* Michx.

A dimple gall with the elevation on the under surface of the leaf. The elevation is a lighter green than the surrounding normal leaf and the folds that occupy the concavity are greenish-yellow or reddish in colour.

In dealing with the anatomical structure it is to be noted that the spongy parenchyma has in this case remained normal. The folds are produced in the same manner as in the preceding species, except that in this form it is the upper epidermis that undergoes the folding process. The nature of this folding can be seen in Fig. 1.

*Eriophyes Sp.*
Chadwick's No. 93.
Host *Prunus nigra* Ait.

A very much elongated pouch gall, greenish or whitish in colour, found on the upper side of the leaf with the opening on the under side.

All the characteristics of the normal mesophyll have been completely altered in the affected part of the leaf. Its cells, which, with the epidermis, constitute the wall of the gall, are larger than the normal and are elongated parallel to the long axis of the outgrowth. The upper epidermis which forms the epidermis of the gall has not been affected, but the cells of the lower epidermis which line the gall cavity have become much enlarged and in addition have produced a large number of closely set trichomes which project into the gall cavity. The nature of these is shown in the upper part of Fig. 5. These structures are often from 2 to 3 cells in length. Around the opening of the gall a circle of closely set acicular hairs occurs. The hairs on the outside of the gall and on the normal leaf are also of the acicular type. The vascular strands are much larger than those of the normal leaf but appear to be simply the stimulated normal veins.

*Eriophyes abnormis* (Garman).
Chadwick's No. 144
Host *Tilia americana* L.

A very much lobed pouch-gall, usually on the upper side of the leaf, found rarely on the under side. The opening which is on the opposite side of the leaf to the pouch is surrounded by a dense growth of acicular trichomes.

The anatomical structure of this gall shows it to combine the characteristics of a pouch-gall with a type in which the epidermis lining the larval cavity is thrown into folds, as described in the case of the galls on Populus. The foldings of the lining of the gall cavity often coalesce, and practically divide the cavity into a number of compartments. Morphologically this is one of the most highly differentiated of our Phytoptocecidia. The lining epidermis has produced a large number of acicular, unicellular trichomes; these in some cases almost fill the cavities. The hairs surrounding the gall aperture are of the same type, as are also the normal hairs of the leaf. Glandular cells are much more abundant in the gall tissues than in those of the normal leaf.

*Eriophyes serotinæ* Beut.

Chadwick's No. 100

Host *Prunus serotina* Ehrh.

This is a club-shaped gall, produced on the upper side of the leaf of the host. The aperture of exit, which is on the lower side, is surrounded by fine white hairs. It varies in length from 5 to 8 mm. and at a distance from the apex of about two-thirds the total length, it is narrowed into a stalk with an average diameter of 1 mm. In color it varies from green to a distinct red.

The stimulation has not produced very marked changes in the structure of the leaf near the origin of the gall. Apart from the fact that the spongy parenchyma has divided more actively, the mesophyll is normal. The upper epidermis of the leaf that forms the outer covering of the gall, has its cell walls abnormally thickened. The lower epidermis that lines the gall cavity has larger cells than the unstimulated epidermis, and from these cells originate elongated, unicellular trichomes with bulbous bases. The hairs on the normal leaf are acicular and unicellular. While the cells in the neck of the gall are arranged in rows parallel to its length, the larger cells that form the main body of the gall are not regularly placed. The vascular strands pass up from the leaf at a distance of about three cell layers from the gall cavity.

*Summary.*

In some forms the effect of the stimulation does not extend beyond the epidermis, on which the producers are located, but in other species it is transmitted throughout the mesophyll of the leaf.

The abnormal activity of the epidermis is expressed in the curving and folding of the tissue as well as in the production by it of various forms of trichomes.

In general, when the effect of the stimulation extends to the mesophyll the distinction between the palisade and the spongy parenchyma is lost. In place of these tissues a compact mass of uniform cells is produced.

The types of the galls constitute a clear phylogenetic series from the simple erineum to the well developed pouch-gall. The distinction between the different members of this series is largely a difference in degree rather than in kind, and seems to be explainable on the assumption of a gradually increasing intensity of stimulus from the lowest to the highest member of the series.

The literature of this group of galls contains a number of statements, to the effect that the host plant under the influence of the gall stimulus can originate entirely new types of hairs. While it is true, as a general rule, that hairs produced by an organ under such a stimulus differ from those originated by that organ under normal conditions, yet in such cases I have found that the abnormal type of hair is being produced on another part of the host plant. Thus the curiously convoluted, glandular hairs, originating in the dimple galls on the leaves of *Acer negundo* L., are duplicated exactly in the hairs occurring on the reproductive axis of the same plant, and the long acicular hairs composing the brown pubescence that fills the concavities on the leaves of *Quercus macrocarpa* Michx have their exact counterparts in those produced on the flowering axis of that plant. In the former example the normal hairs on the leaves of the host are straight and acicular, in the latter they are of the stellate type.

## ORDER HEMIPTERA.

The following Hemipterous galls have been studied:—

Fam. Aphididæ.

Gall on *Populus balsamifera* L. (Unclass.).
*Hormaphis hamamelidis* Fitch.
*Hamamelistes spinosus* Shimer.
Aphid Corrugations on Birch.
*Pemphigus vagabundus* Walsh.
*Pemphigus rhois* Fitch.
*Chermes abietis* Chol.
*Chermes floccus* Patch.

Fam. Psyllidæ.

*Pachypsylla celtidis-mamma* Riley.

Aphid Gall (Unclassified),
Host *Populus balsamifera* L.

A pouch-like gall on the under surface of the leaf, produced by a fold in the blade near the base of the midrib. One edge of the fold is attached along this midrib. The slit-like opening, which is on the upper surface of the leaf, extends the full length of the gall. This species resembles very closely the gall produced by *Cecidomyia majalis* Bass. The general structure is shown in Fig. 7.

Dimensions:—Length along line of attachment to midrib, 10-12 mm.; Width, 4-5 mm.

In the part of the leaf blade that forms the gall all resemblance to the normal mesophyll has disappeared. A compact mass of tissue has taken its place, the cells of which are much larger than normal mesophyll cells. Towards the interior of the gall the cells become smaller and richer in protoplasmic contents. The upper epidermis forming the interior of the gall remains practically normal, except that it produces longer and more abundant trichomes than when unstimulated. These trichomes are usually three cells in length. A cross section of this gall shows two small groups of cells with porous laminated sclerenchymatous walls, one of which is situated near the midrib and the other exactly opposite on the other side of the gall opening. Thus each side of the gall aperture is bordered by a band of sclerified cells, as shown in the transverse section of Fig. 8.

*Hormaphis hamamelidis* Fitch.
Host *Hamamelis virginiana* L.

The gall formed by this species is found on the upper side of the leaf of the host, but the larvæ escape from an opening on the under side. The mature gall is conoidal in shape with the apex usually slightly bent over. General structure is illustrated in Fig. 12. A circular ring of tissue covered with pubescence surrounds the gall opening which is shown in Fig. 12.

Dimensions:—Average length of gall 10.5 mm.; diameter at base 4 mm.

The gall is composed of small cells placed close together, forming a compact and very uniform tissue. The cells are arranged with their longer diameters pointing in the direction of the gall apex. In a longitudinal section the vascular strands are seen to pass up each side at a depth of about three cells from the gall cavity. The beaks of the larvæ, often found imbedded in the wall of the gall, were inserted far enough to almost reach these vascular strands. The hairs that surround the gall aperture are acicular and unicellular.

*Hamamelistes spinosus* Shimer.
Host *Hamamelis virginiana* L.

The galls in this species are modified flower buds. These are somewhat elliptical in outline with gradually tapering stalks. They are covered with spines, which are usually curved. The opening is situated at the union of the stalk and the gall proper. This opening is funnel-shaped and is surrounded by a circular ring of tissue as in the preceding species on the same host. The pubescence is absent in this case.

Dimensions:—Average length including stalk 21 mm.; average width 10 mm.

This gall is covered by an unusually small-celled epidermis. The spines that are so noticeable a feature are found to consist of projections of the epidermis, filled with cells in continuity with the mesophyll. The cells of the gall are almost perfectly circular in outline and packed together very closely. This tissue is very uniform except in the four or five layers adjoining the gall cavity. In that zone the cells are smaller and richer in protoplasmic contents, constituting a fairly well marked nutritive layer.

In cross section of the gall about thirty main fibro-vascular bundles are cut; these are comparatively large and situated near the larval cavity. Two of these have been cut in the section shown in Fig. 10. Other smaller strands are cut further out. The gall receives all the fibro-vascular strands that, under normal conditions, would have passed up into the flower. The interior of both this and the preceding species is almost perfectly glabrous.

### Aphid Corrugations on Birch.

Hosts { *Betula lenta* L.
*Betula alba var. papyrifera* (Marsh) Spach.

The primary folds in the leaf that form this gall run parallel to the main veins, with the latter as boundaries between them. Their crests are on the upper side of the leaf, while the hollows which form the larval chambers are on the under side. The primary folds are divided into secondary folds, and these again into depressions resembling minute Acarina dimple-galls. This complex arrangement is conditioned entirely by the veining of the leaf, since each fold, primary or secondary, is supported along its edges by veins. The folding can be seen in Fig. 9.

The anatomical characteristics of these galls show that the folding of the leaf has not entirely changed the structure of its normal mesophyll. Around the gall cavities the spongy parenchyma is nearly normal throughout and the palisade layer is recognizable in different places. The cells, however, are considerably larger than the cells of the normal mesophyll. The cells of the lower epidermis, that form the lining of the gall cavities, are well filled with food materials for the larvæ. The supporting veins on each side of the fold send out branches that supply the gall with an adequate vascular system.

*Hormaphis hamamelidis* Fitch and *Hamamelistes spinosus* Shimer, as worked out by Pergande,[40] show that they inhabit alternately *Betula nigra* L. and *Hamamelis virginiana* L.

The galls on the birch leaves are produced by the fourth generation of *Hamamelistes spinosus* Shimer. Pergande described them as "pseudo-galls or corrugations".

The witch-hazel galls produced by the stem-mother of this species are plentiful in this locality, but *Betula nigra* L. is not found here. The Aphids have consequently been compelled to extend their list of food plants to include *B. lenta* L. and *B. alba var. papyrifera* Spach.

*Pemphigus vagabundus* Walsh.

Host *Populus deltoides*, Marsh.

All the leaf rudiments of the terminal bud appear to be concerned in the production of this gall. Yet it is in reality a large pouch-gall with its wall thrown into smaller secondary folds. The apex of the stem, from which the gall originates, is usually swollen to nearly twice its normal diameter. These galls often remain on the host plant until the next season's galls begin to appear.

The cells composing this gall form a compact and fairly uniform tissue and a nutritive layer is not clearly differentiated. About one-third of the thickness of the gall wall, on the side next the larval chamber, however, is composed of cells that are somewhat larger than the remaining cells. The epidermal cells, lining the gall cavity, are elongated into short trichome-like structures. The phyllome origin of this gall is revealed by the presence of well defined stomata on its epidermis. These structures are numerous and appear to be quite normal. Glandular cells are plentifully distributed. Vascular strands pass irregularly throughout the wall of the gall.

*Pemphigus rhois*, Fitch.

Host *Rhus typhina* L.

A balloon-shaped gall with the regularity of its outline destroyed by the elongated lobes that cover its surface. A gall is shown in Fig. 14. The epidermis is slightly pubescent and coloured red, shading into yellow and green. It originates from the under side of the leaf, and the point of attachment on the upper side is indicated by a small papilla covered with a dense pubescence. These galls vary in size from very short types less than 1 cm. to those that are 4 to 5 cm. in length.

In the part of the leaf blade folded to form the gall, the mesophyll has been entirely changed. The effect of the stimulation has even destroyed the normal characters in the mesophyll for some distance from the point of attachment of the gall. The gall consists of a compact tissue composed of cells considerably larger than the normal cells of the mesophyll. The cells of this tissue are arranged in layers parallel to the epidermis of the gall. The vascular strands are situated about four cell layers in from the gall cavity. In all pouch-galls the tracheary tissue is

composed of the ordinary vascular elements of the normal leaf that have been stimulated to increased activity. There is not a special tracheary system originated for the gall. In the galls the strands occupy a definite position, since in the normal leaf they occupy a definite place in relation to the spongy and the palisade parenchyma. Large glands are present in the gall tissue, as shown in Fig. 14. A gland is found invariably associated with a fibro-vascular strand and seems to have its counterpart in the very small gland that runs through each vein of the normal leaf. In some cases the abnormal glands have acicular trichomes projecting into their cavities.

*Chermes abietis* Chol.

Hosts { *Picea abies* (L) Karst.
*Picea mariana* (Mill) B.S.P.

A polythalamous gall produced by the swelling of the base of the young shoots. Since the twigs are not usually killed the galls are surmounted by a variable length of normal stem. The galls in general vary from conoidal to nearly spherical in shape, but in some cases, owing to the stimulation not having affected the entire circumference of the stem, the gall does not extend completely around it and is consequently less regular in outline. The surface of the gall is covered with the enlarged bases of the aborted needles. These give a faceted appearance to the gall and produce a likeness to a miniature pineapple.

Dimensions:—Longer diameter 2-3 cm.; shorter diameter 1-2 cm.

The gall in this case is a joint production of the cortex of the stem and the bases of the leaves of the host. The cells of the epidermis, lining the gall cavities, in some cases have been prolonged to form very short trichome-like structures. The hairs at the aperture of exit, as seen in Fig. 11, are composed of one or two cells.

The resin ducts that occur in the normal cortex are found considerably enlarged in the gall. In addition to these, there are out near the gall periphery numerous smaller resin ducts, as shown near the margin of Fig. 11, that do not have corresponding structures in the unstimulated tissues. These additional ducts pass in from the swollen bases of the aborted leaves. A cross section near the base of these leaves cuts from four to six resin ducts, while a normal leaf does not contain more than two of these structures.

*Chermes floccus*, Patch.

Host *Picea mariana* (Mill) B.S.P.

In this species the gall is produced by the swelling of the entire shoot. In comparison with the former species, the leaves are little, if any, swollen at the base but are more numerous on the gall than on an equal

length of normal stem, owing to the shortening of the stem axis. The larval cells are in the cortex of the stem at the bases of the needles.

Dimensions:—Average length 2-6 cm.

The abnormal development of the cortex, especially that part contained in the wings on the stem, produces the entire mass of this gall. The stimulation has increased the number of resin ducts in the cortex. Several cross sections of galls were compared with corresponding sections from normal stems. The average number of resin ducts in the abnormal to that in the normal was in the proportion of 20 to 12. The smaller accessory resin ducts are shown in Fig. 13. In every section examined the additional ducts were in an irregular circle outside the normal ducts. The ducts produced under stimulation were larger than the corresponding normal ducts, but those that were found only in the abnormal tissues were smaller than the normal structures.

Fam. Psyllidæ.

*Pachypsylla celtidis-mamma* Riley.

Host *Celtis occidentalis* L.

A complicated form of pouch-gall produced in the mesophyll of the leaf of the host. On the upper surface of the leaf the gall is indicated by a decided depression in the centre of which is a slight elevation that marks the opening of the gall. The part projecting from the lower surface, is oblate-spheroidal in shape, attached to the leaf by a slightly tapering cylindrical stalk. The average number of galls found on a leaf is usually about ten, but in some cases much higher. The surface of the gall is smooth except for a few fine scattered hairs, glaucous and greenish-yellow in colour.

Dimensions:—Height from point of attachment 6-7 mm.; width 7-8 mm.

The anatomical structure of this gall shows it to be a more complex type than any other of the Hemiptera discussed in this paper.

Besides the folding of the leaf the blade has been further changed in thickness and in the character of the cells. The production of the greater part of the abnormal tissue is due to a wide, well differentiated cambium layer, that extends right across the gall and at its margin passes into the tissues of the normal leaf between the palisade and the spongy parenchyma (Fig. 15). The larval chamber is lined by this cambium sheath which thus functions as a nutritive layer. Bordering this zone is a well developed protective tissue composed of cells with uniformly thickened walls. The sclerenchyma is laminated and penetrated by branched canals, presenting the same character as that found in the galls of the Cynipidæ.

Outside of this protective zone lies the chief mass of the gall, composed of thin-walled irregularly shaped cells, as illustrated in Fig. 15. Typical cells of the protective sheath are also found scattered throughout this tissue. As the gall becomes older these cells increase in number.

*Summary.*

These galls are characterized by a folding and wrinkling of the leaf when they occur on that organ; in this particular they resemble the Phytoptocecidia. This common characteristic is due to the fact that in the orders Acarina and Hemiptera the stimulation is all from one side. The spherical type of the Cynipidæ is produced by a stimulus equally disseminated in all directions.

The tendency to produce trichome structures from the stimulated surface, so marked a characteristic of the Acarina forms, is in this group practically absent; the only hairs produced are those surrounding the gall apertures.

In most species of both groups there is little differentiation of tissues, so that the protective sclerenchyma zones mentioned in the genus Pachypsylla and the unclassified species on *Populus balsamifera* L. mark a distinct advance on the specialization attained by the Acarina galls and an approximation to the more complex types found in the orders Diptera and Hymenoptera.

The increased number of resin ducts in the tissues of the Coniferæ stimulated by species of the genus Chermes is an important feature of these galls.

ORDER LEPIDOPTERA.

The Lepidopterous producers referred to in this paper occupy the following positions in Dyar's List of North American Lepidoptera, United States National Museum, Washington, 1902.

Fam. Sesiidæ.

*Memythrus tricinctus* Harris.

Fam. Tortricidæ.

*Eucosma scudderiana* Clemens.

Fam. Gelechiidæ.

*Gnorimoschema gallæsolidaginis* Riley.

*Gnorimoschema gallæasterella* Kellicott.

Fam. Tineidæ.

*Stagmatophora ceanothiella* Cosens.

The host plants of the various species are:—

*Memythrus tricinctus* Harris.

*Populus tremuloides* Michx.

*Eucosma scudderiana* Clemens.
*Solidago canadensis* L.
*Solidago serotina var. gigantea* Gray (seldom).
*Gnorimoschema gallæsolidaginis* Riley.
*Solidago canadensis L.*
*Solidago serotina var. gigantea* Gray.
*Solidago rugosa* Mill (seldom).
*Gnorimoschema gallæasterella* Kellicott.
*Solidago latifolia L.*
*Solidago cæsia var. axillaris* Gray (seldom).

In speaking of the host plant of this producer Busck[19] makes the following statement: "I have before me specimens from Miss Clarke, which were unquestionably bred from the white wood-aster, *Aster divaricatus* L. (*A. corymbosum* Ait.) near Boston."

*Stagmatophora ceanothiella* Cosens.
*Ceanothus americanus L.*

Tucker[43] states that *C. ovatus* Desf. is also a host plant of this species.

The following dates taken from records of specimens represent approximately the time of emergence of the moths:

*Memythrus tricinctus* Harris—July 4 to 8.
*Eucosma scudderiana* Clemens—June 8 to 20.
*Gnorimoschema gallæsolidaginis* Riley—August 5 to 15.
*Gnorimoschema gallæasterella* Kellicott—August 12 to 19.
*Stagmatophora ceanothiella* Cosens—June 23 to 30.

The two species of the genus Gnorimoschema pass the winter in the imago stage but Eucosma and Stagmatophora in the larval form.

Several galls of the Eucosma moth were opened December 11, and the data collected were as follows:—The larva was in a dormant state in the portion of the stem of the plant immediately below the gall. Before passing into this inactive condition the larva had carefully prepared for the emergence of the imago from the gall. The wall of the gall cavity had been eaten through until the part remaining was thin enough to permit the passage of light. The exit thus prepared was located at the upper end of the gall and was on an average 2 mm. in diameter.

A silk lining covered the whole of the interior of the gall and a partition of especially strong silk crossed the cavity just opposite the opening mentioned above. This partition did not pass straight across the gall but was found always in a slanting direction. It was attached to the gall wall just above the aperture and was always higher on that side.

Galls produced by the Stagmatophora moth were examined a few days later and the larvæ were found to be passing the winter under very similar conditions to those described in the case of the Eucosma species. The Stagmatophora larvæ were not perfectly dormant, however, and soon became quite lively in a warm room. They were found invariably in the gall cavity with their heads a short distance below the prepared exit. This had been constructed as in the preceding case and was situated at the same place. The silk lining covered the interior of the gall but in this case was gradually narrowed to the size of the hole around the edges of which it was attached. As the plant stem was not hollow above the gall, the roof of the gall cavity occupied much the same position as the slanting silk partition in the Eucosma gall.

The silk lining common to both of these galls helps to prevent the loss of moisture and the consequent desiccation of the larva.

The cross partition of the Eucosma gall and the tapering neck found in the lining of the Stagmatophora species seem to have the function in common of guiding the occupant of the gall to the prepared exit.

*Memythrus tricinctus* Harris.

This form has hitherto never been considered a true gall maker—just why it is difficult to understand. Beutenmüller[3] reports it as a borer in stems of poplar and willow and in galls of *Saperda concolor*. I have repeatedly, however, bred this species from swellings on the smaller branches of young trees of *Populus tremuloides* Michx. These swellings were spindle-shaped, gradually tapering at each end to the size of the normal stem. In external form they were quite typical galls of the Lepidopterous class.

A comparison of the larval chamber of this gall with that of the Eucosma species shows that the two have certain features in common. Thus, although the opening in the stem made by the young larva in entering closes in the Eucosma gall, but not in this one, it can be found in the earlier stages of both. The silk lining in the larval chamber is not present, but the slanting silk partition has a similar structure and position to that found in the Eucosma species. This partition shuts off the permanent larval entrance from the part of the chamber in which pupation takes place. The place of exit has the same relation to this partition as that described in the Eucosma gall. The opening is prepared in the same manner. The larva eats through the wall of the gall until the part remaining is translucent just as in the case of the Eucosma or Stagmatophora forms. The larvæ in the two latter species prepare this opening in the fall, but the Memythrus larva does not complete it until shortly before pupation in the spring.

Dimensions:—As the size of the gall varies with that of the stem, an average of several specimens was taken. Length, 60 mm.; width, 20 mm.; diameter of normal stem at place of location of the gall, 12 mm.

A cross section of this gall, when compared with the normal stem, shows an abnormal thickening of the cortex and an increase in width of the bast and wood. Throughout the annual rings of wood are bast fibres, sometimes arranged irregularly in patches, in other cases forming fairly definite zones on the outside of the annual ring. The fibres are shown in Fig. 16.

*Eucosma scudderiana* Clemens.

"The galls are at the top of the main stems of the plants, usually within the flowering panicle, rarely on the branches of the panicle; usually but one gall on a plant, occasionally two, rarely three.

"The galls are spindle-form, varying in size from 10×16 mm. to 12×28 mm.; diameter of stem below gall from 4 mm. to 5 mm.; the average of ten galls collected in ten seasons, 100 specimens, was $9\frac{1}{2}\times21\frac{1}{2}$ mm., diameter of stem below gall 5 mm."—Brodie.[17]

The gall mass in this case is produced from the vascular bundles and the intervening parenchymatous strands. When the larva enters the stem it first eats out the pith. After the exhaustion of this source of nourishment, its food is supplied by the radial thickening of the bundles into the gall cavity. The secondary wood elements thus formed remain somewhat parenchymatous and can scarcely be distinguished from the cells of the medullary rays. The cortex is somewhat thicker than that found in the normal stem but this is not a very marked feature in the gall production.

In the normal stem of *Solidago canadensis* L. there is a gland opposite each bundle both on the side of the cortex and on that of the pith. The glands in the cortex of the gall are the same in number but are very much larger (Fig. 21). Likewise they are not regularly arranged but grouped two or three together. This is due to the fact that since some of the bundles have developed much more rapidly than others, their alignment has been destroyed.

The glands corresponding to the normal inner row were not found in the gall. This is accounted for by the early removal of the pith of the stem by the producer larva.

*Gnorimoschema gallæsolidaginis* Riley.

"Galls usually on the lower third of the stems of *Solidago canadensis* L. occasionally on the upper third, rarely at the summit of the stem. The galls vary in form from spindle-form to prolate and oblate spheroid; and in size from 10×21 mm. to 18×30 mm.

"Some observers say the interior of the gall is lined with silk. I have never found this, but preparatory to the exit, the mature larva before pupating constructs a silken hammock in the upper end of the gall, and opposite the aperture of exit. The larva resting in this hammock bites out a hole to the epidermis of the gall which is carefully left. The hole is bevelled towards the outside, and then neatly filled up with the material gnawed out, mixed with a silk-like substance, doubtless from a gland, which forms a tight-fitting, hard plug which cannot be pushed in from the outside but is easily pushed out from the inside."—Brodie.[17]

The anatomical features of this gall are very similar to those described in the Eucosma species. The gall mass is produced by the radial increase in thickness of the bundles and the growth into the gall cavity of the intervening parenchymatous strands seen in Fig. 20. There is greater proliferation of the cortical tissue in this case than in that of the Eucosma gall and the cells produced are much larger than those found in the normal stem.

The remarks concerning the gland production and distribution of the preceding species are also applicable to this form.

*Gnorimoschema gallæasterella* Kellicott.

"In a collection of galls made May 29, 1890, a few miles north of Toronto, most of them were at the top of the stem, surmounted by a few leaves, occasionally but one, usually two. The galls at this date seemed to be mature, subtriangular, corresponding to stem of plant; from 20 mm. to 32 mm. long, and from 10 mm. to 15 mm. diameter. In size, form and structure the galls closely resemble the galls of *G. gallæsolidaginis* Riley. Rarely they occur on the middle and lower third of the stem of the plant."—Brodie.[17]

"The gall produced on *Solidago cæsia var. axillaris* Gray by this producer is quite unlike the *S. latifolia* gall in appearance, but as both galls are merely spindle-shaped enlargements of the stems of the host plants, this difference in outward form can easily be explained. The glaucous, terete and slender stem of *S. cæsia* produces a gall with glaucous epidermis, circular in cross section and gradually tapering towards each end. On the other hand, the smooth, angled and comparatively thick stem of *S. latifolia* gives rise to a gall with smooth epidermis, somewhat triangular in cross section. This gall has also a greater diameter and tapers more abruptly than the *S. cæsia* gall."—Cosens.[25]

The anatomy of this gall presents the typical structure of a gall of the Lepidopterous class. The cortex of the stem does not play an important rôle in the production of the abnormal tissue; but when the host plant is *Solidago latifolia* L. the gall cortex is thicker than that of the normal stem. As in the case of the gall produced by *G. gallæsoli-*

*daginis* Riley, the bundles and the medullary rays are extended into the gall cavity and furnish the principal part of the tissue proliferation. Only a very shallow seam of normal wood is found in the galls produced on *S. cæs a var. axillaris* Gray.

Glands do not occur in the normal stems of either of the host plants and were not found in the tissues of this gall.

A section through the aperture of exit of a gall on *S. cæsia var. axillaris* Gray showed that the edges of the opening had been prepared by the larva for the reception of the "plug" that closed the opening. The sides of this aperture, roughened by the gnawing away of the tissue, would not admit of the "plug" fitting tightly and at the same time slipping out easily when occasion required. Consequently the gnawed surface is smoothed over by a layer of material that presents a perfectly even surface. This levelling-up material is uniform in character and does not show any trace of vegetable débris. At right angles to its free surface, an effect resembling checking takes place, a change that it has probably undergone in drying. This is illustrated in Fig. 22.

The "plugs" of the galls produced by the genus Gnorimoschema have been reported as consisting of silk and material gnawn out by the larvæ in preparing the openings. My observations incline me to the belief that the material, forming the plug and lining the opening, consists entirely of an exudation from the larva. It seems to be a plastic silk-like substance.

*Stagmatophora ceanothiella* Cosens.

"These abnormal growths are found commonly on a main stem, but rarely on a branch. The flower cluster is sometimes entirely aborted, but usually only partly so, the lower pedicels in the cluster remaining normal. In the majority of cases this gall is terminal, but in a few instances the stem was found to project a short distance beyond it.

"The gall has the relatively simple structure of a spindle-shaped enlargement of the stem. In length it varies from 10 to 15 mm. and in greatest width from 5 to 8 mm. It is roughened on the outside by the stumps of the aborted branches. On account of the shortening of the stem axis and the consequent crowding of the nodes, these branches are more numerous on a gall than on a corresponding length of normal stem. This gives the gall a gnarled surface and forms a strongly protected case for the larva. The gall in some cases is surmounted by a tuft of leaves growing from its apex.

"The aperture through which the moth escapes from the gall is made always near the upper end."—Cosens.[24]

As in the preceding galls described, the principal part of the gall tissues in this species is originated from the vascular bundles and parenchyma strands (Fig. 17). The latter are very wide and the abnormal cell division is more marked in them than in the bundles. The wood elements produced remain undifferentiated and pith-like. The cuticle of the gall epidermis is much stronger than that found in the normal stem. The epidermis itself has responded to the stimulation by the production of an extra layer of cells. The cortex of the gall contains approximately one-third more cell layers than the normal cortex as seen in Fig. 18.

The normal stem of *Ceanothus americanus* L. contains glands in the cortex. These are fairly regularly spaced around the stem but are larger and more numerous at the nodes. Glands occur also in the pith of the stem, the petioles of the leaves and the reproductive axes. But in parts of stems, contiguous with galls, though glands occur in the pith there are none in the cortex, except at the nodes. Glandular cells, however, are plentiful in the cortex of such stems.

A cross section of a gall shows larger and more numerous glands than a corresponding section of the normal stem. The probable explanation of this is that owing to the shortening of the stem axis, nodes are cut more frequently. In the gall cortex there are also narrow, elongated, glandular cavities, that do not seemingly correspond to anything seen in the normal stem. They require further elucidation. They are in groups each containing three or four glands, as illustrated in Fig. 19.

*Summary.*

The galls are all of a comparatively simple type, for while there is considerable proliferation in the tissues there is little differentiation. The medullary rays and vascular bundles respond the most readily to the gall stimulus, yet cell division takes place in the epidermis of the species *Stagmatophora ceanothiella* Cosens.

The highly specialized habits of the larva, developed in caring for the welfare of the imago, make the group very interesting. Thus in each of the forms studied provision is made by the larva for the emergence of the moth from the gall. These habits are seen at different stages of development. In *Stagmatophora ceanothiella* Cosens and *Eucosma scudderiana* Clemens the gall wall is simply gnawn partly through, while in the Gnorimoschema genus an aperture of exit is carefully prepared and plugged. These different methods of procedure are remarkably suited to the habits of the insects. In the former a plugged exit would not be suitable as the insect winters in the larval condition and the drying of the gall would prevent the plug from slipping out easily. In the latter the galls are still green when the insect becomes mature and the plug mechanism is preferable. It is clear then that in these galls the producer

is much more active in providing for its own welfare than in the higher types and the plant renders a relatively smaller amount of assistance. As the stimulation of the animal participant becomes more effective, the plant is coerced into providing more suitable conditions for the maturing of the producer, which consequently becomes less active on its own behalf and more dependent on the host.

While glands are invariably larger in the gall tissues than in the corresponding normal stems, *Stagmatophora ceanothiella* Cosens furnishes the only example where there is a distinct increase in the number of glands.

An unusual cell division occurs in the species *S. ceanothiella* Cosens and *G. gallæasterella* Kellicott. The daughter cells are in clusters, usually four in number, clearly showing they have originated from a common progenitor. The septations between the cells are always very straight and the elongated nuclei are pressed closely against the division walls. This form of mitosis produces only a very small portion of the gall tissues in these species, but in the Dipterous gall *Neolasioptera perfoliata* Felt (Fig. 23) it originates nearly the whole mass. This form will be referred to again and the cell division illustrated in the part of this paper dealing with Dipterous galls.

As I have repeatedly found the opening through which the larva of *Eucosma scudderiana* Clemens has entered the stem, it is certain that this Lepidopterous producer always oviposits on the outside of the host, and this may prove to be true of the entire group.

## ORDER DIPTERA.

The anatomy of the following species is considered:—

Order Diptera,

Fam. Cecidomyidæ.

*Cecidomyia bulla* Walsh.
*Cecidomyia balsamicola* Lintner.
*Cecidomyia impatientis* O.S.
*Cecidomyia majalis* O.S.
*Cecidomyia ocellaris* O.S.
*Cecidomyia pellex* O.S.
*Cecidomyia triticoides* Walsh.
*Lasioptera corni* Felt.
*Lasioptera impatientifolia* Felt.
*Neolasioptera perfoliata* Felt.
*Rhabdophaga batatas* Walsh.
*Rhabdophaga strobiloides* Walsh.

Fam. Trypetidæ.

*Eurosta solidaginis* Fitch.

The classification is as far as possible in accordance with Aldrich's catalogue of North American Diptera, Smithsonian Institute, Washington, D.C., 1905.

*Cecidomyia bulla* Walsh.

Hosts { *Helianthus decapetalus* L.
*Helianthus divaricatus* L.

"Galls found usually on the stem, often from leaf axils, occasionally on petiole and midvein of leaf, rarely on flower disc, protruding from between scales of involucre.

"The galls are attached by an ample base and are very irregular in form and position, usually somewhat compressed, varying from nearly spherical to flask and cone shaped and from equilateral triangular to spur-shaped.

"Dimensions:—The average of twenty galls was, base, 5.5 mm. thick and extending 8 mm. from stem."—Brodie.[16]

On the side of the stem from which the gall originates the vascular bundles are very irregularly arranged and elongated transversely in the direction of the gall axis. From these bundles vascular strands pass out into the gall mass. The principal part of the tissues in this gall originates from the medullary rays, as can be seen in Fig. 39. When the cortex of the stem passes into the abnormal cortex it becomes considerably thicker; this is due chiefly to the increase in size of the cells as the number of rows remains approximately the same as in the normal cortex.

Glands are found in the normal cortex of Helianthus; they are arranged in such a manner that a gland is placed opposite each fibro-vascular bundle. These glands are very much larger in the abnormal cortex of the gall. Besides these glands there are others that have not a counterpart in the normal stem. These are elongated in the direction of the gall axis and are most abundantly produced in the vicinity of the fibro-vascular strands.

There are practically only two zones represented in this gall, the epidermal and the parenchyma. The cells of the latter become slightly smaller towards the larval chamber but a well defined nutritive layer is not differentiated.

*Cecidomyia balsamicola* Lintner.

Host *Abies balsamea* (L) Mill.

A monothalamous gall formed by a folding of the leaf with the upper surface on the inside (Fig. 29). An enlargement ellipsoidal in shape is thus produced. The needles affected are near the apex of the stem and the galls are situated close to the base of the needles.

Dimensions:—Diameter along the leaf average 2.5 mm.; shorter diameter, average 1.5 mm.

The abnormal part of the leaf differs very markedly from the normal. The cuticle is entirely absent from the upper surface of the leaf that lines the interior of the gall. While the epidermis is uniformly thickened the normal cells have much heavier outer walls. The normal mesophyll cells are circular to widely elliptical in outline (Fig. 28), but the abnormal cells are very much elongated (Figs. 29, 30). Since the endodermis is poorly developed the mesophyll is not clearly separated from the pericycle. In this region the transfusion tissue is well represented, but the non-pitted parenchyma is not so abundant as in the normal (Fig. 31). The abnormal resin ducts are increased in size and have the protective layer irregularly developed.

In tabulated form is given a comparison of the anatomical structure of a normal leaf with one infected by the gall producer *Cecidomyia balsamicola* Lintner and one from a witches' broom produced by *Æcidium elatinum* on *Abies balsamea* (L.) Mill.

The data for the last named were obtained from Anderson.[2]

| Leaf Structure. | Normal Leaves. | Affected with *Æcidium elatinum* | Affected with *Cecidomyia balsamicola* Lintner. |
|---|---|---|---|
| Cuticle | Well developed on both surfaces. | Present but less developed. | Abnormally thickened on the lower surface (outside of the gall), not developed on the upper (inside). |
| Epidermis | The outer are thicker than the inside walls. Both are laminated and perforated by pores. | Epidermal cells more irregular than in normal; less thickened and seldom laminated and provided with pore canals. | On the outside of the gall the epidermal cells are irregular and have uniformly thickened walls; they are not clearly laminated but pore canals are more plentiful than in the normal. The inner epidermis is not thickened. |
| Stomata | More numerous on the lower than on the upper leaf surface. | Like the normal but fewer on each surface. | The same as the preceding affected by the fungus. |
| Hypoderm | Well developed at the basal half of the leaf. | Hypodermal cells fewer, but usually larger, thicker walled and more irregular than in normal leaves. | Cells irregularly developed, invariably curved and completely filled with laminated sclerenchyma. |

| | | | |
|---|---|---|---|
| Mesophyll | Usually two layers of palisade parenchyma developed on the upper leaf surface. The remainder of the mesophyll consists of spongy parenchyma (Fig. 28). | No distinction between palisade cells and spongy parenchyma. | The same as the preceding in the fungus, but the cells are very much elongated in a plane perpendicular to the midrib and parallel to the epidermis. |
| Resin Canals | Two circular canals present. These consist of an outer layer of thick walled cells and an inner epithelial layer of thin walled cells. | Irregular; varying in form and size, on account of the absence of the layer of strengthening cells. | Resemble the normal type in shape and are the same in number, but are considerably larger and the strengthening layer consists of very irregularly shaped cells. |
| Endodermis | Consists of a single layer of thin-walled elliptical cells that bound the mesophyll on the inside and separate it from the pericycle (Fig. 31). | Endodermis seldom distinguishable. Cells irregular in form and size. No distinct boundary between mesophyll and pericycle. | Only a few cells differentiated and these are irregular in shape and much enlarged (Fig. 30). |
| Transfusion Tissue of the Pericycle. | Always present: found in two masses, one bordering each phloem area (Fig. 31). | Nearly always present. | Found in from 2-4 layers around the inner side of the endodermis. It comprises the greater part of the pericycle. The cells contain protoplasmic material (Fig. 30). |
| Non-pitted Parenchyma of the Pericycle | Fills between the two divisions of the bundle and projects on each side but more plentifully on the dorsal (Fig. 31). | More irregular in form and size. Larger and thicker walled than in normal. | Developed only between the bundles and in a single row along the edge of the bast (Fig. 30). |
| Fibro-vascular Bundle | Phloem and xylem consist of an average of 7 rows of cells. Medullary rays found between the rows. | Phloem and xylem less developed than in normal. The cells are often larger and thicker walled. Medullary rays are absent. | Phloem and xylem better developed than in the normal, the latter is more irregular. The medullary rays are absent. |

*Cecidomyia impatientis* O.S.

Host *Impatiens biflora* Walt.

A spherical, polythalamous gall attached to the host plant by a tapering stalk. Produced by the deformity of a flower bud.

Dimensions:—Diameter at right angles to stalk axis 6 mm.

There are three well differentiated zones. Immediately inside the small celled epidermis is a mass of large thin-walled cells irregularly arranged. The walls of these cells are seldom straight but usually present a wavy outline. They diminish in size progressively, passing in from the periphery of the gall until they merge into quite a well defined nutritive zone. This tissue is illustrated in Fig. 32. In this zone the cells are very much smaller and are arranged in rows radiating out from the larval chamber. Vascular strands pass irregularly throughout the gall. There is no indication of a protective layer separating the two inner zones.

*Cecidomyia majalis* Bass.

Hosts { *Quercus rubra* L.
*Quercus coccinea* Muench.

A flat pouch-like gall on the under side of the leaf. The opening which extends the entire length of the gall is on the upper side. It is produced by a folding of the blade of the leaf; this fold is parallel with and very close to the midrib or a main vein.

Dimensions:—Along the line of its attachment to the leaf, diameter 4-7 mm.

The gall has been formed in this case by a folding of the blade of the leaf. The resulting type recalls the pouch-like form usually associated with the Eriophyidæ or more rarely the Aphididæ.

The part of the blade included in the fold has not a well defined palisade and spongy parenchyma, the mesophyll being practically uniform throughout. The cells of this region are much larger than those of the normal leaf and are placed together without intervening air spaces. At the apex of the fold the leaf blade is much thicker than at any other part of it (Fig. 27).

The epidermis that lines the interior of the fold seems to remain intact throughout all the developmental stages of the larva.

*Cecidomyia ocellaris* O.S.
Host *Acer rubrum* L.

A circular ridge on the under side of the leaf and a slight convexity on the upper surface constitute the chief part of this gall. In the depression the larva rests covered with a viscid fluid secretion. The effect of the stimulation extending out from this centre is shown in the different coloured concentric rings produced in the leaf blade. These colours change in the course of development of the gall through various shades of red, green or yellow.

The slight depression in the leaf blade that constitutes this gall has been produced in the following way. The part of the leaf blade that forms the bottom of the depression has remained practically normal,

but around this the blade of the leaf has become about five times as thick as the normal organ. A circular ridge is thus formed that produces a saucer-shaped hollow in the leaf blade. This can be clearly seen in Fig. 33. The cells that form this ridge are placed at right angles to the blade of the leaf, in nearly the position of the palisade parenchyma. There has been very little increase in the number of the cells, the accretion in thickness of the blade being due principally to the lengthening of the cells already present in the normal leaf.

Wherever a vein occurs in the gall, the cells are arranged in less regular rows and the individual cells are much larger and not nearly so elongated in outline. Intercellular spaces are not found in any part of the gall tissue. The feeding habits of the larvæ are such as do not necessitate the destruction of the epidermis lining the gall.

*Cecidomyia pellex* O.S.
Host *Fraxinus americana* L.

This gall is formed by a swelling of the blade of a leaflet on each side of the midrib, the cortex of which also undergoes a proliferation that merges insensibly with the mesophyll. Since the production of tissue is unequal on the two sides of the leaf, a folding of the blade occurs with the upper surface on the inside and the midrib at the apex. The depression thus formed constitutes the larval chamber.

Dimensions:—Along the line of the midrib 10-25 mm.

The greater part of the gall mass is produced from the mesophyll of the leaflet but a small part originates from the cortex of the midrib. The epidermal cells have not been stimulated to division. It is possible to determine the origin of the cellular elements from the circumstances that in the gall, as in the normal leaf, the veins mark the boundary between the palisade and the spongy parenchyma. About two-thirds has originated from the spongy parenchyma and the remainder from the palisade layer. The greater amount of tissue thus produced from the lower surface causes the folding of the leaflet with the sinus of the fold above.

The cells produced from the spongy parenchyma are several times larger than the normal. They constitute a tissue in which intercellular air spaces are entirely lacking. On the other hand, the cells that owe their origin to the palisade parenchyma, while larger than the normal cells, are considerably smaller than those originated from the spongy parenchyma. The latter with their epidermal covering constitute the nutritive layer of the gall. Near the surface of this tissue, where the larvæ are feeding, the cells have initiated divisions; here too they show signs of collapsing.

*Cecidomyia triticoides* Walsh.

"On *Salix cordata* Muhl. A polythalamous woody gall .70-1.23 inch long and .30-.37 inch in diameter, bearing a remote resemblance to a head of wheat with the kernels elongated, naked, pointed and very protuberant, its general outline oval or elongate-oval, and formed by the swelling of a twig to 2 or 3 times its former diameter, the swelled portion being very much contracted longitudinally, so as to bring each kernel-like bud nearly or quite into contact with the base of the one that precedes it in the same row, the whole number being arranged in four irregular rows."—Walsh.[44]

The larval chambers in this gall are placed usually along the line of the fibro-vascular bundles of the stem, and wherever a chamber is situated the vascular tissues are not developed.

An examination of a young stage of this gall shows that nearly the whole of the pith and cortex of the stem consists of a well defined aeriferous tissue. It is absent in only a few cell layers that surround the larval chambers. Represented in Figs 34, 35, 36. At this stage there is a well differentiated protective sheath, of about five cells in depth, around each larval cavity. The cells of this layer have uniformly thickened walls and are arranged in concentric rows around the larval chamber. Each cell of this zone contains either a crystal aggregate or a well defined single crystal of calcium oxalate. Inside of this protective sheath is a nutritive layer which consists of about six rows of thin-walled cells. The cells of this zone have the same tangential arrangement as those of the protective sheath. Many of them are empty, this being especially the case in the innermost row. Likewise, many are commencing to collapse on account of the withdrawal of their contents. These zones are represented in Fig. 38.

A section of a gall at a much more advanced stage of development presents several important differences. The aeriferous tissue is very much compressed in the pith and somewhat in the cortex. The cell walls of the protective zone are now much thicker, and a well defined crystal of calcium oxalate completely fills the lumen of each cell (Fig. 37).

Weidel[45] has recorded a phenomenon similar to this occurring in the gall *Andricus corticis* Hart.

"Oft ist das ganze an sich schon grosse Zellumen durch einen einzigen Kristall ausgefüllt, dem anscheinend so ansehnliche Zellulosemassen späterhin waren sie verholzt, aufgelagert worden sind, dass diese die Wand des Behälters erreicht haben und mit ihr verwachsen sind."

My observations differ in one respect from Weidel's, namely, there is no ensheathing mass of cellulose around the crystals found in the gall dealt with here. In deciding this point tests were made at different stages with Schulze's solution.

There is a cambium layer lying just outside the protective zone in the later stages. The nutritive layer consists of a mass of collapsed cells that stain deeply with hæmatoxylin. These layers are shown in Fig. 37.

*Lasioptera corni* Felt.

Hosts { *Cornus alternifolia* L.
*Cornus paniculata* L'Her.

This gall appears on the upper side of the leaf as a circular elevation but does not project on the under side.

The colour is entirely green when young but becomes surrounded by a circle of red at later stages.

In anatomical structure the tissues that compose this gall are the same as those in the normal leaf.

The lower epidermis and the row of mesophyll cells immediately in contact with it remain in the normal position. The upper epidermis and the remainder of the mesophyll become arched and thus separate from the lower epidermis and the part of the mesophyll that adheres to it in the manner shown in Fig. 40. In the space thus formed the larvæ are found.

*Lasioptera impatientifolia* Felt.

Hosts { *Impatiens biflora* Walt.
*Impatiens pallida* Nutt.

A monothalamous gall, projecting chiefly from the under side of the leaf. It consists of an elongated, spindle-shaped swelling of the midrib.

Dimensions:—Longer diameter 8-12 mm.
Shorter diameter 3-4.5 mm.

Practically all the abnormal tissue in this case is produced from the cortex of the midrib of the leaf. The stimulation has extended out only a very short distance into the adjoining mesophyll. The general mass of gall tissue consists of large cells with a few small intercellular air spaces. The epidermal cells are larger than those of the normal epidermis, their increased length being particularly noticeable. The features are shown in Fig. 41.

A nutritive layer is not differentiated.

*Neolasioptera perfoliata* Felt.

Host *Eupatorium perfoliatum* L.

A spindle-shaped swelling of the stem forming a monothalamous gall. It varies in size in proportion to the diameter of the stem or branch from which it originates.

It may be stated as an almost invariable rule, that when a gall and the plant organ from which it originates have a common epidermis the cell walls of that epidermis are thicker in the area covering the gall than

elsewhere. But this gall is an exception to the rule. While the outer walls of the epidermal cells are considerably thickened in the normal they are much less so in the gall. Also the two layers of collenchyma cells underlying the epidermis in the normal stem are absent.

The increase in size of the stem where the gall is situated is due principally to increased cell division in the cortex, since the epidermis produces only two additional layers. The cells produced in the cortex are larger than the normal, but the most peculiar feature to be noted is the mode in which division has taken place and the relative arrangement of the products of division. The location of this tissue is illustrated in Fig. 23. The method of cell division is clearly the same in this species as in the two Lepidopterous types *Stagmatophora ceanothiella* and *Gnorimoschema asterella*. The clusters contain from 2 to 6 members produced from a single cell. The dividing walls are straight, and at the stage examined had the greatly elongated nuclei in close contact with them. These nuclei were seldom exactly opposite but usually diagonally across from one another. These characteristics are represented in Fig. 23. Schürhoff[41] has described the mode of division in callus, contrary to the views in vogue, he states that the nuclei divide mitotically only. There is good reason to believe that the phenomena observed here correspond very closely to those given in his account.

Near the inner edge of the cortex, glands are regularly spaced around the stem. This is a rather remarkable fact as they do not occur normally in this part of the stem. Indeed it was only after a careful search that they were located in the transition region between root and stem. The search was extended to other species with the result that they were found in *Eupatoria purpureum* L. in the roots, the cortex and the reproductive axis, but in *E. urticæfolium* Reichard as in *E. perfoliatum* L. only at the base of the stem.

*Rhabdophaga batatas* Walsh.

"On *Salix humilis* Marsh. A polythalamous gall of very variable shape and size, pale green when young, the colour of the bark when mature, growing on twigs .06-.19 inch in diameter and always some distance from the tip of the twig. Sometimes it resembles a small kidney-potato pierced lengthways by a twig, and has then most generally a smooth polished surface studded with a few buds, one or two of which occasionally give birth to a shoot, and it then reaches 1.35 inch in length and .6 inch in diameter. Sometimes it resembles a young apple pierced lengthways by a twig and attains a diameter of .3 inch."—Walsh.[44]

In this gall the larval cells are situated in the pith of the host plant, just inside the line of the fibro-vascular bundles. The epidermis has a much thicker cuticle than that borne by a normal stem of corresponding

age. The cortex is approximately four times as thick as the cortex of the normal stem. This is due principally to the increased size of the cells. The cells of the nutritive layer are very similar to those of the surrounding tissues but a well marked protective zone defines its outer limits. This is clearly shown in Fig. 25.

The cells of the protective zone present a characteristic very rare in Dipterous galls, although frequently found in the Cynipid galls, namely, the walls of the cells are not uniformly thickened but are much heavier on the side next the nutritive zone. This unequal sclerification is illustrated in Fig. 26. Crystals of calcium oxalate, that were so characteristic a feature of this zone in *C. triticoides* Walsh, seem to be entirely absent in this gall.

*Rhabdophaga strobiloides* Walsh.

Host *Salix cordata* Muhl.

"The galls are very uniform in size and form, usually top-shaped, some inclining to spherical, a little oblate below and prolate above, and as the female oviposits but one egg in the terminal bud of the willow shoot, the galls are terminal and monothalamous.

"The gall is a rather tightly and regularly arranged mass of from 70 to 80 aborted leaves, representing perhaps about 1 m. of the leafage of a normal branch.

"The average measurement of 200 galls was 12 mm.×15 mm., and the length of the deformed part of the branch included in the gall around which the aborted leaves were packed was 6 mm."—Brodie.[18]

The leaves that constitute the principal mass of this gall do not take any part in supplying the larva with food. The tissue that has this function is composed of a mass of small, thin-walled cells. It terminates the stem axis and the larva is in immediate contact with it (Fig. 24). This tissue which really furnishes a nutritive layer seems to originate from a cambium-like tissue at the base of the mass of cells.

An important factor in the production of this gall is the practical cessation of growth of the bud axis.

The aborted leaves that compose the gall exhibit very slight anatomical aberrations.

*Eurosta solidaginis* Fitch.

Host *Solidago canadensis* L.

A monothalamous gall produced by the swelling of the stem of the host plant. Very rarely it is found on a branch of the flowering panicle. A separate gall is almost perfectly spherical in form but occasionally two are produced together forming a common gall, prolate-spheroidal in shape.

Dimensions:—Average diameter of fifteen galls, 23 mm.

The cells that compose the principal mass of this gall are slightly smaller than those of the normal pith but in other respects they resemble them very closely. The irregularity in position of the fibrovascular bundles and their imperfect development are well marked features. Yet a sufficient water supply is ensured to the tissues by vascular strands that are given off from the bundles. These strands extend in a radial direction towards the centre of the gall.

The cortex is considerably thicker than that present in the normal stem. This is due in part to the greater number of cell layers, but also to an increased size.

The glands that are present in the normal stem of *Solidago canadensis* L. occupy certain fixed positions. One gland is present in the cortex outside each bundle and one inside in the region of the pith. The glands found in the cortex of the gall are very much enlarged and have not their characteristically regular arrangement. In the gall pith they are abundant throughout (Fig. 42), but decidedly more plentiful in the vicinity of the fibro-vascular strands. This is the most striking example of gland proliferation found in the galls studied.

The tissues that supply the larva with food are not differentiated into a nutritive zone.

*Summary.*

The galls produced by this order of insects vary very much in their degree of complexity. Some forms such as *Cecidomyia ocellaris* O.S. are as simple in structure as an Acarina, "Dimple", gall; other species as *Rhabdophaga batatas* Walsh present all the specialized anatomical characteristics of a Cynipid gall.

The abundant production of glands in tissue under stimulation is very clearly exemplified in *Eurosta solidaginis* Fitch. At first sight it appeared as if glands were not present in the host of *Neolasioptera perfoliata* Felt, but they were located at the base of the stem and in other species of Eupatoria.

The unequal thickening of the tangential walls of the sclerenchyma protective layer in *Rhabdophaga batatas* Walsh is a very unusual phenomenon in this group.

*Cecidomyia triticoides* O.S. is the only gall of this group in which a well defined crystal layer was found. In it each cell lumen is entirely filled with a single crystal of calcium oxalate.

The production of the aeriferous tissue, that occupies practically the entire pith in the gall *Cecidomyia triticoides* Walsh, is one of the most interesting phenomena exhibited in this group. The nature of this will be discussed in the biological section of the paper.

The collapsing of the nutritive zone after the cell contents are withdrawn is well exemplified in *Cecidomyia triticoides* Walsh (Fig. 37).

The unusual type of cell division in the cortex of the hosts infected by certain Lepidopterous forms, e.g. *Stagmatophora ceanothiella* Cosens and *Gnorimoschema gallæasterella* Kellicott and described in that group, is also found in the Dipterous gall *Neolasioptera perfoliata* Felt (Fig. 23). It was not found in any Cynipid form.

A comparison of a leaf of *Abies balsamea* (L.) Mill infected by *Cecidomyia balsamicola* Lintner with one from a witches' broom produced on the same host by *Æcidium elatinum* (*Melampsora Caryophyllacearum*) brings out a number of interesting points. These are given in the tabulated form following the description of the species *C. balsamicola* Lintner.

ORDER HYMENOPTERA.

Following Marlatt's Revision of the Nematinæ of North America, U. S. Dept. of Agriculture, Washington (1896), the species considered in this paper are comprised in the Subfamily Nematinæ, Family Tenthredinidæ. They are included in two genera, Pontania and Euura. The species referred to are:—

*Euura S. gemma* Walsh.
*Euura S. ovum* Walsh.
**Euura* (undescribed).
*Pontania pisum* Walsh.
*Pontania pomum* Walsh.
*Pontania desmodioides* Walsh.
*Pontania hyalina* Norton.
**Pontania* (undescribed).
*Gall on *Salix lucida* (undescribed).
Gall on *Salix humilis* (undescribed).

*Specimens of the first three producers marked (undescribed) were sent to S. A. Rohwer of the Smithsonian Institution.

I have been successful in rearing the producers of all of the undescribed forms except in the case of the one on *S. humilis* Marsh. This was accomplished in the following manner. The galls were collected at the time of the falling of the leaves of the host plants and were placed on earth in breeding jars which were kept under conditions of heat and moisture approximating as closely as possible to that of the natural habitat. Pupation took place at a distance of about a couple of inches below the surface of the soil and the adults emerged the following spring. The dates of emergence were:—

*Pontania* (undescribed), April 14 to April 24.
*Euura* (undescribed), May 2 to May 6.
Gall on *S. lucida* (undescribed), April 20 to April 22.

*While this paper was in press, Rohwer[46] published the description of these producers. Following the order above the names assigned are,—*Euura serissimæ* Rohwer, *Pontania crassicornis* Rohwer, *P. lucidæ* Rohwer.

The close restriction of sawfly gall producers to definite species of Salix can be illustrated by means of the forms mentioned in this paper. The host plants of the species are:—

*Euura S. gemma* Walsh
*Pontania desmodioides* Walsh
*Pontania* (undescribed)
found on *Salix humilis* Marsh.
*Pontania hyalina* Norton
on *S. alba* L.
*Pontania pomum* Walsh
on *S. cordata* Muhl.
*Euura* (undescribed)
on *S. serissima* Fernald.
Gall (undescribed)
on *S. lucida* Muhl.
*Pontania pisum* Walsh
on *S. discolor* Muhl.

In this locality I have not found the above species on any other host than that mentioned. When the type of gall is higher it would seem to be axiomatic that the relations between the host plant and producer would be more intimate than when the gall does not stand so high in the scale and as a consequence the restriction to one host plant would be a necessity. Yet the galls produced by the Cynipidæ are often found on two or three different hosts; as, for example, *Amphibolips inanis* O.S. on both *Quercus rubra* L. and *Q. coccinea* Muench, *Dryophanta palustris* O.S. on *Quercus rubra* L. and *Q. coccinea* Muench. *Aulax nabali* Brodie on *Prenanthes alba* L. and *P. altissima* L.

*Euura S. gemma* Walsh.

"On *Salix humilis*. The lateral bud of a twig enlarged so as to be twice or thrice as long, wide and thick as the natural bud before it begins to expand in the spring; its external surface otherwise entirely unchanged both in texture and colour. Internally, instead of the normal downy embryo leaves, it contains early in the autumn a homogeneous grass-green fleshy matter, which is afterwards gradually consumed by the larva, leaving nothing at last but a mere shell partly filled with excrement. The gall is monothalamous, sometimes one only on a twig, sometimes two or three or more at irregular intervals, very rarely as many as three or four formed out of three or four consecutive buds.

Length .17 to .36 inch
Breadth .10 to .17 inch.—Walsh.[44]

The anatomy of this gall presents little differentiation of tissue. A cross section shows that the entire mass of the gall consists of small

thin-walled cells, shown in Fig. 68. The bud scales surrounding this group of cells resemble those of the normal bud except that the cuticle of the epidermis is abnormally thickened.

*Euura S. ovum* Walsh.

"On *Salix cordata*. An oval or roundish, sessile, monothalamous swelling, .30 to .50 inch long, placed lengthways on the side of small twigs, green wherever it is smooth, but mostly covered with shallow longitudinal cracks and irregular rough scales which are pale opaque brown. Its internal substance fleshy in the summer like that of an apple, but with transverse internal fibres. When ripe in the autumn filled with reddish-brown spongy matter, with close-set transverse internal fissures at right angles to the axis of the twig. On cutting down to the twig at any time a longitudinal slit about .20 inch long becomes plainly visible."—Walsh.[4]

As already noted the host of this gall in this locality is *Salix humilis*, it remains to be determined whether there are two distinct species of producers or one species with two hosts. Walsh's description of the gall on *Salix cordata* corresponds to the form occurring here on *S. humilis*.

The ovipositor of the producer has in this case made a longitudinal cut in the stem. A transverse section at the place where the gall is located shows that this wound extends in from the epidermis to the boundary of the pith. The activity of the young tissues, abnormally stimulated, soon fill this fissure with a mass of small, angular parenchyma. The rapid division of these cells forces the exposed edges of the cortex and central cylinder apart so as to form a wedge-shaped opening which is filled up with the gall mass (Fig. 71). It should be stated that the newly formed cells originate mainly from the division of a cambium bordering the pith at the bottom of the fissure. But other tissues also respond to the stimulation initiated by the ovipositor of the insect. Thus a section of the stem at a short distance from the gall shows that the outlying cambium has become abnormally active and has produced a layer of bast nearly one-third thicker than that found in the normal stem. Likewise the activity of a cork-cambium layer has thrown off a strongly cuticularized epidermis present in the earlier developmental stages.

Undescribed Sawfly Gall (*Euura N.S.*) on *Salix serissima* Fernald.

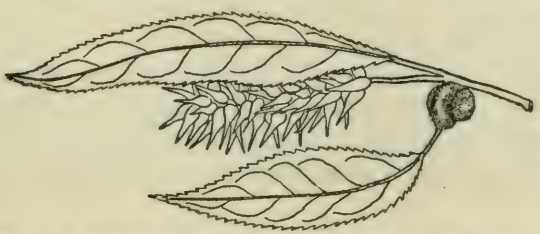

Fig. 1.—A nearly mature gall attached to a leaf of the host plant.

This gall is produced by the abnormal swelling of the petiole of the leaf. The leaves infected are those borne on the branchlets from which spring the pistillate catkins. In the majority of cases, the leaf that bears the gall is the one from the axil of which the peduncle of the catkin arises. The swelling is so close to the branchlet that after the leaves have fallen, the gall appears to have originated from it. This misleading appearance is due to the petiole of the leaf breaking just above the gall. The galls are cone-shaped with the apex towards the blade of the leaf. They are marked deeply by three or four grooves that meet at the tip.

Dimensions:—Height of gall 7-8 mm.; diameter at base 6-7 mm.

The anatomy of this gall presents a very compact tissue, owing to the cells being placed close together without intervening air spaces. The very much thickened cuticle of the epidermis is the greatest departure from the normal tissue. Of the three bundles of the normal petiole two are lacerated by the ovipositor of the insect, as shown in Figs. 69, 70. In the mature gall the halves of these two are found in four widely separated regions (Fig. 69), owing to the abundant production of tissue between them. Indeed practically all the abnormal tissue is produced from the undifferentiated cells stimulated by the cutting of the bundles.

*Pontania pisum* Walsh.

"On *Salix discolor*. A subspherical, pea-like, hollow, pale yellowish-green gall, always growing on the under side of the leaf and almost always from one of the side veins (in one case from the midrib), and attached to the leaf by only a minute portion of its surface; 0.18 to 0.28 inch in diameter and a few miniature, only 0.08 inch in diameter. Almost invariably there is but one gall to the leaf, but on four leaves there were two, and occasionally two are confluent. Surface in some smooth and even without pubescence; in others a little shriveled, generally studded in the medium-sized ones with four to twelve small, robustly conical nipples, which in the larger ones have burst into a scabrous brown scar. Only in three out of sixty-two was there any rosy cheek as in *P. pomum*. The point of attachment is marked on the upper side of the leaf by a brown sub-hemispherical depression."—Walsh.[44]

Walsh is incorrect in supposing that this gall originates from a midrib or vein. A section shows that it is clearly a product of the mesophyll and is attached to that part of the leaf. The side vein, near which it is always placed, is cut by the ovipositor, however, and vascular strands pass out from it into the gall body.

The mature gall consists of a peripheral layer of thin-walled cells, irregular in outline surrounding a central cavity (Fig. 81). This tissue is clearly derived from the mesophyll and epidermis of the leaf, but a stage was not secured young enough to show the relative amounts

produced from each. The epidermis bears numerous lenticels, organs which Küster[35] mentions as occurring on the gall produced by *Pontania salicis*.

At the point of attachment of the gall the blade of the leaf is strengthened by several rows of cells derived from the upper epidermis and the palisade parenchyma, as shown in Fig. 81. These cells seem to have remained unmodified in any way, since their arrangement in rows is still clear in fairly old stages of the gall. Consequently they differ very markedly from the irregularly arranged cells of the main part of the gall body.

*Pontania pomum* Walsh.

"On *Salix cordata* (and very rarely on *S. discolor*). A smooth, fleshy, sessile, globular or slightly oval, monothalamous gall, resembling a miniature apple, .30 to .55 inch in diameter, growing on one side of the midrib of a leaf, and extending to its edge or sometimes a little beyond it. The principal part of the gall generally projects from the under side of the leaf, and only about one-sixth of its volume from the upper side, although very rarely it is almost equally bisected by the plane of the leaf. Scarcely ever more than one gall on a leaf and very rarely two of them, more or less confluent so as to seem like one kidney-shaped gall. External colour greenish-yellow, generally with a rosy cheek like an apple especially on the upper surface and often with many dark little dots on its surface."—Walsh.[44]

The ovipositor of the producer of this gall has been thrust laterally through the midrib of the leaf into the mesophyll. The wound has completely severed the bundle of the midrib, as seen in Fig. 76.

The full-grown gall presents an epidermis with a very thick cuticle. The remainder of the gall consists of a complex of thin-walled cells arranged so as to constitute a typical aeriferous tissue (Fig. 77). A similar arrangement of cells is not found in the normal leaf, the mesophyll of which consists of a fairly compact tissue. The vascular strands growing out from the wounded bundle form a complete ring around the gall, situated about half way between the epidermis and the centre.

I was successful in obtaining this gall at such an early stage that the egg membrane was still unbroken (Fig. 76). This phase shows that the epidermis, the palisade and the spongy parenchyma mutually take part in the gall production. Counting along a line passing through the centre, four of the cell layers are seen to have arisen from the lower epidermis and six from the upper, eight from the palisade and fifteen from the spongy parenchyma of the leaf. Hence it is noteworthy that the new tissues are not the product of a cambium but have been contributed to by every morphological region of the leaf. The cells that are

produced at this stage are in rows generally in exact alignment with the cells from which they have arisen. They thus do not have the arrangement of the aeriferous tissue of later stages to which they give rise. The cuticle, so marked a feature of the older stages of the epidermis, is exceedingly thin. The epidermis bears trichomes springing from the bottoms of deep pits (Fig. 76). This condition has arisen through the circumstance that the primary epidermal cells from which hairs have grown out have not experienced the periclinal divisions participated in by their fellows and so have been left far below the general surface as shown in the text fig. below.

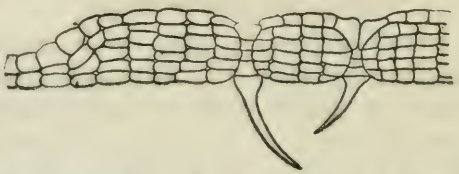

Fig. 2.—Hairs originating from pits in the epidermis of *P. pomum* Walsh.

*Pontania desmodioides* Walsh.

"On *Salix humilis*. A smooth, flattish, fleshy, sessile, yellowish-green monothalamous gall of a semicircular outline, the chord of the semicircle adjoining the midrib of a leaf; its general shape like the seed of a Desmodium or like the so-called "quarter" of an orange, the thin inside edge of the "quarter" closely hugging the midrib of the leaf, and the robust outer surface not biangulated but rounded off. No rosy cheek. The volume of the gall is generally about equally divided between the upper and lower sides of the leaf but sometimes the lower portion is rather the larger. Usually there is but a single gall on a single leaf, but occasionally there are two of them, either on the same side or on opposite sides of the midrib."—Length .23 to .50 inch Walsh.[44]

When mature this gall shows in cross section a cavity surrounded by a peripheral layer of little differentiated tissue. The epidermis has given rise to a very thick cuticle that is not present in the normal leaf. The bundle of the midrib has been injured only slightly by the ovipositor of the producer. The vascular strands given off from it almost encircle the gall along a line half way in from the epidermis.

A stage of the gall so young that the larva was unhatched shows the gall tissue to have been produced by cell division in the upper epidermis, the spongy parenchyma and the palisade parenchyma of the normal leaf (Fig. 80). At the thickest part of the gall, when it is in this stage, the upper epidermis has produced four layers of cells, the spongy and palisade parenchyma seven layers each. The lower epidermis has not divided as yet, and probably takes no part in the production of the

gall. The abnormal cells from the palisade parenchyma show clearly their origin by their arrangement in rows at right angles to the surface of the leaf. The cells produced by the spongy parenchyma, on the other hand, are not regularly placed but include air spaces. The result is that the abnormal tissue in this case also resembles the normal tissue from which it is derived.

This stage of the gall shows that the cavity present in the mature gall has arisen between the tissue produced by the spongy and that derived from the palisade parenchyma of the normal leaf.

*Pontania hyalina* Norton.

"Fleshy galls occurring in two parallel rows, one on either side of the midrib, sometimes touching but not originating from the latter, and rarely extending to the edge of the leaf; sometimes as many as twenty on a single leaf; in other cases confined to a row on one side of the leaf or occasionally occurring singly; shape irregular elongate-ovate, projecting equally on both surfaces of the leaf; length 7 to 10 mm.; the abortive ones smaller. Colour on upper side more or less brownish red; beneath white, with slight purplish tinge."—Marlatt.[37]

The anatomy of this gall presents scarcely any differentiation of tissue. When mature it consists of a mass of thin-walled chlorophyll-bearing cells, the innermost of which are arranged in rows almost at right angles to the blade of the leaf, as seen in Fig. 75. Cells are so much alike that they afford no clue as to their origin.

Again I was able to obtain material so young that the larva was still confined within the egg membrane (Figs. 73, 74). It shows that the spongy parenchyma, the palisade parenchyma and the epidermis of the normal leaf were jointly concerned in the production of the abnormal tissue. The spongy parenchyma has contributed nearly half of the entire mass, the epidermes three layers each, and the row of cells immediately overlying the lower epidermis three layers. The remainder has been derived from the palisade parenchyma.

Undescribed Sawfly Gall (*Pontania N.S.*) on *Salix humilis* Marsh.

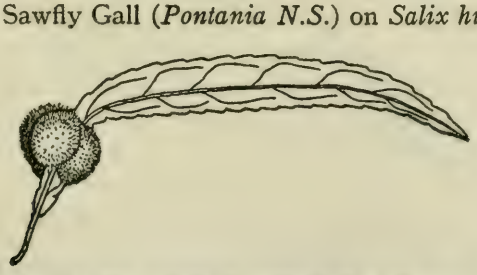

Fig. 3.—Two galls attached on opposite sides of the midrib.

This is a monothalamous gall found on the leaves of *Salix humilis*.

It is spherical in shape and in that feature resembles *P. pomum*, but in other respects it differs very markedly. It extends from the side of the midrib almost out to the margin of the leaf and is divided into two hemispheres by the leaf blade. In consequence the gall protrudes nearly equally from each leaf surface. Usually there are two or three galls on a leaf. When two are present they come in contact with the midrib at the same place but on opposite sides, as illustrated in Fig. 79. In a few cases four and even five galls were found on one leaf. The galls are pubescent but not as densely as the under surface of the leaf.

Dimensions:—Average diameter 1 cm.

The mesophyll of the leaf and the upper epidermis are mutually concerned in the production of this gall. In one, sufficiently immature to show the relative amount of tissue arising from each source, it was found that the upper epidermis had produced two cell layers, while the lower had not responded to stimulation; and that the palisade and spongy parenchyma had each produced one-half of the remaining mass. The hollow in the gall, present from the earliest stages, has been formed between the tissue arising from the palisade and the spongy parenchyma respectively.

When only one gall originates from the midrib at any point, the vascular bundle is cut approximately half through (Fig. 78). But more frequently two galls are located opposite one another, one on each side of the midrib, in which case the two incisions meet and completely sever the bundle, as seen in Fig. 79.

Vascular strands pass almost completely around the gall, along a line half way between the epidermis and the gall cavity. These strands originate from the midrib in the neighborhood of the injury and pass in opposite directions.

Undescribed Sawfly Gall on *S. lucida* Muhl.

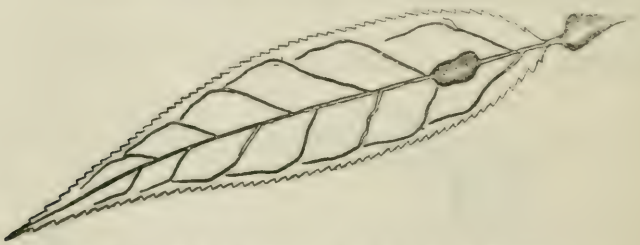

Fig. 4.—Two galls produced on the same leaf of the host.

This gall consists of an enlargement of either the petiole or midrib of *S. lucida*. Neither of these organs bears, as a rule, more than one gall

at a time, but occasionally the petiole of a leaf carries two or even three and the midrib in rare instances two.

The midrib galls are fairly regularly elliptical in outline with the shorter diameter across the leaf. The swellings in most cases are nearly equally divided between the upper and the lower leaf surfaces. The petiole galls vary from spherical to ovoid in shape. In the latter case the smaller end of the gall is towards the apex of the leaf.

Dimensions of the gall:—Longer diameter 6-12 mm.; shorter diameter 3-7 mm.

The very marked proliferation of tissue in this gall is not accompanied by a differentiation that presents many points of interest. The cells are larger than those of the normal leaf and the nuclei are correspondingly larger. The bundle is cut nearly through by the ovipositor (Fig. 82). The free ends of the bundle thus stimulated grow out until in some cases they almost surround the gall. This elongation is produced in part by the increased diameter of the vessels but also by the production of new cellular elements.

The pith, exposed by the cutting of the bundle, produces almost all the abnormal tissue (Figs. 83, 84), but the cortex contributes some. The cells are arranged in curved lines that pass across from one elongated end of the bundle to the other. Between these rows are many air spaces which are elongated in the direction of the lines of cells (Fig. 84).

Undescribed Sawfly Gall on *Salix humilis* Marsh.

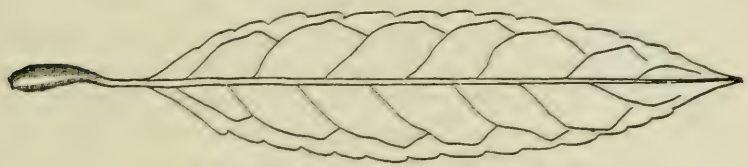

Fig. 5.—Leaf of the host with attached gall.

This is a monothalamous gall produced by the abnormal swelling of the leaf petiole of S. *humilis*. It is conoidal in shape with a long tapering apex which is towards the blade of the leaf. As it is situated at the base of the petiole the uniform enlargement of that organ is often prevented by the axillary bud. This causes the gall to project to a greater extent on the outside of the petiole and produces an irregularity in the outline of the gall. The surface is quite glabrous in spite of the fact that the epidermis of the leaf is decidedly pubescent.

Dimensions:—Length of gall 6-9 mm.; width 3-4 mm.

Nearly the entire mass of this gall originates from the vascular bundle of the petiole which has been stimulated to activity by the insect's ovipositor. The small thin-walled cells of the gall substance

spring from a cambium layer produced in the pith of the bundle near the ovipositor wound. The cells arising from this tissue force the severed ends of the bundle apart until the vascular elements form only a narrow line of cells between the gall proper and the cortex of the petiole; it is shown at this stage in Fig. 85. This cortex is not materially thickened but shows signs of stimulation in that there appears a small amount of aeriferous tissue located near the place of entrance of the ovipositor. This tissue is shown on each side of the wound in Fig. 85. This tissue was not found in the normal cortex of the petiole. The cuticle is very much thickened.

*Summary.*

Great proliferation of tissue with little differentiation is a common characteristic of all sawfly galls.

All the leaf tissues of the genus Salix appear to be susceptible to stimulation by sawfly producers.

The pith of the bundle produces practically the whole mass of the gall when the place of origin is the petiole or the midrib of the leaf. In the case of a stem gall a layer of cells bordering the pith produces the chief proliferation.

When the gall originates from the mesophyll of the leaf the bundle of the midrib produces relatively only a very small part of the total gall mass.

Of the remaining tissues of the leaf the upper epidermis responds more readily to stimulation than the lower and the spongy parenchyma more actively than the palisade parenchyma.

In some cases the abnormal tissues exhibit the characteristics of the normal from which they have originated. The cells produced to form a solid base of attachment for the gall *Pontania pisum* Walsh never lose their arrangement in vertical rows, and the cells that originate from the same tissue in *P. desmodioides* Walsh are also in vertical series (Figs 80, 81).

Adler[1] secured specimens of *Nematus vallisnierii* in which the larva was still within the egg. I have been equally fortunate with the species *Pontania pomum* Walsh, *Pontania hyalina* Norton and *Pontania desmodioides* Walsh. At this early developmental stage considerable proliferation of tissue had already occurred. Adler even reports that the gall was nearly full grown. My experience has been that the larvæ are invariably found feeding unless the material is secured almost as soon as the galls are visible to the unaided eye. At this time little swelling of the leaf tissues is apparent, but a discoloration of the leaf enables the wound of the ovipositor to be detected. Owing to cell proliferation preceding the emergence of the larva, Adler concluded that the immediate

cause of cell activity, productive of sawfly galls, is the wound caused by the act of ovipositing. But there is a slight possibility that secretions or excretions from the developing larva may be active through the egg membrane.

The protective sclerenchyma sheath of the more advanced types of galls is absent in this group, and the only protective device appears to be the cuticularizing of the epidermis and the presence of tannin in the cells. The cuticle has also a more important function in preventing the desiccation of the underlying thin-walled tissues of the gall.

The possible significance of the aeriferous tissue found in *Pontania pomum* Walsh will be discussed later in this paper.

Lenticels on galls seem to occur very rarely. They were found in this group only in the one species, *Pontania pisum* Walsh, a leaf gall.

The restriction of the various species in many cases to single hosts seems noteworthy when the minor specific differences between the members of the Salicaceæ are considered.

A series of the undescribed species of Euura on *Salix serissima* Fernald furnished undoubted examples of cell proliferation produced by the excrement of the larval producer (Fig. 72). This fact is discussed in the biological section of the paper.

### LOCALIZATION OF TANNIN-BEARING TISSUE IN SAWFLY GALLS.

Küstenmacher[33] has discussed the question of tannin in certain Cynipid galls and Cook[22] has detected it in different stages of a number of galls, but no attempt has been made up to the present to work out its distribution in the family Tenthredinidæ.

*Pontania pomum* Walsh.

Tannin-containing cells are abundant in the epidermis of this gall. They are found also in the "Aeriferous tissue," but are not so numerous there. They are plentiful in the vascular strands, but can scarcely be demonstrated in the tissue next the larva.

In the normal leaf of *Salix cordata* Muhl. these cells are abundant, especially so in the vascular tissue and the epidermis.

Undescribed Pontania Gall on *S. humilis* Marsh.

Tannin-containing cells are very plentiful in the epidermis and in six or seven rows of cells that immediately underlie that tissue. They are also present in the vascular strands and in the tissue next the larva.

In the normal leaf of *S. humilis* Marsh these cells are not present in the epidermis of the midrib but are found in the bundle of the midrib especially in the bast portion.

*Pontania hyalina* Norton.

Tannin cells are found abundantly in the epidermis and in the gall tissue generally, except the cells on which the larva is feeding.

In the normal leaf of *S. alba* L. on which this gall is found, these cells are not present in the epidermis of the midrib but are plentiful in the wood and bast of the bundle.

*Pontania desmodioides* Walsh.

Tannin cells are plentiful in the epidermis and in the underlying tissue, but gradually diminish in number in the tissues nearer the larva.

This gall also is found on *S. humilis* Marsh.

Undescribed gall on petiole of *Salix lucida* Muhl.

Tannin cells are found practically all through the tissues of the gall, but tannin is most plentiful in the epidermis and in the petiole that is involved in the gall swelling.

Tannin cells are found throughout the normal petiole except in the parenchyma tissue immediately underlying the epidermis.

Conclusions concerning Tannin-bearing Tissue.

(1) Tannin is more plentiful in gall tissue than in the normal tissue from which it originates.

(2) In gall tissues it is most abundant in the epidermis and the bast.

(3) It is more abundant in the older than the younger stages of galls.

(4) It does not appear to function as food for the larvæ as the tannin cells are less abundant in the tissue on which the larvæ feed.

(5) As tannin is always plentiful in the gall epidermis it may serve for protection by rendering the gall tissues unpalatable.

Technique used in Testing for Tannin.

The test substance used was a saturated solution of ammonium chloride saturated with ammonium molybdate.

Razor sections of the galls were used for testing.

NOTES ON OVIPOSITING BY SAWFLY GALL PRODUCERS.

*Pontania hyalina* Norton.

The leaves of *Salix alba* L. are folded in the bud with the under surfaces towards the outside. The ovipositing takes place before the leaf selected has separated from the others in the same bud. As a consequence of this the ovipositor is inserted from the ventral surface of the leaf (Fig. 74).

May 27th, 1911.—On this date a producer was observed ovipositing in young leaves, but other leaves on the same stem lower down bore galls that were almost full size.

June 3rd and 4th.—On these dates producers were seen ovipositing.

June 18th.—Producers ovipositing.

August 11th.—Galls were found in which the ovipositing could have taken place only a few hours before. The ovipositing in this species must continue over a period of at least two months.

*Pontania pomum* Walsh.
*Pontania desmodioides* Walsh.

These producers begin to oviposit at about the same date as the above species. The period of ovipositing must comprise a comparatively short space of time as the galls of these species are all at about the same stage of development on the same date.

The galls produced respectively on *Salix cordata* Muhl. and *Salix humilis* Marsh. by these producers are not found near the tips of the young stems, but the galls on *Salix alba* L. produced by *Pontania hyalina* Norton are found on leaves along the whole length of the young stems. This difference in the location of the galls is caused by the period of ovipositing being much longer in *Pontania hyalina* Norton than in the other two species.

The first effect of ovipositing by *Pontania pomum* Walsh, visible to the unaided eye, is a dark red colour produced in the leaf blade surrounding the spot where the ovipositor has entered. This colour is also visible for a short distance along the midrib of the leaf. When the gall is opened at this stage the egg of the producer can be found with the aid of a lens. It is elliptical in outline and of pearly lustre. The membrane is translucent and the egg contents can be distinguished through it.

## ORDER HYMENOPTERA.

Whenever possible the specific names have been selected from the monographs by Wm. Beutenmuller,[5-14] that are now being issued from the American Museum of Natural History, New York. These publications give full synonymy and bibliography of the different species.

The following species are here described:—

Fam. Cynipidæ.

*Holcaspis globulus* Fitch.
*Holcaspis bassetti* Gillette.
*Philonix erinacei* Beut.
*Philonix hirta* Bassett.
*Philonix nigra* Gillette.
*Amphibolips confluens* Harris.
*Amphibolips inanis* O.S.
*Dryophanta palustris* O.S.
*Andricus imbricariæ* Ashmead.
*Andricus singularis* Bassett.

*Andricus piger* Bassett.
*Andricus petiolicola* Bassett.
*Andricus* (undescribed).
*Rhodites multispinosus* Gillette.
*Rhodites lenticularis* Bassett.
*Rhodites ignotus* O.S.
*Rhodites bicolor* Harr.
*Rhodites gracilis* Ashm.
*Rhodites nebulosus* Bassett.
*Cynips? constricta* Stebbins.
*Solenozopheria vaccinii* Ashm.
*Aulacidea nabali* Brodie.
*Neuroterus majalis* Bassett.
*Aylax glechomæ* Linné (referred to the section on Cytology).

*Holcaspis globulus* Fitch.
Host *Quercus alba* L.

A monothalamous, spherical gall produced at the nodes of the stem of the host.

It occurs singly or in groups of from two to four. The colour is yellowish-green usually with a reddish tinge. When mature the oval larval chamber is free from the remainder of the gall. The aperture of exit of the producer is placed at the end of this larval cell.

Dimensions:—Average diameter 13 mm.

When this gall is so young that it is still soft, it has the following anatomical characteristics. The larval chamber is in organic continuity with the remainder of the gall. Beneath the small celled epidermis are four or five layers of cells with their long axes parallel to the periphery of the gall. Inside this tissue is the more typical part of the parenchyma zone. Here the cells are in radial rows forming a fairly compact tissue with only a few small air spaces. Their radial walls are more elongated the nearer they are to the larval chamber. Inside of the parenchyma zone is a poorly defined cambium tissue that passes gradually into a crystal layer. Each cell of this zone contains a large crystal mass. A second cambium tissue, in this case well defined, bounds the crystal layer on the inside. From this cambium the nutritive layer is produced. This consists of cells, almost square in outline, arranged in radial rows (Fig. 65).

The protective zone is differentiated only in the later stages of development. It is found, however, when the gall is mature, forming the entire wall of the free larval chamber and extending a short distance beyond it. Its cells are of the usual sclerenchyma type with uniformly thickened, laminated walls perforated by branched simple pores.

*Holcaspis bassetti* Gillette.

Host *Quercus macrocarpa* Michx.

A monothalamous gall occurring singly or in clusters around the stems of the host. When grouped the galls often cover completely 4 to 5 inches of the stem.

When the gall is not deformed by crowding, it is irregularly circular in outline at the base, gradually tapering to a distinct point that is recurved in most cases. The gall is attached to the host by a small stalk at the centre of the base. Colour green, often tinged with pink when young; becoming brown when more mature. The larval chamber resembles closely that found in the former species in being oval and free at maturity, but it differs in being placed nearer the base of the gall and in tapering to a point at the end nearer the twig.

Dimensions:—Diameter at base, average, 16 mm.

Except in a few details the anatomical structure of this gall is the same as that found in the species just described. In this species the outer part of the parenchyma zone is composed of cells almost square in outline, but towards the larval chamber the cells become more elongated and arranged in distinctly radial lines. Rays of from one to three cells in width pass in radial lines throughout this zone. The cells composing them are much smaller than the ordinary cells of the zone. The cells of the nutritive layer are much more elongated radially than those of *H. globulus* Fitch (Figs. 58, 65). The cambial layers and the crystal-bearing tissue hold the same relative positions as in the preceding species. The relation of the crystal layer to the nutritive zone is shown in Fig. 58. The protective sheath in the mature gall extends out almost to the epidermis. Except in its distribution it cannot be distinguished from the corresponding zone in *H. globulus* Fitch.

*Philonix erinacei* Beut.

Host *Quercus alba* L.

A polythalamous gall springing usually from the midrib but rarely from a principal vein of the leaf. It originates from the under or occasionally the upper surface of the leaf.

The gall is spherical or ellipsoidal and slightly flattened on the surface in contact with the leaf. The point of attachment is narrow and elongated in the direction of the vein. The epidermis of the gall is divided up into numerous facets, each of which is drawn out at the centre into a trichome structure that becomes more spiny as the gall approaches maturity. The colour of the surface of the gall is yellowish with occasional red tints. The trichomes vary in shade from pink to red.

Dimensions:—Longer diameter 10-15 mm.; shorter diameter 5-10 mm.

In the earliest stage examined the gall was 2 mm. in diameter. At this time none of the cell walls are sclerenchymatous and the nutritive zone is only about four narrow cells in width. Outside of this layer is a part of the parenchyma zone in which each cell contains a large crystal mass.

At a stage in which the gall is full grown but still soft, all the zones are differentiated. The epidermis is thrown into folds and is covered with a heavy cuticle (Fig. 64). This is absent in the sinuses of the folds and on the epidermis covering the spines. The parenchyma zone is gradually converted into a protective tissue of porous sclerenchyma. The thicker deposit is usually on the walls of the cells nearer the periphery of the gall. Along the outside of the nutritive zone and throughout the protective layer generally are lines of small cells almost square in outline. The walls of these cells are very thick and the lumen of each is filled with a single crystal or a mass of crystals. In galls that had become hard all the cells of the parenchyma zone were found to have sclerified. The sclerification is partially complete in Fig. 64.

The nutritive layer of this gall differs very little in appearance from the parenchyma zone. Its cells do not contain the rich protoplasmic contents common to the nutritive zones of typical Cynipid galls.

*Philonix hirta* Bassett.

Host *Quercus macrocarpa* Michx.

A monothalamous, spherical gall originating from a principal vein of the leaf. Found somewhat irregularly spaced along the vein and about equally distributed between the upper and lower surfaces of the leaves.

The epidermis has the same faceted appearance found in the preceding species, but in this form the trichomes are represented only by short points. Colour greenish yellow. When the leaves become tinted in the autumn the galls assume a reddish brown colour.

Dimensions:—Diameter 2-3 mm.

The anatomical structure of this gall differs from *P. erinacei* Beut. only in the distribution and nature of the protective zone. This tissue is limited to a layer 3 to 4 cells in thickness, just outside the nutritive zone. The sclerifying deposits are limited almost entirely to the outside tangential walls of these cells and gradually entirely fill them. As a result of this the pores pass completely across the cells in the older stages. The small square crystal-bearing cells are, in this species, just outside the regular protective sheath.

*Philonix nigra* Gillette.

Host *Quercus alba* L.

A monothalamous gall attached to the principal veins on the under side of the leaf.

This species is spherical in form and has an epidermis covered with a short dense pubescence that gives a felty appearance to the exterior of the gall. A fibrous mass of cells surrounds the centrally placed larval chamber. Colour gray turning darker when dry. Individuals of this species are so numerous that the ground, under the trees infested by them, is often covered thickly with galls.

Dimensions:—Average diameter 8 mm.

Outside the nutritive zone is a wide crystal layer, each cell of which is completely filled with a crystal mass. The sclerenchyma of the protective zone is formed in a very unusual manner. The sides of contiguous cells are thickened in such a way that there is an almost spherical deposit at the points where the cells are in contact.

Radiating out from the protective layer are long narrow cells which form the minor part of the parenchyma zone. The remainder of this zone consists of irregularly elliptical, thin-walled cells. The epidermis is covered with a dense growth of trichomes with thick laminated and sclerified walls.

*Amphibolips confluens* Harris.
Host *Quercus coccinea* Muench.

A monothalamous gall attached to the petiole or midrib of the leaf. The midrib is never continued beyond the point of origin of the gall.

Globular to prolate spheroidal in shape and invariably terminating in a minute point. The thick walled larval cell at the centre of the gall is surrounded by a sponge-like mass of fibres that is at first white but becomes dark brown when the gall is dry. At a very early stage of development the epidermis of the gall is pubescent but later it becomes smooth. The colour is at first green but this changes to a lustrous light brown when the gall is old.

Dimensions:—Average diameter 40 mm.

(*a*) Stage in which the gall is 2 mm. in diameter.

Almost the entire gall consists of a compact tissue, which is composed of small uniform cells. Lines of narrow elongated cells, however, pass in a radial direction throughout this tissue. These cells do not extend into the gall cavity nor out to the epidermis, they traverse about two-thirds of the gall radius. As they approach the epidermis the lines curve around and run parallel to its surface. Spiral vessels are in some cases differentiated in these rays and the elements are more numerous near the point of attachment of the gall.

(*b*) Older stage 9 mm. in diameter.

The gall wall can now be divided roughly into three sections. That part lying next the larval cell resembles closely the compact tissue described in the preceding stage, except that immediately adjoining the

cavity a typical nutritive layer has been formed by the elongation of the cells in a radial direction. In the centre zone the lines of cells containing the vessels are much more apparent at this stage, since the intervening tissue has become loose and skeleton-like. The cells composing it are long, very narrow and frequently branched. In many cases a branch is attached to the main cell without the formation of an intersecting partition between the two. The outside zone of the three is composed of somewhat elliptical cells. These form a fairly firm tissue constituting the rind of the gall.

(*c*) Mature stage.

The protective zone is now the most characteristic feature of the anatomical structure. The part of the protective sheath adjoining the larval cavity consists of a few layers of elliptical cells arranged in tangential rows. The sclerenchymatous deposits on the outside walls of these cells are much heavier than those on the inside. Further out the protective cells are formed in radial rows and their walls are uniformly thickened. This protective strengthening of the cell walls extends even into the loosely arranged filament-like cells, some of which are heavily sclerified.

*Amphibolips inanis* O.S.

Hosts *Quercus coccinea* Muench.
*Quercus rubra* L.

Resembles the preceding species in external appearance and in its attachment to the midrib or the petiole of the leaf.

In shape it is more nearly spherical than *A. confluens* Harr. and it has a much thinner rind than is found in that species. The epidermis of the gall, which is at first green with dark spots, becomes light brown with darker patches at a later stage. The larval cell in this case is held in position by a number of fine radiating fibres.

Dimensions:—Average diameter 35 mm.

In the earlier stages the anatomical structure of this gall is practically the same as *A. confluens* Harr. The vascular strands surrounded by elongated cells are present, but as the gall becomes older the connecting tissue from between the strands disappears.

In the mature gall the protective zone is very apparent. It consists of 8 to 10 rows of comparatively small elliptical cells. The walls of these cells are uniformly thickened, constituting a porous sclerenchyma.

*Dryophanta palustris* O.S.

Hosts { *Quercus rubra* L.
*Quercus coccinea* Muench.

A monothalamous gall produced singly or in groups of two or more on the leaves of the host plant. It is spherical in form and extends almost

equally on each side of the leaf. In the majority of cases the gall extends out almost to the margin of the leaf and only the edge of the blade rims its outer side. Rarely this gall is found originating from the peduncle of the staminate catkin of the host.

The very young gall of this species is densely pubescent, while the well-grown specimens are usually quite smooth. In galls collected when the leaves are just beginning to unfold from the bud the larval cell and the outer zones of the gall are united, but very soon a separation occurs and the larval cell is left rolling freely around in the outer gall. Colour of mature gall green with patches of red in some places.

An average of about three weeks elapses from the time of the opening of the buds until the producers emerge from the galls. After another week the galls are wrinkled, dried up and brown. About ten days before the time of emergence of the producers the larval chambers were removed from several galls and placed under dry conditions. While the time of emergence of these producers was not appreciably changed, the insects in almost every case had difficulty in freeing themselves from the larval cells and one wing usually remained shrunken. It would appear that the outer gall during the later stages of development functions only as a moist chamber for the prevention of the desiccation of the larval cell.

The youngest galls examined were obtained from leaves that were just breaking out of the bud. At this stage the larval chamber still has organic connection with the remainder of the gall (Fig. 49). A well-defined cambium zone, in which mitosis is taking place, divides the gall wall into nearly equal parts. The parenchyma layer on the outside extends from the cambium to the epidermis. It consists of small cells that resemble closely those of the cambial zone. The inner half of the gall, forming the nutritive layer, is composed of much larger cells arranged in rows radial to the larva. A canal passes from the outside into the larval chamber. The epidermal lining of this canal is continuous with that of the epidermis of the gall and is covered with the same class of trichomes (Fig. 49). It gradually passes over into the innermost layer of the nutritive zone.

In a very short time after the opening of the buds, the larval chamber is severed from the remainder of the gall. The break occurs near the outside of the cambium zone, and separation has commenced in Fig. 50. At this stage the protective layer is not yet differentiated. Soon after the separation occurs it is produced, however, and the four zones of a typical Cynipid gall are complete.

The cells of the protective sheath are placed tangential to the larva. There are two layers of these cells, both of which have one tangential wall thicker than the other. In the outer row the thicker wall is towards

the larval chamber, but in the inner row the reverse is the case. On the outside of the protective zone are about two layers of round, loosely connected parenchyma cells (Fig. 51). The canal mentioned in the early stage is still visible, penetrating the outer wall of the gall and that of the larval chamber. A layer of collapsed tissue is now clearly defined around the inside of the nutritive zone (Fig. 51). The inner layer of the parenchyma zone is also showing this same tendency to collapse.

In the mature gall the nutritive zone is represented by only a narrow layer of shrunken tissue (Fig. 52), the individual cells of which cannot be distinguished. The inner layer of the parenchyma zone is now almost completely collapsed and the cell walls of the whole zone are wrinkled.

*Andricus imbricariæ* Ashmead.

Host *Quercus coccinea* Muench.

A globular gall issuing from the stem of the host plant. Several galls are found near each other on the stem but they are never crowded.

It is usually monothalamous, but occasionally dithalamous forms are found, the larval cells are closely connected with the remainder of the gall. When the gall drops off its point of attachment is marked by a small, elliptical, depressed area surrounded by thin scales of tissue. These scales represent tissue forced aside by the emergence of the young gall.

Dimensions:—Diameter 6-9 mm.

This species has the four zones well differentiated. The most striking features of the anatomical structures are the following:—

The cells of the protective layer contain large crystal masses and have their walls uniformly thickened. Radiating lines of cells pass out from this protective zone (Fig. 48), through the parenchyma sheath and end near the epidermis. These bands are composed of narrow, elongated cells and are from 1 to 3 cells in width. These rows of cells contain a great deal of starch and a substance that takes a very deep stain with saffranin. Large cells of the parenchyma zone separate these bands of cells from each other, as seen in Fig. 48.

*Andricus singularis* Bassett.

Host *Quercus rubra* L.

In the majority of cases this gall originates from the mesophyll of the leaf blade but rarely it is found attached to the petiole. It is situated near the margin of the blade of the leaf and projects about equally from the upper and lower surface.

It is a monothalamous gall closely resembling in external form *Dryophanta palustris* O.S., but its outer wall is much firmer and it does not wither so quickly after the producer emerges. The ellipsoidal, larval chamber is suspended at the centre of the gall by radiating bands of

tissue which pass inwards from the gall rind; this gives the species a superficial resemblance to small specimens of *Amphibolips inanis* O.S.

Dimensions:—Diameter 10-15 mm.

The larval chamber in this species is suspended at the centre of the gall by fine strands of tissue. These are composed of long, narrow, filament-like cells interspersed with spiral vessels. These fibres represent the inner part of the parenchyma zone. The outer part of this zone resembles closely that found in the gall produced by *Dryophanta palustris* O.S. The cell walls of the epidermis are strongly thickened and this is the case also in the underlying layer of cells of the parenchyma sheath. The protective zone, when the gall is full grown, consists of two rows of porous, laminated sclerenchyma cells. The outside tangential walls of these cells are much more thickened than the inside walls. The cells of the nutritive layer are unusually large and almost square in outline. By the time the gall is nearly mature many of them have been emptied of their contents and a wrinkling in the radial walls shows that the whole tissue is collapsing (Fig. 59).

*Andricus piger* Bassett.

Host *Quercus coccinea* Muench.

A polythalamous gall produced by the swelling of the petiole or midrib of the leaf. It is situated always near the distal end of the petiole or the proximal end of the midrib.

It is an irregular, elongated structure, somewhat triangular in cross section. When it originates from the midrib the projection is almost entirely from the under surface of the leaf, the broad flattened part of the midrib above rising very little above the general surface of the blade. On the under surface of the leaf along each side of the gall is a row of small openings. The larval cells are in two rows following the line of the openings. The total number in the gall varies from 20 to 30.

Dimensions:—Length of longer diameter 20-25 mm.

A nearly mature specimen shows the following anatomical characteristics. The four typical zones are well defined. Surrounding the nutritive zone are three rows of cells that form the protective zone. The walls of these cells are porous laminated and uniformly thickened. Outside of the protective sheath is a zone of tissue of about the same width, each cell of which contains a large crystal aggregate. These masses of crystals alone distinguish this tissue from that of the parenchyma zone into which it gradually passes by the crystal groups becoming less plentiful.

Connected with the openings mentioned in the macroscopic description are remarkably straight canals that extend in as far as the protective sheath. At this point they are closed by cone-shaped plugs of sclerenchyma (Figs. 43, 44), that extend out from the protective zones of the

larval cells towards which the canals are passing. The cells of this tissue are identical with those of the ordinary protective zones.

From analogy with *Dryophanta palustris* O.S. (Fig. 49), and with other species of Andricus it would seem safe to infer that this canal opens into the larval chamber at earlier stages in the development of this gall. This also appears more likely to be the case since the protective zone that blocks the way, is differentiated only in the later stages.

*Andricus petiolicola* Bassett.

Host *Quercus alba* L.

This gall is produced in the same manner as *A. piger* Bassett by the swelling of the petiole or midrib. It is also located at the same place on the leaf as that species.

It has an irregular, spherical shape drawn out at some place on its surface into a short tapering projection. At the summit of this elongated part of the gall is an opening surrounded by a dense ring of coarse, brown trichomes. The larval cells are numerous and very variable in number. They are arranged around the axis of the gall at about the same distance from the epidermis.

Dimensions:—Diameter of the swollen basal part 10-12 mm.

In this species the protective layer is much thicker than in *A. piger* Bassett, but the individual cells composing it are the same in both species. The galls sectioned were nearly mature but the crystal layer of the former species was not found.

In this species also there is a canal passing towards each larval chamber (Fig. 46). These canals do not open directly to the outside but into a main canal of larger bore that extends a considerable distance into the mass of the gall (Figs. 45, 46). The branch canals are blocked by the protective sheath as in the preceding species. All of the canals are lined with a cuticularized epidermis, continuous with the gall epidermis (Fig. 45). The lining of the main canal produces abundant trichomes but these structures do not appear to be present in the tributaries. A tubular outgrowth of the protective zone surrounds the main canal. This sclerenchymatous sheath is separated from the canal by several layers of parenchyma cells. Outside of this protective tube a cork cambium is differentiated.

Andricus (Undescribed).

Host *Quercus macrocarpa* Michx.

The swelling of the midrib of the leaf produces this gall. It resembles closely *A. piger* Bassett, but is always found within the blade of the leaf, although close to its base in most cases.

The openings mentioned in the two preceding species are in this case found on the surface of the gall which appears on the upper side of

the leaf. They correspond to the larval cells, varying from 3 to 7 in number.

Dimensions:—Length parallel to axis of midrib 10-15 mm.

Although nearly mature specimens of this gall were sectioned, a protective zone was not found.

In this species each larval chamber has a canal related to it. In this respect it resembles *A. piger* Bassett. A section of the gall at a very early stage of development shows that the canals open into the larval chambers. When the gall becomes older, each canal is blocked by two plugs of sclerenchyma. One of these occupies the same position relative to the larval chamber as in the two preceding species; the other is formed near the external opening. These masses of sclerenchyma are shown in Fig. 47. Trichomes do not appear to be produced in the canals.

*Rhodites multispinosus* Gillette.

Host *Rosa blanda* Ait.

A globular to ovoid polythalamous gall produced by the swelling of the stem or branches of the host plant. Since the larval cells are arranged around the stem axis at about the same distance from the periphery of the gall, the abnormal swelling completely encircles the stem.

The gall is reddish brown in colour and has its surface usually densely covered with fairly stout prickles.

Dimensions:—Average diameter 25 mm.

The principal mass of this gall is formed from the cortex of the stem. The larval cells are embedded in it and a common parenchyma zone is thus formed. A well-marked protective tissue, composed of cells with porous, sclerenchymatous walls, separates this parenchyma zone from the nutritive tissue that lines each larval cell.

The response of the gall epidermis to stimulation is shown in the production of the numerous prickles that are so marked a characteristic of this gall. Since the stem of the host is usually unarmed this feature appears the more remarkable.

*Rhodites lenticularis* Bass.

Host *Rosa blanda* Ait.

A monothalamous, lens-shaped, thin-walled gall produced in the mesophyll of the leaf of the host. They sometimes occur singly but usually several are located on one leaflet. They often are so crowded that they lose their circular outlines.

This gall projects chiefly from the under side of the leaflet.

Dimensions:—Longer diameter 2-3 mm.; shorter diameter 1-2 mm.

Since it is possible to trace a considerable part of the unaltered mesophyll of the leaf along the upper surface of this gall, proliferation must have commenced in the spongy parenchyma of the leaf. The

normal epidermis of the leaf passes over the surface of the gall without modification. On the upper surface of the leaf a protective layer of about five cells in depth separates the normal part of the leaf from the gall tissue. On the under surface a corresponding protective layer occurs at a distance of three rows of cells below the epidermis. The cells of this protective zone have uniformly thickened sclerenchymatous walls. The general structure of the gall is shown in Fig. 63. Inside this layer a cambial tissue is differentiated, from which the cells of the nutritive zone are produced directly. The nutritive cells are rectangular in outline and arranged in radial lines, presenting very much the same appearance as the cambium from which they have originated.

*Rhodites bicolor* Harr.

Hosts { *Rosa blanda* Ait.
*Rosa carolina* L.

A monothalamous, spherical, hollow gall with a wall 1 to 2 mm. in thickness.

They originate singly or several close together from the upper surface of the leaf.

The gall bears numerous stiff prickles that average about the same length as the diameter of the gall. Colour green with red tints, turning brown at maturity.

Dimensions:—Average diameter 11.5 mm.

The anatomical structure of this gall presents very little differentiation of tissue. The parenchyma zone consists of large irregularly shaped cells. This tissue passes into the nutritive layer with little change in the shape or size of the cells. The protective zone is entirely absent.

*Rhodites ignotus* O.S.
Host *Rosa blanda* Ait.

A polythalamous or occasionally monothalamous gall attached to the under side of the leaves by a small extent of surface. These galls are generally found clustered together and often deform the entire leaf.

Dimensions:—Average longer diameter 11 mm.; average shorter diameter 3-5 mm.

While somewhat variable, the shape approximates usually to an irregular oblate-spheroid. At the apex of the gall is a shallow depression containing a small scale-like patch of tissue. The epidermis is glaucous and light brown in colour.

The anatomical structure of this species presents the rare feature of two protective layers. These are each of about five cells in thickness in the full-grown gall. One of them is found in the usual position separating the parenchyma and the nutritive zones. The other is situated in the

parenchyma just beneath the small-celled epidermis. This outside protective sheath gradually passes into the parenchyma zone by the constituent cell walls becoming thinner. The large size of the cells in the parenchyma layer marks them out from the rounder and smaller cells of the nutritive zone.

Below the depressed area, mentioned in the macroscopic description, is a small patch of sclerenchymatous cells. In position and character these cells appear to be homologous to the groups of cells that block the canals in different species of Andricus. Only the mature stage of this gall was examined, but in all probability the depression at the top is the remains of a canal that connected the gall cavity with the outside in the early stages of development. A part of the normal epidermis of the leaf was held fast by the closing of this canal, and when the gall was forced out beyond the leaf tissues a small patch of the epidermis of the leaf was carried out on it. This persists in the later stages of development as the scale of tissue in the depression.

*Rhodites gracilis* Ashm.
Host *Rosa blanda* Ait.

A thin-walled, monothalamous gall produced from the mesophyll of the under surface of the leaf of the host. Occurs singly or in clusters on the leaflets.

It is irregularly spherical with a broadened top, in the centre of which is the same shallow depression and scale-like patch found in *R. ignotus* O.S. Numerous ridges radiate out from the point of attachment of the gall, pass up its sides and project as short blunt tubercles around the top.

Dimensions:—Diameter 5-6 mm.

This species resembles closely *R. bicolor* Harr. in anatomical structure. It presents little differentiation of tissue. The protective sheath is not present, and the parenchyma and nutritive zones are marked out from each other only by the cells of the latter being slightly smaller and more circular in outline. The observations on the preceding species concerning the depression at the summit of the gall and the discussion of them also apply to this species.

*Rhodites nebulosus* Bass.
Host *Rosa blanda* Ait.

This species, as the preceding, is monothalamous and thin-walled. It also originates from the mesophyll of the leaf of the host. It occurs usually in dense clusters deforming the entire leaflet.

The gall is spherical in form, bearing at the summit the depressed area and scale-like patch characteristic of the two preceding species.

The surface of the gall is smooth or covered with short weak spines. Colour green, tinted strongly with red.

Dimensions:—Diameter 5-6 mm.

This gall resembles closely the preceding species in anatomical structure. The cells of the nutritive and parenchyma layers differ in much the same way and to the same extent. Further, the protective zone is again absent. The explanation given in the two preceding forms, to account for the scale in the depression at the summit of the galls, is applicable also in this case.

*Cynips? constricta* (Stebbins).

Host *Quercus coccinea* Muench.

A monothalamous gall originating from the midrib or a principal vein of the leaf. Its origin from a vein is shown in Fig. 53. It is usually found on the under side of the leaf but occurs occasionally on the upper side.

This gall has the form of a sphere surmounted by a short cylindrical neck, which is slightly constricted at the base. The general form is shown in Fig. 54. A very small portion of its surface attaches it to the leaf. The epidermis on the main body of the gall is smooth, shiny and green in colour. The neck is red at the tip.

Dimensions:—Diameter of spherical part 2.5-3.5 mm.

At an immature stage of the gall the parenchyma zone in the spherical part consists of a mass of cells that gradually decrease in size from the epidermis to the inner limit of the layer. At the epidermis the cells are nearly circular in outline but become square or rectangular in proportion to their proximity to the centre.

Bounding this zone on the inside is a crystal layer of about three cells in thickness, each cell containing a large crystal mass. Around the inside of this tissue is a nutritive zone, the cells of which are regularly rectangular.

At the top of the main part of the gall is a well-defined cambium tissue which produces the cylindrical projection that caps the spherical portion (Fig. 54). The anatomical structure of this part shows clearly that it functions as an outer nutritive zone. Its walls are thin and the cell contents take the same stain as those in the nutritive zone surrounding the larva. Large starch grains are also scattered throughout the cells. This zone is separated from the cambium tissue in the later developmental stages by a protective layer of typical porous sclerenchyma. These cells are filled with protoplasmic material, and the system of canals between the individual cells is very complete and clearly defined. This feature is very important since the nourishment from the outlying nutritive zone has to pass through this tissue to reach the larva.

*Solenozopheria vaccinii* Ashmead.

Hosts { *Vaccinium pennsylvanicum* Lam.
*Vaccinium canadense* Kalm.

A polythalamous gall originating from the lower part of the stem of the host plant.

In the majority of cases this gall is reniform in shape but rarely it is irregularly spherical. The surface is depressed where it is attached to the stem, which is almost invariably bent at that point. The colour is green, often with red tints turning to brown as the gall becomes older.

Dimensions:—Longer diameter 10-30 mm.

At an early stage, while the tissues are still soft, the anatomical structure of this gall presents practically no differentiation. It consists of a mass of dense tissue, the cells of which are small and placed very close together. The small-celled epidermis is covered with an exceedingly heavy cuticle. At regular intervals small papillæ occur on the epidermis which seem to secrete a glandular material from small openings at their tips.

When the gall is mature all the cells, except a few layers below the epidermis, have sclerified walls. The thickenings are decidedly heavier on one wall than on the opposite.

*Aulacidea nabali* Brodie.

Hosts { *Prenanthes alba* L.
*Prenanthes altissima* L.

A polythalamous gall originating from the stem or the main root of the host plant. It occurs at or near the base of the stem, usually just below the surface of the ground but in some cases it is situated some distance above the ground.

The single galls are irregularly spherical, but these are generally clustered in such a way as to form roughly cylindrical masses. In some cases these completely surround the stem, but in others they only partly encircle it.

Dimensions:—Diameter of single gall 5-10 mm.

The cambium of the stem stimulated to unusual activity produces the abnormal tissues in this case (Fig. 66). Along the line of contact of the gall with the normal stem, the cambium produces wood and bast, but in abnormally large amounts, as can be seen in Fig. 66. In the gall tissue proper, in place of wood, radial lines of nucleated thin-walled cells occur. A few rows of vessels are interspersed among these cells. The stimulated cambium produces these parenchyma cells also on the side where the bast would normally occur. In the gall tissue on the outside of the line of the cambium, small patches of vessels are found. These have arisen from clumps of cells detached from the original cambium.

Associated with these isolated masses of vessels, often occurs a small amount of bast that appears normal. When the detached cambium is curved, wood is almost invariably produced on the inside of the curve and bast on the outside, giving rise in some cases to almost perfect concentric bundles.

The club-shaped cells of the nutritive zone do not follow the general rule and radiate out from the larval cell, but are oriented with their long axes at right angles to the cambium. The nuclei of the cells in this zone are abnormally large and often present good examples of amitosis (Text Fig. 6).

The normal cortex passes over the gall with little alteration during the early stages of development, but later a cork cambium is differentiated that throws off the cortex and covers the gall with its characteristic corky layer.

*Neuroterus majalis* Bassett.

Host *Quercus alba* L.

A polythalamous gall originating in the mesophyll of the leaf and divided into two nearly equal parts by the blade. The galls are found usually in contact with the side of the midrib and extending out to the margin of the leaf.

This gall is characterized by a flat, irregular shape and a finely granular epidermis. It is translucent and of a light green colour until the producers emerge when it becomes light brown and opaque.

The apertures of exit of the mature insects seem to occur invariably on the upper surface of the gall.

Dimensions:—Diameter parallel to leaf blade 12-24 mm.; diameter at right angles to leaf blade 7-9 mm.

Only the mature gall was examined. At this stage the nutritive zone consists merely of a narrow line of collapsed tissue (Fig. 67). From two to three rows of cells constitute a protective layer. The tangential walls of these sclerified cells are unequally thickened, the heavier deposit being on the wall nearer the larval chamber. The parenchyma zone consists of large thin-walled cells, the majority of which are empty and devoid of nuclei. A small-celled epidermis continuous with that on the normal leaf passes over the gall.

*Summary.*

All the galls in this group have three tissue zones developed and only very seldom is the fourth absent. The three always present are the epidermal, the parenchyma or tannin and the nutritive. The parenchyma zone, as shown by Cook[23] is subject to a great amount of variation. The fourth, not always present, is the protective or sclerenchyma zone.

Even in one genus there may be considerable variation in the degree of development of the protective zone. It is entirely absent in *Rhodites gracilis* Ashm. and *R. bicolor* Harr., but two distinct layers are found in *R. ignotus* O.S.

In several species of the genus Andricus canals were found passing from the exterior towards the larval chambers. In the early developmental stages these opened into the gall cavity, but later were blocked by outgrowths of sclerenchyma from the protective zone. They were located in the species *Andricus piger* Bassett, *A. petiolicola* Bassett and *Andricus* N.S. (Figs 43-47).

A canal similar to those in the Andricus genus was found also in *Dryophanta palustris* O.S. In this species the plug of sclerenchyma is not developed (Fig. 49).

An epidermal scale was found in the bottom of a depression at the apex of the galls produced by certain species of Rhodites. Below each scale a small mass of sclerenchyma is differentiated. These structures seem to be homologous to the canals in the genus Andricus. They are present in the following species: *Rhodites ignotus* O.S., *R. gracilis* Ashm. and *R. nebulosus* Bassett.

The gall *Solenozopheria vaccinii* Ashmead has the sclerified tangential walls of its protective zone much thicker on one side than on the opposite. This is very unusual in stem galls, although a common feature in leaf galls.

The collapsing of the cells of the nutritive zone after the withdrawal of the contents is exemplified in almost any gall studied. It is, however, particularly noticeable in *Dryophanta palustris* O.S. (Fig. 52).

Empty cells were found throughout the nutritive zones in the later stages of nearly all the galls examined. Good examples of this phenomenon are furnished by *Andricus singularis* Bassett and *Aylax glechomæ* Linné.

The separation of the tissues so as to produce a free larval chamber gall is shown in the species *Holcaspis globulus* Fitch and *H. bassetti* Gillette; also in *Dryophanta palustris* O.S. (Fig. 50). In the last species, as Cook[23] has shown, the separation of the larval chamber takes place at a very early developmental stage.

Mitotic phenomena were observed in the cambium and near it in the adjoining parenchyma zone of *Dryophanta palustris* O.S. The number of chromosomes remains as in the normal. Good examples of amitosis were located in the nutritive tissues of *Aylax glechomæ* Linné, and *Aulacidea nabali* Brodie (Text Fig. 6).

The parenchyma zone of *Amphibolips confluens* Harris furnishes an example of a tissue consisting of long filamentous cells from which

branches are given off without the formation of intercepting walls. It seems to represent an exaggerated form of the spongy parenchyma.

Proliferation of glandular tissue is shown in *Aulacidea nabali* Brodie.

*Cynips? constricta* Stebbins furnishes an example of an outer accessory nutritive zone that clearly assists in supplying the larva with nourishment (Fig. 54).

## NOTES ON THE PROTECTIVE ZONE.

This zone is typical for the Cynipid galls, but as already stated it is differentiated in certain Dipterous forms, such as *Rhabdophaga batatas* Walsh (Figs. 25, 26) and *Cecidomyia triticoides* Walsh (Figs. 37, 38), and also in the Hemipterous gall *Pachypsylla celtidis-mamma* Riley (Fig. 15).

In the Cynipidæ it usually bounds the nutritive zone on the outside, but it does not invariably occupy that location. When two layers are present the inner occupies that position, but the outer is situated nearer the periphery of the gall.

The term, "protective," has been applied to this tissue without a very clear idea as to what it protects from. The common notion appears to be that it forms an inner line of defence against parasites and small animals other than insects. The latter class of enemies appears to interfere very seldom with galls. Cook[23] mentions one example—he found birds tearing open the galls of *Pemphigus vagabundus* Walsh. Very few examples of such cases have come under my notice. Galls of *Holcaspis bassetti* Gillette are occasionally opened by woodpeckers, and the larvæ of *Eurosta solidaginis* Fitch are sometimes taken from the galls by field mice. Chipmunks will also tear open the galls of *Pemphigus rhois* Walsh to get at the inhabitants. Not only are galls seldom attacked by such animals but a sclerenchymatous tissue would be a very poor defensive device against them.

Adler[1] has advanced the idea that this zone protects against insects that are parasitic on the producer-larva. This appears very unlikely since the parasites oviposit at a comparatively early stage, and the sclerenchyma is differentiated relatively late in the development of the gall. The same writer cites the large size of the gall and the thickened epidermis as other protective devices against parasites. The same argument is applicable in this case; the gall is not large nor is the epidermis abnormally thick at the time the parasites are ovipositing. Were Adler correct the gall *Amphibolips confluens* Harris should be almost immune against parasites, as it is large, has a thick epidermis and a well-developed protective sheath. In spite of all these apparent advantages this gall has a heavy casualty list owing to parasitism. During last season hundreds of this species were opened and on an average about 75% were found to

be parasitized. In some cases a tree would not yield a single perfect producer, although a couple of dozen galls were examined.

It seems safe to conclude that if this zone has ever functioned as a means of defence against parasites, it is no longer operative. Apparently the only protective function that can be ascribed to this tissue is the prevention of injury to the producer by desiccation during its later larval and pupal stages of development. A thick or cuticularized epidermis would also afford protection in the same manner.

Concerning the form of the elements comprising this zone, Weidel[45] has recently made some interesting observations. He makes the following too sweeping statement in his summary,—

"Auch das gallentragende Organ der Mutterpflanze hat einem Einfluss auf die Gestaltung der Elemente in der Galle, denn die blattbürtigen Gallen führen in der Schutzschicht einseitig verdickte, die übrigen allseitig gleichmässig verdickte Zellen."

That this can be accepted only as a general rule, and at least requires further study, is indicated by the fact that our American galls furnish undoubted exceptions. Thus the gall produced on the stem of *Vaccinium pennsylvanicum* Lam. by *Solenozopheria vaccinii* Ashmead has cells that have a much thicker deposit of sclerenchyma on one tangential wall than on the other. In some cases practically the entire cell lumen is filled with sclerenchyma and the deposit has grown entirely from one side of the cell. Also a number of leaf galls have their protective zones composed entirely of cells with uniformly thickened walls. The following species furnish examples of this: *Amphibolips inanis* O.S., *Rhodites lenticularis* Bass. and *Neuroterus majalis* Bassett. The statement quoted is true, however, in the majority of cases and is important as indicating a possible effect of environmental conditions on the elements composing the gall.

## CYTOLOGY OF GALLS.

Cell division in the Cynipid galls was not found to present any unusual phenomena. In the cambial layer of *Dryophanta palustris* O.S. in which mitosis was taking place the chromosomes were found to be eight in number. They are slightly curved and show a decided tendency to group in pairs when moving out from the equatorial plate. The root tips of the host *Quercus coccinea* Muench. were found to give the same chromatic count and the chromosomes present the same feature of moving out to the poles of the spindle in groups of two.

In several galls amitosis was noted and very marked examples in the nutritive zones of the galls *Aulacidea nabali* Brodie (Text Fig. 6) and *Aylax glechomæ* Linné. Cell division did not appear to accompany the phenomenon in any of the cases examined.

In galls other than the Cynipidæ, the only cytological phenomena that presented unusual features were found in the orders Diptera and Lepidoptera. An unusual type of cell division was observed in the cortex and epidermis of *Neolasioptera perfoliata* Felt (Text Fig. 7) and in the cortex of *Gnorimoschema gallæasterella* Kellicott and *Stagmatophora ceanothiella* Cosens. This has been already referred to in the descriptive part of the paper and only the main features will be noted here. The mother cells produce from 2 to 5 daughter cells and these remain in groups that are easily recognizable. The elongated nuclei are found in contact with the septating walls but not exactly opposite each other. In the Dipterous genus Neolasioptera this was the only form of cell division that occurred in the gall, but in the Lepidopterous genera it was found only in a limited area of the abnormal tissue.

Fig. 6.—Nuclei from the nutritive layer of *Aulacidea nabali* Brodie.

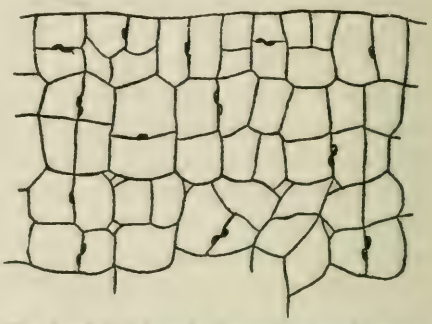

Fig. 7.—Cell division in the epidermis and cortex of *Neolasioptera perfoliata* Felt.

## THE BEGINNING OF GALL DEVELOPMENT.

In some species of galls that originate from stems, veins or petioles, the eggs of the producer are deposited within the tissues of the host in or near the cambium zone. Adler and Küstenmacher have detected eggs actually placed in that region, and while my own observations in the case of *Cynips? constricta* Stebbins were made on older developmental stages, yet the nature and arrangement of the tissues were such as would seem to preclude any other conclusion. The origin of this gall from the vein is shown in Fig. 53. In leaf galls that are produced in the blade as *Rhodites lenticularis* Bass. (Fig. 63), a cambium is differentiated in the mesophyll into which, in this case, the egg is inserted. Two independent observers, Beyerinck and Weidel, have demonstrated beyond a doubt, however, that the egg is in some cases placed on the epidermis of the host, and consequently there are at least two distinct methods of ovipositing.

The two observers, who deal with the development of galls from eggs deposited on the outside of the host, hold entirely different opinions concerning the early stages. Beyerinck[15] supposes that after the egg is placed at the selected spot, the tissues under it grow very little, if at all, but those immediately adjacent undergo rapid proliferation until the egg is completely enclosed, forming a gall of the "Umwallung" type. According to his view the larva possesses the power to stimulate the tissues through the egg membrane without rupturing it.

Weidel, on the other hand, holds an entirely different opinion concerning the enclosing of the larva by the tissues of the host. He has been able to convincingly demonstrate that in the gall *Neuroterus vesicator* Schlecht, the cuticle of the leaf is punctured before the larva is completely free from the egg membrane. "Die in der Eihaut noch vollständig eingeschlossene Larve durchbricht diese an einer Stelle und senkt in die Epidermis des Blattes ein Organ ein durch das die Cuticula durchbrochen und das pflanzliche Gewebe verletzt wird."—Weidel.[45] The influence of the larva[45] soon produces proliferation in the tissues of the leaf, and following this a degeneration commences at the epidermis and extending quickly forms a cavity of sufficient size to hold the larva. Into the larval chamber thus prepared the producer gradually passes, and the opening through which it entered is soon closed by the growth of the stimulated tissues.

While the excellent work of Weidel cannot be questioned concerning this particular gall, it is not necessary to assume that this is the only method by which a larval cavity is formed. In my opinion the method as described by Beyerinck is found in some of our American species of Andricus and Dryophanta, reference to which will be made later. Weidel's objections contained in the following quotation do not seem serious enough to warrant the setting aside entirely of the "Umwallung" type of development. "Gerade diese Stelle war es, die mich zu meinen Untersuchungen anregte, denn eine grosse Anzahl von Fragen bleibt bei diesen Ausführungen Beyerinck's unaufgeklärt: Wie kommt es, dass an der Stelle, wo das von der Larve abgesonderte Enzym am stärksten wirken muss, keine Vergrösserung der Zellen stattfinden soll, sondern nur in einiger Entfernung? Was wird aus Epidermis unmittelbar unter dem Ei? Aus Beyerinck's Figuren muss man annehmen, dass sie in Nährgewebe umgewandelt wird, da sie die Larve unmittelbar berührt. Wie kommt das, "Sinken," oder, "Vergraben," zustande, Vorgänge, für die ihn seine Erklärungen selbst nicht befriedigen?"—Weidel.[45]

Concerning the first question, as to why the proliferation is more pronounced around the larva than in immediate contact with it, it may be stated that this is a usual occurrence in the lower groups of galls in

which the stimulus is applied in one direction only. The stem mothers of the genus Chermes thus become surrounded by a ring of tissue that grows out around the point of attachment of the insect (Fig. 11). The Dipterous gall *Cecidomyia ocellaris* O.S. also furnishes a very striking example of this phenomenon. In this species the leaf is scarcely at all thickened under the larva, but the proliferation is so marked around it that the producer ultimately lies in a concavity, not formed by the leaf becoming depressed, but by the outgrowth of the circular ridge of tissue (Fig. 33). Any explanations offered to account for these facts are merely conjectural, but it seems likely that the enzyme content requires a certain degree of concentration in order to exhibit its maximum activity, and that immediately in contact with the larva it has not the requisite dilution to cause the greatest proliferation. It is a well-known fact that the amount of growth of plants in culture solutions varies with the degree of concentration of the nutrient substance in the medium; thus there is an optimum quantity and as this is exceeded growth is more and more inhibited. An example of this is furnished by the checking of the growth of Penicillium when the culture solutions are too concentrated.

With regard to the question, "What becomes of the epidermis under the egg?" I agree with Weidel that there is little likelihood of abnormal cell production until the larva punctures the egg membrane, but when this occurs the epidermis becomes part of a nutritive zone and will undergo such rapid changes that its epidermal characteristics will soon disappear. The chief alterations will be expressed in the much richer contents of the cells and in their steady collapsing as these contents are withdrawn (Figs. 49, 51, 52). The latter change makes it extremely difficult to follow the normal into the abnormal epidermis unless at an extremely early stage. While the enclosing of the larva is due chiefly to the growth of the surrounding tissues, yet the collapsing of the nutritive layer will assist it to a certain extent.

Weidel's photographs show that in Neuroterus there is not at any stage an opening into the larval cavity that is lined with the epidermis of the leaf, and that after the larva enters its prepared chamber the opening is very soon closed. In the method of development as stated by Beyerinck we would expect to find such an opening persisting for some time, and if we do, that must be accepted as confirmatory evidence of the truth of his hypothesis. In two different genera, namely Dryophanta and Andricus, I have found canals leading into the gall cavity in the early developmental stages (Figs. 43-49). The epidermis of these structures is continuous with the gall epidermis and it bears the same class of trichomes as the latter. The canal is very marked in *Dryophanta palustris* O.S., and its lining which is the same as the gall epidermis, can be

traced until it passes over into the inside layer of the nutritive zone (Fig. 49). This canal can still be detected in well-grown specimens.

Only mature material of *Andricus piger* Bassett and *Andricus petiolicola* Bassett was obtained, and while the canals with the epidermal lining are well marked, they are shut off from the larval cavity by outgrowths of sclerenchyma from the protective sheath (Figs. 43, 44). There is little doubt, however, but that in early developmental stages they open into the gall cavity as in the genus Dryophanta. This view, indeed, I have practically confirmed in the examination of an undescribed species of Andricus on *Quercus macrocarpa* Michx. In this form the canal is blocked at maturity by sclerenchyma (Fig. 47) as in the former species, but at an early stage I have found it extending into the larval chamber.

*Summary.*

The evidence seems conclusive that there are two types of early developmental stages of galls when the egg is deposited on the epidermis of the host. The method of formation of the larval chamber as described by Beyerinck is found in certain genera, as Dryophanta and Andricus, while the method worked out by Weidel occurs in Neuroterus and in all probability other forms.

## FEEDING HABITS OF THE LARVÆ OF GALL PRODUCERS.

With the exception of the family Tenthredinidæ, all gall-producing larvæ have started to feed before the abnormal production of tissue commences. The narrowing of the problem of gall production to the influence of the larvæ on the tissues of the host has given additional importance to the problems dealing with the feeding habits of these larval producers.

Order Arachnida.

Fam. Eriophyidæ.

The members of this family have mouth parts of the sucking type. With their cone-shaped beaks they pierce the cell walls and withdraw the liquid contents. The cell walls are not used as food.

Order Hemiptera.

Fam. Aphididæ.

Fam. Psyllidæ.

The feeding habits of these families are similar to the preceding. The possession of a suctorial proboscis makes it possible for them to obtain the liquid contents of the cells by merely puncturing the walls.

Order Lepidoptera.

The larvæ in this case consume the entire cells that line the interior of the galls.

Order Coleoptera.

Feeding habits as in the preceding order.

Order Diptera.

Fam. Cecidomyidæ.

Fam. Trypetidæ.

Concerning the feeding habits of this order, Packard[38] states that the Cecidomyia larvæ must absorb their nourishment through the skin or suck it in at the mouth. He bases his conclusion on the facts that the larvæ are devoid of jaws and that excrement is not found in the mature galls.

Walsh[44] from the same data has come to the conclusion that the larvæ abrade the interior of the galls with the chitinous structure, the so-called breast bone, on the ventral surfaces of their bodies. The irritation produces a flow of liquid from the cells and upon this the larvæ feed. He further states that the mouth of the larva of *Eurosta solidaginis* Fitch possesses a horny, black termination that probably serves the same purpose of abrasion as the breast bone of the Cecidomyidæ.

Both of these observers have concluded that the nourishment is obtained by the larvæ without the destruction of the cell walls, and that these do not form a part of the food of the larvæ. My observations confirm this view. In several species such as *Lasioptera corni* Felt and *Cecidomyia ocellaris* O.S. (Figs. 33, 40), the walls of the cells, through which the larvæ were obtaining food, were apparently uninjured. In other forms as *Cecidomyia triticoides* Walsh (Fig. 37), and *Cecidomyia pellex* O.S., the cells of the nutritive zone had collapsed as the contents were withdrawn.

Order Hymenoptera.

Fam. Tenthredinidæ.

By the time the larvæ in this family are full fed, nothing remains of the galls but a thin rind on the outside of each. Both the cell walls and contents are swallowed indiscriminately.

Fam. Cynipidæ.

In this family the larvæ are invariably surrounded by a layer of thin-walled cells which usually present a radial elongation especially in the innermost rows (Fig. 58). The cells of this nutritive zone contain sugar, starch, oil emulsion and albumen. The amount of starch varies directly and the sugar inversely with the distance of the cells from the larvæ.

With regard to the manner in which this zone is used as food by the larva at least two views are current. The following statement of Kerner[32] may be presented as an adequate expression of one of these theories: "The larva when hatched finds the inner wall of the chamber which has been fitted for its temporary abode always provided with the necessary

food, and it immediately attacks and devours the juicy tissue with great avidity. The cells which are demolished, wonderful to relate, are replaced almost at once. The cells of the gall pith remain capable of division as long as the larva in the chamber requires food, and the surface cells which have been devoured in the gall chamber are soon replaced by new cells."

Küstenmacher[33] has advanced an entirely different view and his opinion may be taken as representing the theory of the other school of observers.

He states,—"Die im Innern entschlüpfte Larve, welche ihren Tisch reichlich gedeckt findet, beisst die innern Zellen des Nahrungsgewebes, welche lose, von der Eiweiss-Zucker-Oel-Emulsion strotzend, hervorragen, an und saugt dieselben regelmässig ringsherum aus, während die sehr dünnen Wandungen schmal schlauchartig übrig bleiben."

In deciding between these two theories the question to be answered is, does the larva eat both the walls and contents of the cells as stated by Kerner, or does it extract in some way the contents of the cells, leaving the walls practically intact? Several different points are involved in the discussion of this question. (*a*) The absence of frass in the larval chamber. (*b*) The completeness of the alimentary canal in the larva. (*c*) The nature of the stomach contents. (*d*) The presence of collapsed tissue and empty cells in the nutritive zone.

When a mature Cynipid gall is examined the larval chamber, in which the producer has passed through its early stages, is found unsoiled by excrement. Concerning this matter my observations agree with those made by Walsh in respect to the Cecidomyia larvæ. By way of comparison, if a mature gall is examined, the larva of which is known to eat the entire cells, a comparatively large quantity of excreted material is found (Fig. 68). The mature larva and its frass from a gall of *Pontania pomum* Walsh were dried in a desiccator and weighed. The following result was obtained:

Larva .0115 gm.
Frass .0319 gm.

In view of the comparatively large amount of frass in the sawfly gall, its absence in those of the Cynipidæ appears significant.

This fact concerning the larvæ of the Cynipidæ has not received attention since it has been supposed that the intestine of the Cynipid larva ends blindly. Comstock[21] makes the following statement on this point: "The larvæ are maggot-like and without a caudal opening to the alimentary canal." Serial sections were made of the larvæ of the producers *Philonix nigra* Gill (Fig. 61), and *Amphibolips confluens* Harris (Fig. 62). These sections prove conclusively the completeness of the

intestinal tract throughout, and that therefore if Kerner's theory be correct frass should be found as in the sawfly galls.

Further evidence in favor of Küstenmacher's view is furnished by a comparison of the stomach contents of a Cynipid and an inquiline larva. The former consists of a mass of extremely fine particles, among which can be detected nothing that is recognizable as having formed a part of a cell (Fig. 55). As this material passes along the digestive tract it becomes less dense the nearer it is to the posterior opening, and is entirely absent in the last part of the canal (Figs. 61, 62). The latter consists of much coarser material in which crystals, similar to those in the surrounding cells, and parts of cell walls can be easily detected. These contents are shown in Fig. 56, and at a higher magnification in Fig. 57. So characteristic is this difference between these two classes of stomach contents that by means of it alone a Cynipid can be easily distinguished from an inquiline larva.

The data already presented furnish indirect proof that only the contents of the cells form the food of the Cynipid larva. An examination of the walls of the cells immediately surrounding the larva gives direct evidence in favour of this hypothesis. The nutritive layers of a large number of Cynipid galls were examined at different stages of development, and in none of the examples did the walls of the cells appear to have been eaten away by the larva. A layer of collapsed tissue (Figs. 52, 59, 60), especially in the older specimens, is often found around the inside of the larval chamber and there are also many empty cells throughout the nutritive zone. These are shown in the inner row of cells in Figs. 59, 60. In some cases the radial walls of the cells are wrinkled, indicating that these cells are gradually contracting. This can be seen with the aid of a lens in Fig. 59. The folds are not found in the tangential walls of the cells. The majority of the empty cells are found in the row that lines the interior of the larval chamber (Fig. 60), but others are distributed irregularly throughout the entire nutritive zone. These can be seen in Figs. 59, 60. There does not seem to be the slightest possibility of doubt that the larva withdraws the contents from the cells of the nutritive zone without destroying the walls, and that in consequence the cells surrounding the larva gradually collapse.

If an inquiline larva is feeding in the gall, a ragged, broken edge of tissue is found lining the cavity in which it is living, a marked contrast to the smooth interior of the Cynipid larval chamber. This uneven edge is shown in Fig. 56, compare with Fig. 55. Neither of these views takes into account the possibility of enzyme action in rendering more soluble the contents of the nutritive zone.

A number of investigators have suggested that some form of enzyme is secreted by the larvæ of the Cynipidæ. Küstenmacher[33] indeed states in this connection that he could detect a distinctive odor from these larvæ, but enzyme action has always been considered in relation to the gall-producing stimulus and never with the feeding habits. The gradual decrease in the proportion of sugar to that of starch, in the contents of the cells, from the inside of the nutritive zone to the outside, would seem to indicate a relation between the relative amount of sugar and the proximity of the larva. Experiments were accordingly undertaken with the purpose of deciding whether the larva was capable of producing this change and of thus rendering the cell contents more easily soluble.

FIRST SERIES OF EXPERIMENTS.

Forty larvæ of *Amphibolips confluens* Harris just removed from the galls were placed in about 7 c.c. of starch solution made of corn meal. The test tube containing the larvæ was placed in a bath at 50° C., along with a control.

This starch was tested for sugar with Fehling's solution. No sugar was found at the end of 2 hrs. but after 20 hrs. a test for sugar was readily obtained.

SECOND SERIES OF EXPERIMENTS.

Forty-two larvæ were placed in the same quantity of starch solution and treated as in preceding case.

No sugar was found at the end of 8 hrs. but after 12 hrs. from the beginning of the experiment sugar was detected, and again at the end of 24 hrs.

THIRD SERIES OF EXPERIMENTS.

Thirty-five larvæ were placed in 7 c.c. of water and left for about 3 hrs. This water was then placed in an equal quantity of starch solution and kept at about 50° C. as before. The water was tested before it was poured into the starch and found to give an acid reaction. In this case sugar was detected in 50 hrs. and a very decided reaction was obtained after 70 hrs. The larvæ that had been washed were placed in starch and kept at 50° C. as before but sugar could not be detected.

In all the cases cited above, as a control experiment, starch without the larvæ was kept in the bath under the same conditions as that which contained the larvæ. This starch did not give the slightest indication of sugar at any time. From these experiments we conclude that the Cynipid larvæ must secrete an enzyme that has the property of changing starch to sugar. It seems quite possible that other ferments may be employed by the larva for similar purposes. To my knowledge

no tests have been made, but the observations of Weidel[45] point conclusively in this direction. He noted that the walls of the protective sheath become delignified; this is strongly suggestive of the presence of a hadromase or allied ferment.

With the purpose of discovering the source of the enzyme a number of species of Cynipid larvæ were examined for glandular structures. An enlargement of the first two segments immediately below the mouth was found to be a common characteristic of all these specimens. Regularly arranged on these projections are two pairs of openings as shown in Text Fig. 8. Longitudinal serial sections of *Philonix nigra* Gillette and *Amphibolips confluens* Harris show that these openings are connected by ducts with cavities lined by a glandular epithelium composed of large cells. From these cells the enzyme containing material passes into the cavity and from thence to the outside by means of the duct. There seems little reason to doubt but that these structures are salivary glands opening externally, and that they are the source of the enzyme. A gland with the connecting duct is shown in Text Fig. 9. Only the two species mentioned have been examined by serial longitudinal sections, but the external openings were noted in several forms and in all probability these glands are a characteristic common to all the Cynipidæ.

Fig. 8.—Head of Cynipid larva showing external openings of the salivary glands just below the mouth.

Fig. 9.—Longitudinal section of the larva of *Philonix nigra* Gillette, passing through a salivary gland and its associated duct.

Concerning the feeding habits of the larvæ of the Cynipidæ, we can state positively that the cell contents alone furnish the nourishment and that these are withdrawn from the cells without destroying the walls. An enzyme secreted by the salivary glands of the larva partially predigests this food. This ferment must act through the *cell membrane

*I have found that the froth on plants in which the "Spittle Insects" of the Family Cercopidæ develop, also contains an enzyme that rapidly changes starch to sugar. Experiments by Miss J. McFarlane that are not yet fully completed seem to indicate a larger amount of sugar in the stems surrounded by the froth than in corresponding parts of unaffected stems.

lining the interior of the larval chamber. None of the nourishment, taken into the alimentary canal, passes from it as excrement; it is either completely absorbed or remains in the digestive tract until the completion of the larval stage.

The invariable inert appearance and partially coiled condition of the larva would seem to indicate inactive feeding habits, but the theory of food absorption through the body wall is quite untenable; since the complete digestive tract, containing often large quantities of nourishment, as in Fig. 62, shows conclusively that the food enters the canal through the mouth.

## GALL-PRODUCING STIMULUS

All actively growing tissues are capable of responding to a gall-producing stimulus; the growth energy already present in them is controlled and compelled to expend itself in a definite direction. These abnormal tissues that result have the common characteristic of remaining longer in a plastic state than if they had been produced under normal conditions of growth.

The stimulating influence produces an effect on tissues at a considerable distance from the centre of application. Thus in the Acarina galls this influence extends to tissues other than the epidermis on which the mites are located, and in such a case as *Stagmatophora ceanothiella* Cosens the epidermis of the stem undergoes division, although the larva is feeding in the pith (Fig. 17).

The power to stimulate tissues to abnormal activity is not confined to gall-producing larvæ. Certain inquilines likewise exhibit this ability to a limited extent. By a fortunate chance I have been able to establish this fact in the case of an inquiline larva found in the gall of *Holcaspis globulus* Fitch. Reference to Fig. 65 will show that a nutritive layer has been developed around the inquiline. That it possesses the power of stimulation to a less extent than the producer is obvious from the fact that it was unable to originate a cambium of its own, and in consequence the nutritive zone is incomplete on the side opposite to the producer-larva. This is shown in Fig. 65. Yet it is equally obvious that, feeding as it was in proximity to the cambium of the producer, it was able to excite that zone to the production of typical nutritive cells instead of the parenchyma zone cells that would have resulted had the producer alone been in control. Küster[35] records a similar instance of inquiline-produced galls in *Rhodites eglanteriæ*.

Küster[35] states that the excrement of the larval *Pontania salicis* is capable of producing cell division. I have found this phenomenon occurring also in *Pontania pomum* Walsh and particularly good examples

in the undescribed sawfly gall on *Salix serissima* (Bailey) Fernald (Fig. 72). While I have made no attempt to determine by experiment the cause of this unusual example of cell proliferation, yet it would seem highly probable that the enzymes, introduced into the protoplasm by the ovipositor of the producer and swallowed by the larva, have not entirely lost their power by passing through the digestive tract but are still able to excite cell division.

The gall producer's influence works remarkable changes in the affected part of the host; even apparently new tissues, glands, trichomes, etc., make their appearance. The activity of its protoplasm is so much increased that hypertrophy or hyperplasia is an invariable accompaniment of gall production. The conventional view to account for these phenomena is that the protoplasm has been endowed with entirely new characteristics and power to produce something foreign to the normal host. But this is probably true only in a very limited sense, for according to my experience at least the prototypes of such apparently new tissues, etc., have been found elsewhere in the host or its relatives. Seemingly the correct explanation is that not only are dominant characteristics in the protoplasm stimulated but also in certain cases latent properties are called into activity, and thus apparently new structures appear in the host. Attention has already been drawn to examples confirming this opinion, but the evidence will now be more fully elaborated in the case of glands, trichomes and aeriferous tissue.

It may be stated as an unvarying rule, that when glands are present in the normal tissue they are always more plentiful or larger in the gall originating from that tissue. This is exemplified in the galls produced by *Eurosta solidaginis* Fitch (Fig. 42), *Aulacidea nabali* Brodie (Fig. 66), and numerous other species to which attention has been directed in the descriptive part of this paper.

But glands also occur in certain galls on parts of the host that are normally glandless; thus they are plentiful in the gall produced by *Neolasioptera perfoliata* Felt on *Eupatoria perfoliatum* L. (Fig. 23), but are not found at the same location in the normal. At first sight they appeared to be new structures, but were finally discovered in the normal host at the base of the stem. In *E. urticæfolium* Reichard they likewise occur in the transitional region between stem and root, while in *E. purpureum* L. they are present in the roots, petioles, and flowering axes as well as in the cortex and pith of the stem. In the case of gland production it is clear that not only have active characteristics of the protoplasm in that direction been stimulated to an activity greater than the normal maximum but nearly dormant properties have sometimes been aroused into action.

The trichomes worked out in a manner very similar to the glands. When the gall produced types different from the normal they were searched for successfully on the reproductive axes of the host. The unicellular, acicular hairs of *Eriophyes querci* Garman (Fig. 6) are totally unlike the stellate hairs of the leaf, but their exact counterparts are found on the reproductive axes of the host *Quercus macrocarpa* Michx. The much convoluted type of hair present in the Acarina dimple gall on the leaves of *Acer negundo* L. (Fig. 4) are found plentifully distributed over the reproductive axes, although the normal leaf hairs are straight.

The production of aeriferous tissue in certain Salicaceous galls substantiates in quite as striking a manner the view I have advanced. These galls contain examples of a typical aeriferous tissue, comparable indeed to that found in such aquatics as Nymphæa, Potamogeton or Saururus, while in the corresponding part of the host it does not occur. Indeed, this statement may be extended to include all the species of the host genus. A cross section of the gall originated on *S. cordata* Muhl. by *Cecidomyia triticoides* Walsh shows this tissue surrounding each larval cell. It is present throughout the cortex of the stem and extends entirely across the pith (Figs. 34, 35, 36). This tissue is found also in the gall originated on the leaf of the same willow by *Pontania pomum* Walsh (Fig. 77), but is not found in the normal tissues; indeed, the mesophyll of the leaf of *S. cordata* Muhl. is peculiarly compact in structure. It is figured by Cook[22] in the cortex of the stem gall produced on *S. discolor* Muhl. by *Cecidomyia rigidæ* O. S.

With the purpose of determining the distribution of this tissue in the normal stem a number of species of Salicaceæ were examined by Mr. T. A. Sinclair and myself with the following results, a detailed description of which will be published later. It was found in the primary cortex of the stems of the following species and invariably more plentiful at the nodes,—*Salix humilis* Marsh., *S. alba* L., *S. rostrata* Richards, *S. lucida* Muhl., *S. discolor* Muhl., *S. nigra* Marsh., *S. longifolia* Muhl., *S. serissima* (Bailey) Fernald, *S. cordata* Muhl., *Populus deltoides* Marsh., *P. balsamifera* L., P. *tremuloides* Michx. and *P. grandidentata* Michx. It was also differentiated to some extent in the pith of the stems of *P. balsamifera* L. and *P. deltoides* Marsh., *P. grandidentata* Michx. and *P. tremuloides* Michx. The only indication of this tissue found in the stem pith of Salix was in sections through the bases of branches of *S. cordata* Muhl. and *S. alba* L. Possibly it may be present in the corresponding region in other species. It can be traced a greater distance from the growing tip in the cortex of Populus than in Salix before it becomes unrecognizable owing to compression. It is apparently nearly always present in the pith and cortex of the reproductive axes of Populus and

Salix. The leaf petioles of the following species were found to contain it,—*Populus balsamifera* L., *P. deltoides* Marsh., *Salix humilis* Marsh., *S. alba* L. and *S. cordata* Muhl. The tissue is developed much more plentifully on the side adjacent to the stem.

In general then this tissue is indicated in the pith of the stem of Populus but is restricted in Salix to the bases of the branches. It is well represented in the primary cortex of the stems of both Populus and Salix, rather better so in the case of the former genus. It is abundant in such primitive regions as the reproductive axes, nodes and leaf traces. Thus the unexpected appearance of this tissue in the galls cited is readily explainable on the same grounds as in the case of glands and trichomes, namely, the power to produce this tissue is latent in the protoplasm of the host and it becomes sufficiently active to reinstate the tissue only when the gall-producing stimulus gives rise to unusual conditions.

Concerning the nature of this powerful stimulating agent there is at present a growing tendency to ascribe it to enzymatic action. It is difficult to say just how wide the application of this method of stimulus may be, but as plants present so many features in common in their reactions to produce the different types of galls, universal enzyme action would seem to be at least a safe working hypothesis. It is only, however, in the case of the Cynipidæ that we have any experimental evidence concerning enzymatic action. As described in a previous part of this section, I have been able to prove, in the case of the gall *Amphibolips confluens* Harris, that the larva secretes an enzyme capable of changing starch to sugar. It is now my purpose to discuss this fact in its relation to gall production.

Küster,[34] after experimenting with Cynipid galls in culture solutions, arrived at a conclusion that furnishes some experimental data on the subject. "Bei normaler Entwickelung wird der Inhalt der Nährgewebe von den Gallentieren verzehrt; unter abnormalen Verhältnissen kann aber das Nährmaterial von den Pflanzenzellen selbst verbraucht werden. Gallen von Pediaspis Aceris (Cynipide), die von ihren Bwohnern befreit und auf nährstoffarmen Lösungen oder auf gewöhnlichem Leitungswasser belassen werden, bleiben wochenlang am Leben; der Inhalt der Nährgewebe schwindet dabei. Werden Gallen gelicher Art ceteris paribus auf Zuckerlösung verbracht, so bleibt der Inhalt der Nährgewebe unverbraucht oder erfährt noch eine geringe Vermehrung."

These experiments prove that a gall is able to extract nourishment from the nutritive zone to assist in its growth in general. It appears axiomatic then that the greater the quantity of soluble food there is in the nutritive layer, in excess of what the larva requires, the larger is the supply the gall has at its command and the more marked will be the

proliferation of gall tissue. The larva consequently by accelerating the rate of change from starch to sugar is indirectly stimulating the protoplasm and thus controlling the growth of the gall. The general principle is applicable here that the available food supply governs very largely the size of an organ and consequently must influence the activity of its protoplasm. It is interesting to note in this connection that the size of the gall and the contained larva are directly proportional to each other. The relations between the two are reciprocal. The larger larva ensures a greater enzyme production and hence a more abundant food supply and presumably a larger excess for the stimulation to cell proliferation. The amount of enzyme action appears clearly to be proportional to the size of the larva. The evidence seems conclusive that the nutritive zone functions as an organ for preparing soluble food materials for both the larva and the gall. This evidence receives further confirmation from the fact that in addition to the empty cells lining the larval chamber there are others scattered throughout the nutritive zone often to its outermost layers (Fig. 59). This also seems to point to the conclusion that the contents of these cells have been used in supplying food for the proliferation of tissue in the other parts of the gall.

Summing briefly, the larva secretes an enzyme, capable of changing starch to sugar, which acts on the starchy constituents of the nutritive zone and accelerates the rate of their change to sugar. The material thus prepared supplies nourishment for both the larva and the gall. The protoplasm of the latter is thus rendered unusually active since it receives an abnormal quantity of available food material in a limited area. The hypertrophy and cell proliferation and probably also the appearance of vestigial tissue or other primary characters are the response of the protoplasm of the host to the additional food supply.

Attempts were made to substantiate this theory by further and more direct experiment. Diastase in solution was injected into seedling Windsor beans at different points with the purpose of stimulating the tissues to increased cell proliferation. When the place selected was just below the arch of the hypocotyl, a decidedly large callus was obtained in some of the experiments. These were not conclusive, however, owing to the variation in size of the normal plant in that region and the very great if not insurmountable difficulty of detecting increased callus formation when only differences in amount are to be expected. It is further very difficult to simulate the action of the producer-larva in bringing the diastase into contact with the proper tissue.

The discovery of an enzyme as an exudation from gall-producer larvæ recalls the statement of Laboulbène[36] that he had induced cell proliferation by injecting into plant tissue the water in which larvæ had been washed.

The theory, just stated, furnishes an explanation intended to account only for the stimulation of the protoplasm expressed in cell proliferation, hypertrophy and the production of unusual structures. There are other gall characteristics, however, that can scarcely owe their origin to the action of enzymes alone on the protoplasm of the host. For example, the colour of galls appears to be controlled partly at least by the intensity of the illumination. Thus the galls produced on *Salix cordata* Muhl. by *Pontania pomum* Walsh are little, if at all, coloured when the host is growing in deeply shaded stations. Besides this environmental effect, however, there is another factor that may also have an influence on the colour of this gall. De Vries[26] states that the red colour in plants is a dormant characteristic in the protoplasm that can be reinstated by stimulation. As a distinct confirmation of his view he found that red tints were produced in the leaves of *Viburnum opulus* L. as a consequence of bruising. This experiment seems to be closely paralleled in the sawfly gall *Pontania pomum* Walsh, where a red colour is apparent in the leaf of *Salix cordata* Muhl. in a very short time after oviposition. It seems very probable that in this case as in that of Viburnum the dormant red characteristic has been reinstated by the mere mechanical injury. It is noteworthy in this connection that shades of red are the predominating tints in gall structures, so that in the production of colour enzymatic action may frequently be operative in reinstating the dormant character red, especially in the case of galls in which the mechanical injury is negligible.

Further, the shape of the gall and the relation of the various zones to each other are not explainable by reference to any one factor. They doubtless result from a combination of factors. Just what all of these may be is yet not apparent but this much is certain that there appears to be an entire lack of evidence supporting the view that the protoplasm of the host has become endowed with a property that enables it to produce a fairly definitely shaped but withal abnormal structure. Such a pronounced change would surely be expressed in the hereditary characteristics, yet there is not a vestige of proof tending to show that insect galls ever produce the slightest variation in the descendants of the host. Not only so, but in the case of stems growing beyond the gall, there is no certainty that the prolongations are abnormal except for the slight dwarfing which is possibly explainable on the basis of an interrupted food supply. Examples of such stems are furnished by *Cecidomyia triticoides* Walsh on Salix, *Chermes abietis* Linn. on Picea, or *Eurosta solidaginis* Fitch on Solidago. Küster also found in his regeneration experiments that the roots produced from specimens of *Pontania salicis* were perfectly normal. There is still another argument to be cited in opposition to this view, in the fact that one gall may be parasitic on another. Thus when

*Biorhiza forticornis* Walsh is produced on *Neuroterus batatus* Fitch the stimuli from the different producers are exerted on nearly the same region of the host at the same time, as both these species are stem galls and commence to develop just as the buds are opening. In such a case as this if we assume that the protoplasm of the host has acquired characteristics necessary to the production of a certain form of gall, it seems unlikely that it could also possess the characteristics that would enable it to originate an entirely different type at the same time.

With the exclusion of the likelihood that the genetic characteristics of the protoplasm have been modified in any way, we must turn to the environmental factors to account for the shape of the gall and the relations of its various zones. Among these one feature that must have a certain amount of controlling effect is the direction in which the stimulus is applied. The various types of dimple and pouch galls, in which a curving of the affected organ is a very marked feature, are originated by stimuli disseminated in one direction only, while a Cynipid gall with its characteristic, spherical inner gall arises when the influence is about equally distributed in all directions. In some species it is also clear that the location of the egg has produced an effect on the external form of the gall. If the egg is deposited on the epidermis of the host and the tissues grow up around it, a gall of the type produced by *Cecidomyia ocellaris* O.S. results (Fig. 33). Even in the Cynipidæ this factor has been in operation. In species of Andricus the openings of the canals give a characteristic appearance to the galls, and in *A. petiolicola* Bassett the gall is drawn out to a decided tip in the region of the canal. These canals owe their origin to the fact that the galls are of the "Umwallung" type, and the larvæ have been enclosed by the growth of the surrounding tissues.

In some galls such as *Dryophanta palustris* O.S. a cambium is differentiated at a very early developmental stage, and has a very marked influence on the general relation of the zones in the gall. This cambium layer is shown in Fig. 49. The cells produced from the inside of this cambial tissue constitute the nutritive and sclerenchyma zones, while those given off from the outside form the parenchyma zone and epidermis. The former that are under the immediate control of the larva and less exposed to external conditions come to differ more markedly from the normal than do the latter that are nearer the outside limit of the larva's sphere of influence.

*Summary.*

The idea that the gall-producing stimulus must of necessity be applied directly to the cambium layer is not true in all cases, as any actively growing tissue will respond to a producer's influence.

The effect of this stimulus is operative on tissue at a considerable distance from the centre of application.

Certain inquilines in Cynipid galls possess the gall-producing power but to a less extent than the real producer.

Cynipid producers and probably others secrete an amylalytic ferment that pre-digests food for the larva and may indirectly stimulate cell proliferation by storing the nutritive zone with an unusually large quantity of available nourishment which can diffuse to all parts of the gall.

The gall-producing stimulus renders the protoplasm of the host more active and awakens in it dormant characteristics, but apparently does not endow it with power to produce entirely new structures. This has been demonstrated in the case of glands, trichomes and aeriferous tissue.

The red colour of galls is perhaps a dormant characteristic that may be reinstated by enzymatic action but there are other possible inducing factors such as the light relations and in sawfly galls mechanical injury by the act of oviposition.

The shape of galls is controlled partly at least by the direction of the stimulus and the location of the egg of the producer. In galls such as the Lepidopterous types, where the larva burrows into the tissues after leaving the egg, this feature has no effect.

The relation of the various zones in the Cynipid galls is influenced in some cases by the early differentiation of a cambium layer.

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## EXPLANATION OF PLATES.

### PLATE I.

Fig. 1. *Eriophyes Sp.* (*Populus tremuloides* Michx.). Section showing the folding of the upper epidermis of the leaf. ×50.

Fig. 2. *Eriophyes Sp.* (*Populus grandidentata* Michx.). Section showing the nature of the folding produced on the lower surface of the leaf. ×60.

Fig. 3. *Eriophyes Sp.* (*Fagus grandifolia* Ehrh.). Section through a number of capitate trichomes. The almost normal character of the leaf is shown. ×50.

Fig. 4. *Eriophyes Sp.* (*Acer negundo* L.). Section through the gall, showing a large number of convoluted trichomes. ×35.

Fig. 5. *Eriophyes Sp.* (*Prunus nigra* Ait.). Longitudinal section in which is shown the elongation of the cells in the direction of the long axis of the gall. The peculiar nature of the trichomes is also shown. ×30.

Fig. 6. *Eriophyes querci* Garman (*Quercus macrocarpa* Michx.). Section in which the long acicular trichomes are shown, as also the thickening of the leaf blade. ×30.

Fig. 7. Unclassified gall on the leaf of *Populus balsamifera* L. The uniform nature of the abnormal cells is apparent. The gall cavity is occupied by the mycelium of a fungus. ×10.

Fig. 8. Transverse section of band of sclerenchyma from the preceding gall, showing the pores that traverse the tissue. ×120.

### PLATE II.

Fig. 9. Aphid corrugations on the leaf of *Betula lenta* L. produced by the fourth generation of *Hamamelistes spinosus* Shimer. The formation of the larval chambers by the closing of the folds is shown. ×22.

Fig. 10. *Hamamelistes spinosus* Shimer on the leaf of *Hamamelis virginiana* L. Transverse section that passes through a spine, cross sections of two bundles are shown. ×35.

Fig. 11. *Chermes abietis* Chol. on the stem of *Picea abies* (L) Karst. Longitudinal section, showing two larval chambers with their apertures of exit. A number of resin ducts are cut near the margin of the section. ×30.

Fig. 12. *Hormaphis hamamelidis* Fitch on the leaf of *Hamamelis virginiana* L. Longitudinal section passing through the aperture of exit. ×10.

Fig. 13. *Chermes floccus* Patch on the stem of *Picea mariana* (Mill.) B.S.P. Transverse section, showing the smaller accessory resin ducts. ×25.

Fig. 14. *Pemphigus rhois* Walsh on the leaf of *Rhus typhina* L. Longitudinal section of a young gall, showing a number of glands. ×15.

PLATE III.

Fig. 15. *Pachypsylla celtidis-mamma* Riley on the leaf of *Celtis occidentalis* L. Section through the larval cavity, showing the cambium tissue and the sclerenchyma sheath bordering it on the outside. ×15.

Fig. 16. *Memythrus tricinctus* Harris on the stem of *Populus tremuloides* Michx. Cross section through the thickened annual rings, showing the clumps of bast fibres. ×20.

Fig. 17. *Stagmatophora ceanothiella* Cosens on the stem of *Ceanothus americanus* L. Cross section, showing secondary growth in the wood and the abnormal glands in the cortex. ×50.

Fig. 18. Normal stem of the host of the preceding species. Section taken near the gall; glands are not present in the cortex. ×50.

Fig. 19. Glands found in the cortex of *S. ceanothiella* Cosens; these correspond to those shown in Fig. 17. ×150.

Fig. 20. *Gnorimoschema gallæsolidaginis* Riley on the stem of *Solidago canadensis* L. Transverse section showing an abnormally large gland in the cortex. ×45.

PLATE IV.

Fig. 21. *Eucosma scudderiana* Clemens on the stem of *Solidago canadensis* L. Transverse section showing the proliferation in the bundles and the medullary rays and also the enlarged glands in the cortex. ×75.

Fig. 22. *Gnorimoschema gallæasterella* Kellicott on the stem of *Solidago latifolia* L. A transverse section through one side of the aperture of exit, the material used by the larva in smoothing the sides of the hole for the reception of the plug is shown. The cross checking in this material can also be seen. ×120.

Fig. 23. *Neolasioptera perfoliata* Felt on the stem of *Eupatorium perfoliatum* L. Transverse section showing an unusual type of cell division in the cortex and epidermis of this gall. The glands shown near the inner boundary of the cortex are not found in the normal stem at the same height. ×55.

Fig. 24. *Rhabdophaga strobiloides* Walsh on the stem of *Salix cordata* Muhl. Longitudinal section showing the larva in contact with the small celled tissue at the apex of the stem. ×25.

Fig. 25. *Rhabdophaga batatas* Walsh on the stem of *Salix humilis* Marsh. A transverse section that shows the larval chamber surrounded by a nutritive zone which is bounded on the outside by a well defined protective sheath. ×22.

Fig. 26. A part of the protective sheath of the preceding species enlarged to show the unequal thickening of the tangential walls. ×200.

Fig. 27. *Cecidomyia majalis* O.S. on the leaf of *Quercus coccinea* Muench. Section at right angles to the midrib. The folding of the leaf is shown and the uniform character of the mesophyll of the gall. The epidermis lining the gall cavity is shown intact. ×15

## PLATE V.

Fig. 28. *Abies balsamea* (L.) Mill. Transverse section of normal leaf. ×35.

Fig. 29. *Cecidomyia balsamicola* Lintner on the leaf of *A. balsamea* (L.) Mill. Transverse section showing the folding of the leaf and the elongation of the mesophyll cells. The irregularity of the cells in the strengthening layer of the resin ducts can also be seen. ×35.

Fig. 30. *C. balsamicola* Lintner on the leaf of *A. balsamea* (L.) Mill. Transverse section through the midrib. The chief points shown are the irregularity in the development of the endodermis, the large amount of the transfusion tissue and the relatively small amount of the non-pitted parenchyma. ×100.

Fig. 31. *Abies balsamea* (L.) Mill. A section, through the midrib of a normal leaf, corresponding to the preceding section (Fig. 30). ×100.

Fig. 32. *Cecidomyia impatientis* O.S. on *Impatiens biflora* Walt. Section through a larval chamber, showing the general nature of the cells of the gall and the smaller cells of the nutritive layer. The two dark masses attached to the nutritive tissue in the lower part of the gall cavity consist of the mycelium of a fungus. ×30.

Fig. 33. *Cecidomyia ocellaris* O.S. on the leaf of *Acer rubrum* L., showing the almost unchanged character of the leaf immediately below the larva and the great amount of proliferation in the region surrounding it. The general arrangement of the cells at right angles to the leaf blade is also shown. ×30.

Fig. 34. *Cecidomyia triticoides* Walsh on the stem of *Salix cordata* Muhl. Transverse section in which is shown the general arrangement of the larval chambers and the distribution of aeriferous tissue throughout the cortex and pith of the gall. ×10.

## PLATE VI.

Fig. 35. *Cecidomyia triticoides* Walsh on the stem of *Salix cordata* Muhl. Transverse section showing the character of the aeriferous tissue. ×50.

Fig. 36. *Cecidomyia triticoides* Walsh on the stem of *S. cordata* Muhl. Transverse section of a young gall, showing the well defined nutritive layer lining the larval cavity and the protective zone bounding this tissue on the outside. The aeriferous tissue is also shown. ×60.

Fig. 37. *Cecidomyia triticoides* Walsh on the stem of *S. cordata* Muhl. Transverse section through the nutritive and protective zones of a mature gall. At the top of the figure is a dark band of collapsed nutritive cells; below that a lighter coloured and wider band of sclerenchymatous cells, the lumen of each filled with a crystal of calcium oxalate; and below that again a layer of cambium of nearly the same width as the preceding zone. ×150.

Fig. 38. *Cecidomyia triticoides* Walsh on the stem of *S. cordata* Muhl. Transverse section of a young gall, corresponding to the preceding mature form. The nutritive zone is at the top of the figure, its cells are filled with rich protoplasmic contents, with the exception of those in the upper row and a few scattered ones throughout the zone. The protective zone is shown below this tissue, but the cambium layer is not differentiated in the early stages. ×150.

Fig. 39. *Cecidomyia bulla* Walsh on the stem of *Helianthus divaricatus* L. Transverse section through stem of host and attached gall, showing the elongation of the fibro-vascular bundles in the direction of the gall axis and the very marked proliferation in the medullary rays. At the upper part of the figure, in the gall cortex, an enlarged gland is partly shown and also other glands at the junction of the gall and the stem of the host. ×18.

Fig. 40. *Lasioptera corni* Felt. on the leaf of *Cornus alternifolia* L. The section shows the lower epidermis and one row of mesophyll cells in normal position and also the strongly curved character of the upper epidermis and the remaining mesophyll cells. The normal appearance of all the cells is also apparent. ×18.

Fig. 41. *Lasioptera impatientifolia* Felt. on the leaf of *Impatiens biflora* Walt. Section at right angles to the midrib, showing the generally uniform character of the cells. Cells containing the mycelium of a fungus are shown a short distance in from the

gall cavity and above it. These cells give a false appearance of a protective zone. ×20.

Fig. 42. *Eurosta solidaginis* Fitch on the stem of *Solidago canadensis* L. Transverse section, showing the proliferation of glandular tissue and the general arrangement of the glands along the lines of the fibro-vascular bundles. ×25.

## PLATE VII.

Fig. 43. *Andricus piger* Bassett on the leaf of *Quercus coccinea* Muench. At the lower part of the figure a thick nutritive layer is shown, bordering the larval chamber; outside of this tissue is the protective zone from which a cone-shaped projection originates that blocks the canal leading in from the outside. The epidermis lining this canal is shown continuous with the general epidermis of the gall. ×25.

Fig. 44. *Andricus piger* Bassett on the leaf of *Q. coccinea* Muench. Section showing a nearly complete larval chamber. The other parts correspond to those in the preceding figure. The epidermis lining the canal is not shown so well in this case, as the section passes along the edge of the canal. ×25.

Fig. 45. *Andricus petiolicola* Bassett on the leaf of *Q. alba* L. Section passing through the main canal. The epidermis of the gall is shown passing into the trichome bearing lining of the gall. ×25.

Fig. 46. *Andricus petiolicola* Bassett on the leaf of *Q. alba* L. Section passing through the termination of the main canal, showing a number of trichomes and two larval chambers blocked by masses of sclerenchyma. ×45.

Fig. 47. *Andricus* (undescribed) on the leaf of *Quercus macrocarpa* Michx. Section passing through the edge of a canal and showing the two masses of sclerenchyma almost united. ×35.

Fig. 48. *Andricus imbricariæ* Ashmead on the stem of *Q. coccinea* Muench. Section showing the numerous bands of cells radiating out from the boundary of the protective sheath, the light coloured layer in the figure. The darker coloured nutritive zone bounds the protective layer and lines the gall cavity. ×20.

## PLATE VIII.

Fig. 49. *Dryophanta palustris* O.S. on the leaf of *Quercus coccinea* Muench. Section of a very early stage in which the inner and outer galls are still in contact. The following points are shown, a canal passing from the outside into the larval chamber, the

trichome bearing epidermis of the gall continuous with the lining of this canal and passing into the inner row of cells of the nutritive zone, the bay-like depression in this zone where the canal enters. ×30.

Fig. 50. *Dryophanta palustris* O.S. on the leaf of *Q. coccinea* Muench. Section of a somewhat more mature stage than the preceding, showing the commencement of the separation of the inner gall from the outer in the region of the cambium layer. ×12.

Fig. 51. *Dryophanta palustris* O.S. on the leaf of *Q. coccinea* Muench. Section of the wall of the inner gall, showing the nutritive zone with a line of collapsed cells next the larval chamber and a row of empty cells just inside the collapsed tissue. The protective layer borders the nutritive on the outside and a few round cells of the parenchyma adhere to the protective zone. ×70.

Fig. 52. *Dryophanta palustris* O.S. on the leaf of *Q. coccinea* Muench. Section of the larval chamber of a mature specimen, showing the insect breaking out of the inner gall. At this stage the nutritive layer has entirely collapsed. ×15.

Fig. 53. *Cynips ? constricta* Stebbins on the leaf of *Q. coccinea* Muench. Longitudinal section showing the origin of the gall from the midrib of the host in the region of the cambium layer. ×100.

Fig. 54. *Cynips ? constricta* Stebbins on the leaf of *Quercus coccinea* Muench. Longitudinal section of an early developmental stage showing the general structure of the gall. The dark mass at the top of the figure represents the supplemental nutritive zone of the gall; it is separated from the spherical part of the gall by a cambium tissue. The protective sheath that separates the nutritive from the cambium in later stages is not yet differentiated. A nutritive zone is also shown lining the larval chamber. ×40.

## PLATE IX.

Fig. 55. *Holcaspis bassetti* Gillette on the stem of *Quercus macrocarpa* Michx. Section through the gall cavity with enclosed larva. The character of the cells of the nutritive zone is shown and the unbroken edge of its inside boundary. The finely divided material of the stomach contents of the larva is also shown. ×60.

Fig. 56. Section of a larval inquiline from the gall *Holcaspis bassetti* Gillette. The broken edge of the tissue on which the larva has been feeding is shown, also the comparatively coarse material of the stomach contents. ×60.

Fig. 57. Contents of the stomach of the preceding inquiline. The black masses are parts of cell walls, while the lighter roundish particles are crystals. ×300.

Fig. 58. *Holcaspis bassetti* Gillette on the stem of *Q. macrocarpa* Michx. Section through the nutritive zone of a nearly full grown specimen. The nutritive zone is shown to consist of elongated cells next the larval chamber and elliptical further out. The dark zone is a crystal layer that bounds the nutritive zone on the outside. A cambium is differentiated between the nutritive and the parenchyma layers but it is not well shown in the figure. ×60.

Fig. 59. *Andricus singularis* Bassett on the leaf of *Quercus rubra L.* Section through the nutritive and protective zones, showing empty cells throughout the nutritive layer and the wrinkling of the radial walls of its cells in general. ×100.

Fig. 60. *Aylax glechomæ* Linné on the leaf of *Nepeta hederacea* (L.) Trevisan. Section through the nutritive and protective zones, showing the unbroken lining of the gall cavity and the row of empty cells that borders the larval chambers. ×80.

## PLATE X.

Fig. 61. *Philonix nigra* Gillette. Longitudinal section of the larva, showing the external opening of the alimentary canal. ×15.

Fig. 62. *Amphibolips confluens* Harris. Longitudinal section of the larva, showing the completeness of the digestive tract. ×15.

Fig. 63. *Rhodites lenticularis* Bassett on the leaf of *Rosa blanda* Ait. Section through the larval chamber, showing the nutritive layer lining it. Bordering this tissue on the outside is the cambium zone from which practically the entire gall is originated. The dark band shown plainly at the right of the figure is the protective sheath. ×20.

Fig. 64. *Philonix erinacei* Beut. on the leaf of *Q. alba* L. Section in which the four typical zones of a cynipid gall are shown, namely nutritive, protective, parenchyma or tannin, and epidermal. The sclerification can be seen to have passed out into the parenchyma zone. ×25.

Fig. 65. *Holcaspis globulus* Fitch on the stem of *Q. alba* L. Section through adjoining larval chambers of a producer and an inquiline, a complete section of the latter is shown. The nutritive tissue that supplies the inquiline with nourishment can

be seen to have originated entirely from the cambium differentiated by the producer of the gall. Its irregularity on the side opposite to the producer is very marked. ×30.

Fig. 66. *Aulacidea nabali* Brodie on the stem of *Prenanthes alba* L. Section through the stem of the host and the attached gall. The relation of the cambium of the host to the cells of the gall tissue is shown. ×30.

## PLATE XI.

Fig. 67. *Neuroterus majalis* Bassett on the leaf of *Quercus alba* L. Section through a larval chamber containing the pupa of the producer. The dark band around the inside of the gall cavity consists of the collapsed nutritive zone and the protective layer. At the upper right of the figure the general nature of the cells of the parenchyma zone is shown. ×50.

Fig. 68. *Euura S. gemma* Walsh on *Salix humilis* Marsh. Section through a mature gall, showing a larval chamber containing a pupal producer and several large masses of excrement. The general nature of the small celled tissue of the gall is also shown. ×30.

Fig. 69. *Euura* (N.S.) on the leaf of *Salix serissima* Fernald. Transverse section, showing one uninjured bundle of the petiole and the parts of two others widely separated by the proliferation of the tissue stimulated by the laceration of the bundles. ×35.

Fig. 70. *Euura* (N.S.) on the leaf of *Salix serissima* Fernald. Section of a younger stage than the preceding, showing as before one uninjured and two injured bundles. ×35.

Fig. 71. *Euura ovum* Walsh on the stem of *Salix humilis* Marsh. Transverse section through the stem of the host and the gall originated from it. It shows the wedge-shaped cavity occupied by the gall mass and the origin of the latter from a cambium tissue at the boundary of the pith of the host. ×18.

Fig. 72. *Euura* (N.S.) on the leaf of *Salix serissima* Fernald. Section of the gall showing proliferation induced by the excrement of a larval producer. ×18.

## PLATE XII.

Fig. 73. *Pontania hyalina* Norton on the leaf of *Salix alba* L. Section of gall with larva still within the egg membrane. Proliferation is shown well advanced in all the tissues of the leaf. ×35.

Fig. 74. *Pontania hyalina* Norton on *Salix alba* L. Section showing the wound of the ovipositor. ×25.

Fig. 75. *Pontania hyalina* Norton on the leaf of *Salix alba* L. Section of a more mature stage than numbers 73 and 74. The amount of gall tissue derived from the various sources can no longer be distinguished. The uniformity of the tissue is very marked. ×25.

Fig. 76. *Pontania pomum* Walsh of the leaf of *Salix cordata* Muhl. Section through an early developmental stage in which the larva had not freed itself from the egg membrane. There is shown the laceration of the bundle of the midrib by the ovipositor and the proliferation in the various tissues of the leaf. ×20.

Fig. 77. *Pontania pomum* Walsh on *Salix cordata* Muhl. Section of a nearly full-grown gall, showing the distribution of the aeriferous tissue and the location of the vascular strands. ×18.

Fig. 78. *Pontania* (N.S.) on the leaf of *Salix humilis Marsh.* Section at right angles to the midrib of the leaf to which the gall is attached. The general character of the gall tissue is shown and the distribution of the vascular strands from the wounded bundle. ×20.

Fig. 79. *Pontania* (N.S.) on the leaf of *Salix humilis* Marsh. Section through the midrib from which two galls had originated. The bundle is shown completely severed. ×8.

## PLATE XIII.

Fig. 80. *Pontania desmodioides* Walsh on the leaf of *Salix humilis* Marsh. Section through a chamber containing an unhatched larva. Proliferation is well marked in all the tissues of the leaf. ×40.

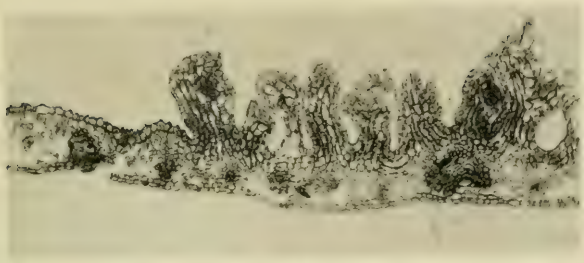
Fig. 81. *Pontania pisum* Walsh on the leaf of *Salix discolor* Muhl. Section of a mature gall, showing the general character of the tissues. The dark band at the upper right of the figure marks the line of attachment of the gall to the blade of the leaf. Just beneath this line the proliferation in the palisade parenchyma is shown. .×20.

Fig. 82. Undescribed gall on the leaf of *Salix lucida* Muhl. Section of a young gall on the midrib, showing the commencement of the proliferation in the pith of the bundle. ×20.

Fig. 83. Normal leaf of *S. lucida* Muhl. Section through midrib for comparison with the preceding. ×25.

Fig. 84. Undescribed gall on the leaf of *Salix lucida* Muhl. Section of a well-grown gall on the leaf petiole. The two arms of the bundle are shown widely separated by proliferation set up in the pith. The arrangement of the cells of the gall tissue in curved rows is shown and the presence of elongated air spaces between these. ×15.

Fig. 85. Undescribed gall on the leaf petiole of *Salix humilis* Marsh. Section showing the ovipositor wound. Around the edge of the incision is shown the cambium tissue from which the greater part of the gall tissue has been produced. ×18.



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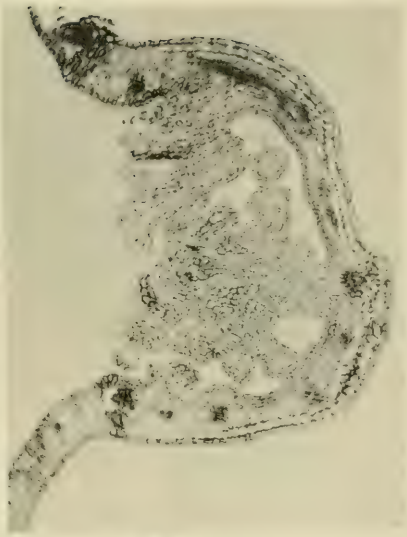

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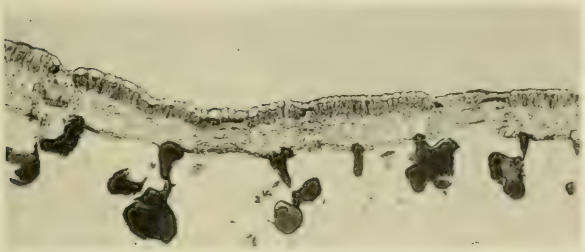

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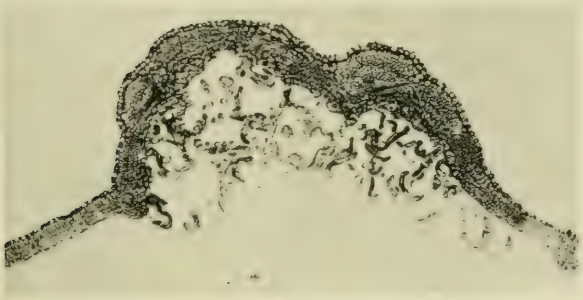

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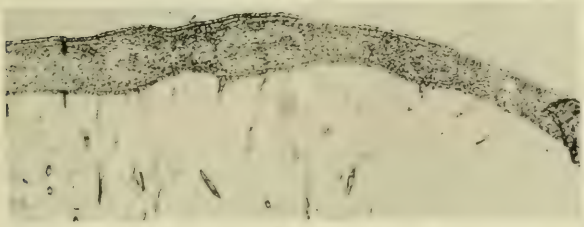

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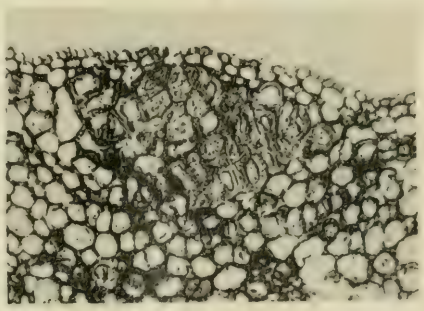

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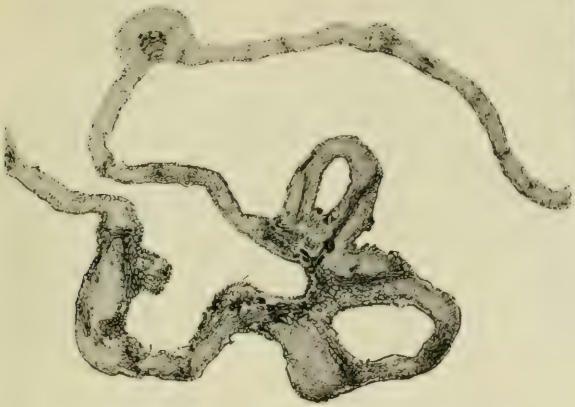

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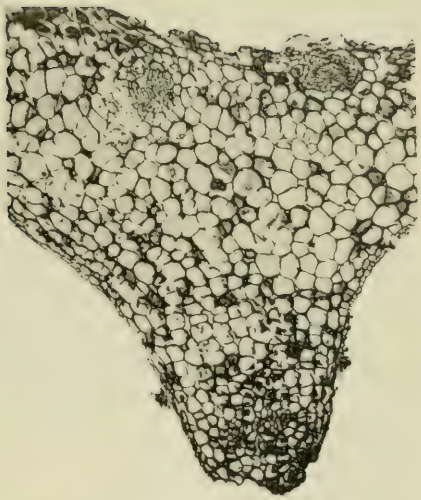

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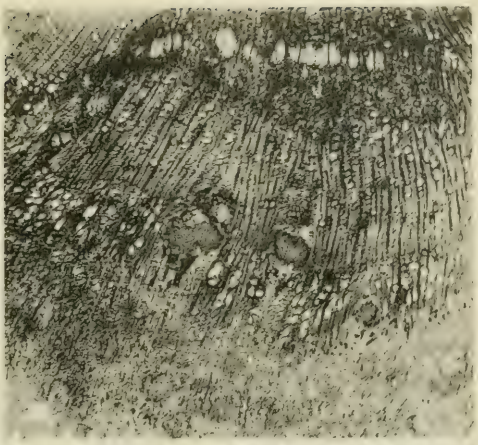

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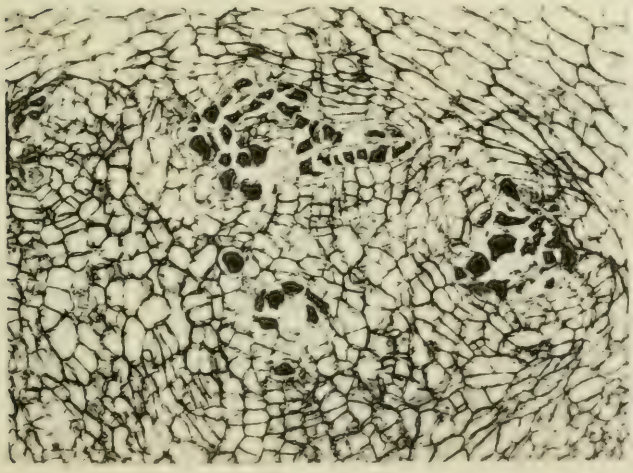

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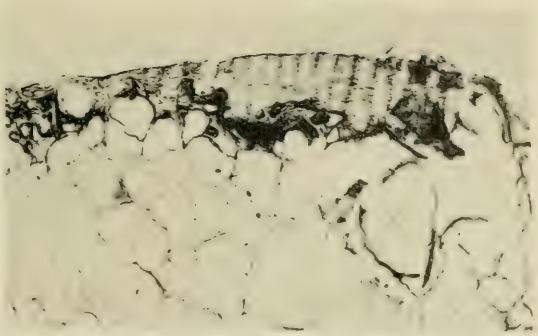

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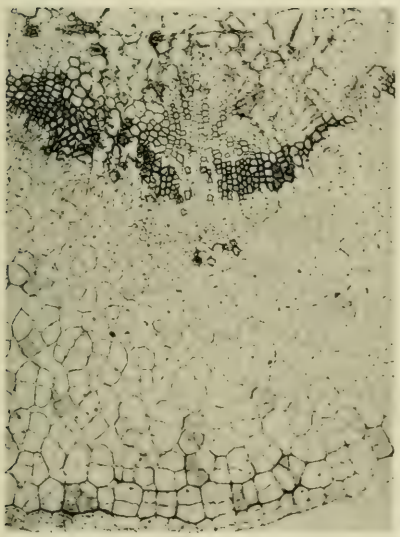

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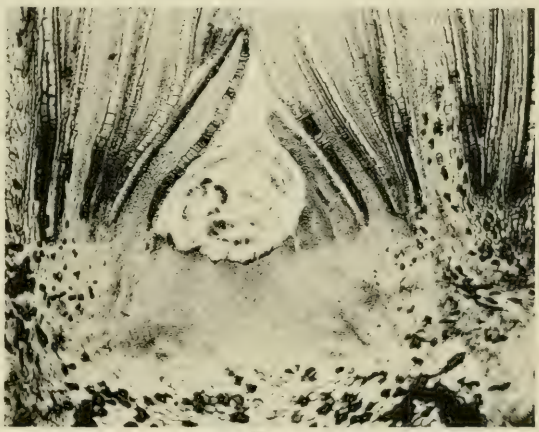

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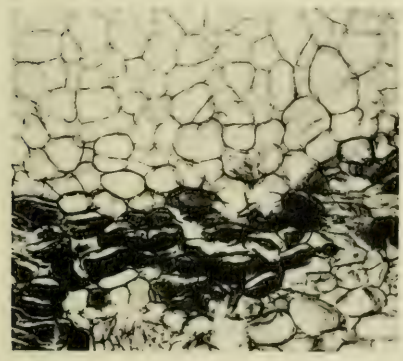

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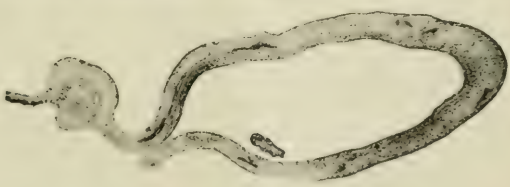

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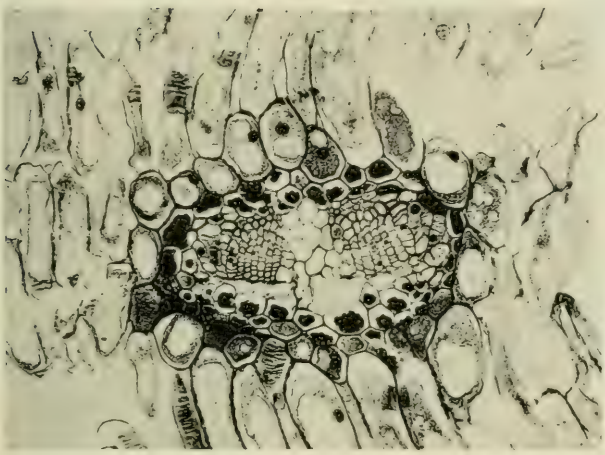

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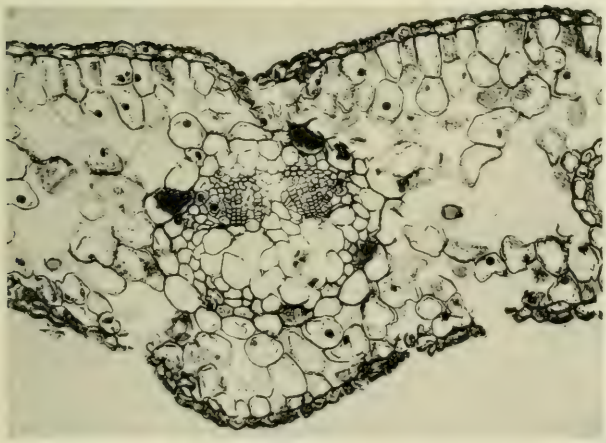

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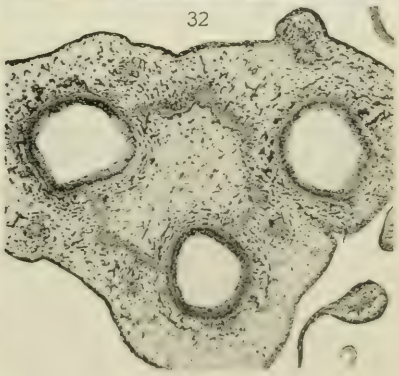

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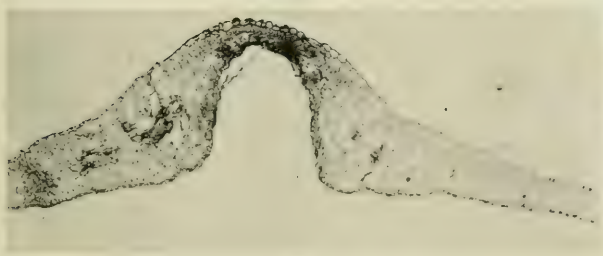

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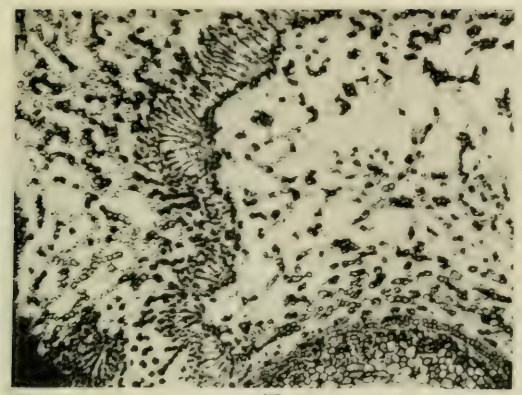

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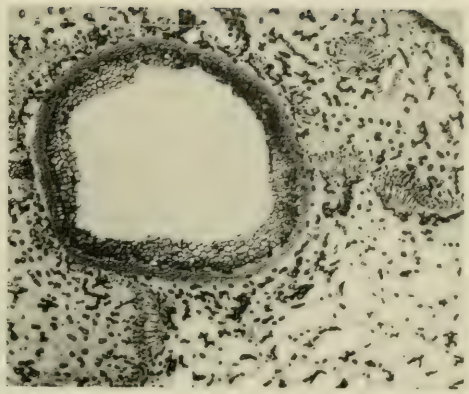

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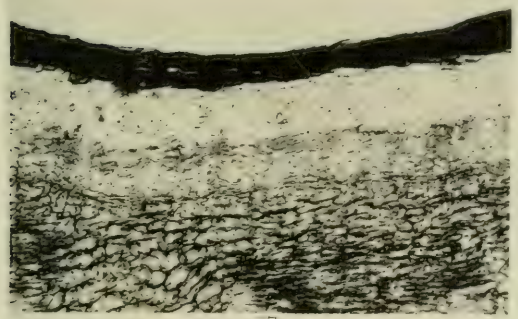

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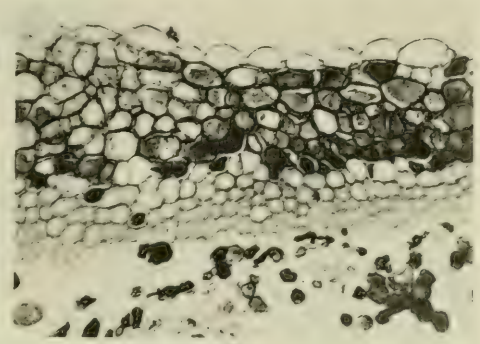

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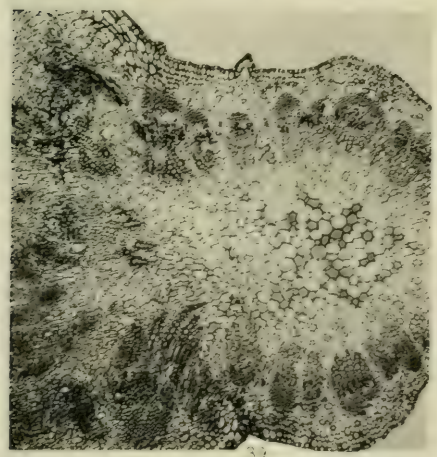

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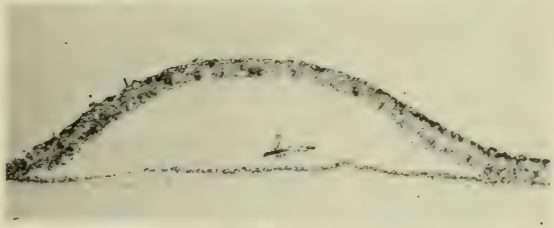

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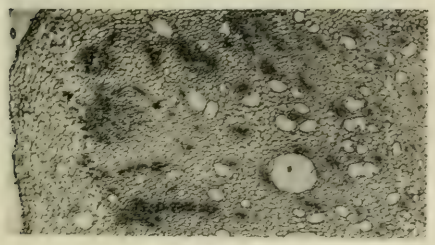

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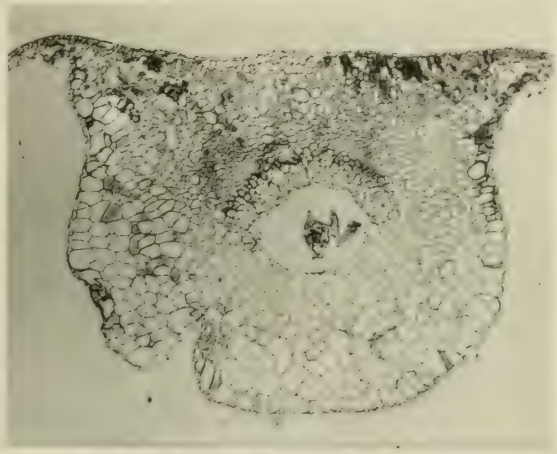

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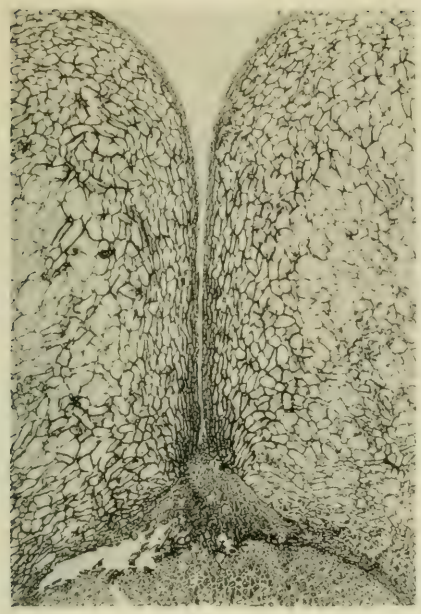

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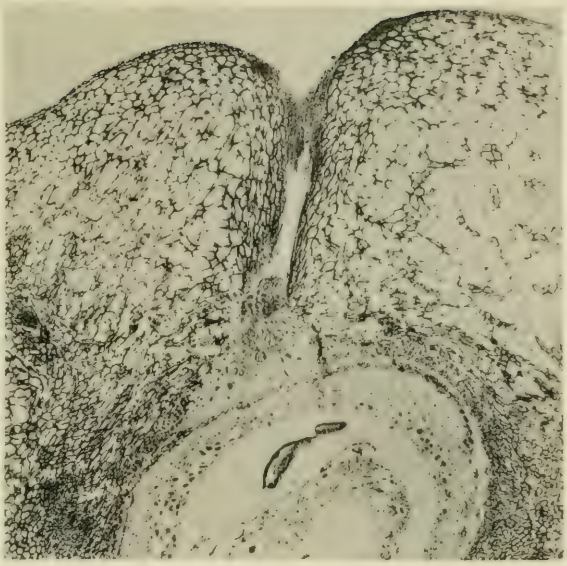

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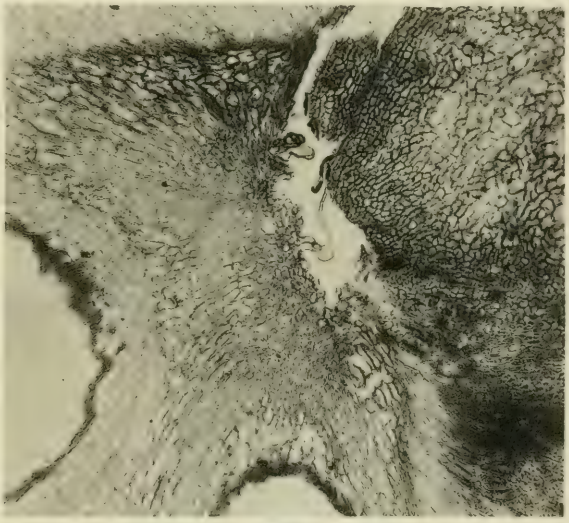

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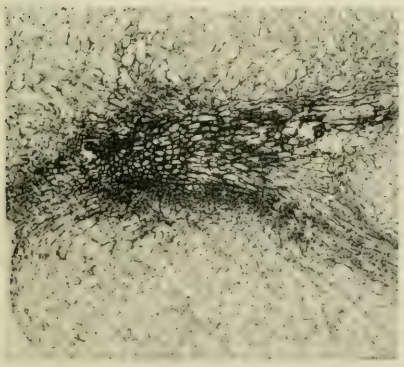

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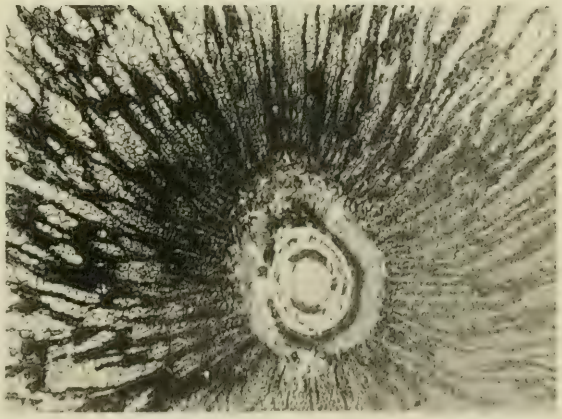

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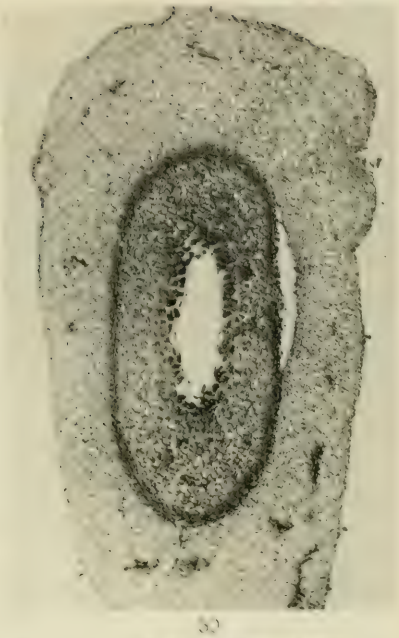

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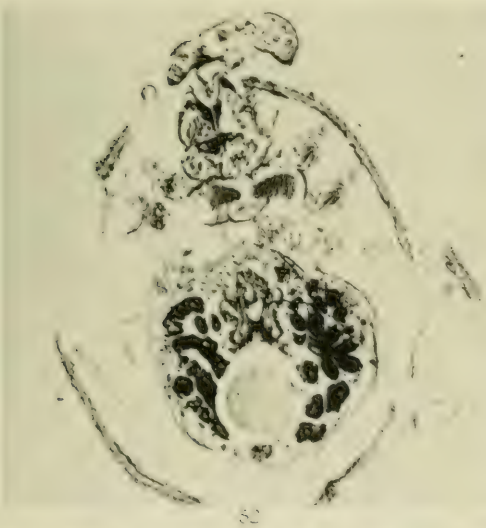

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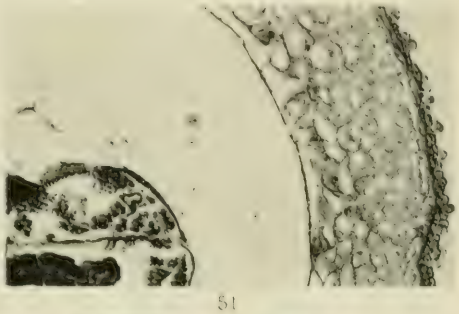

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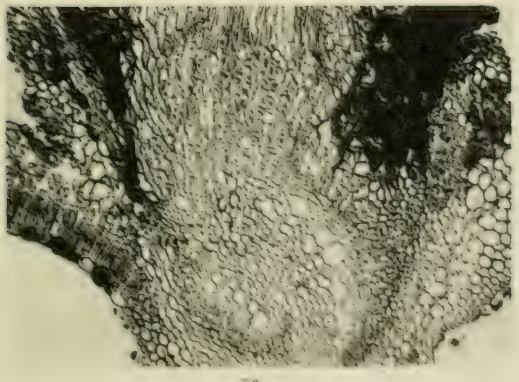

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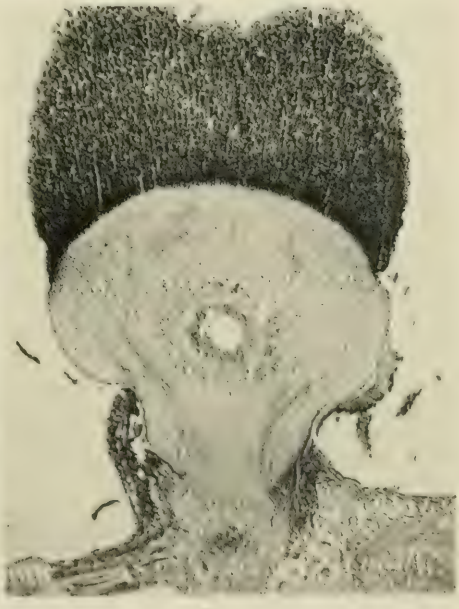

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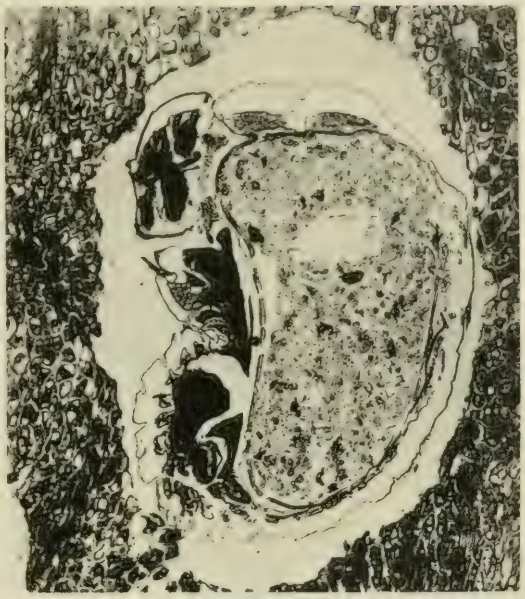

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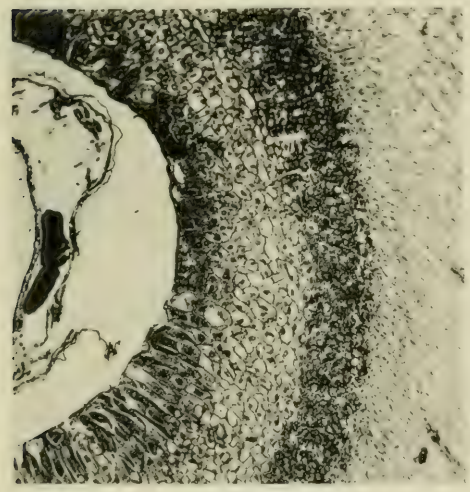

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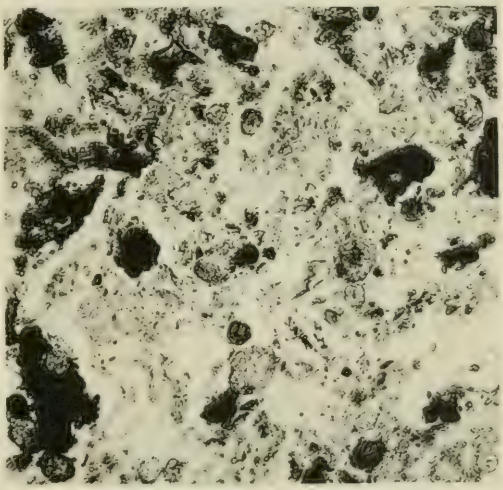

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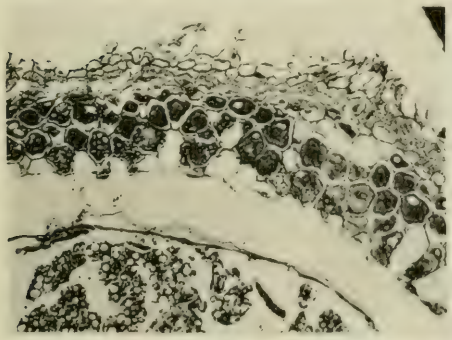

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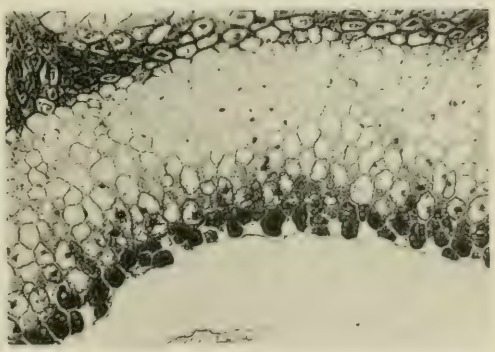

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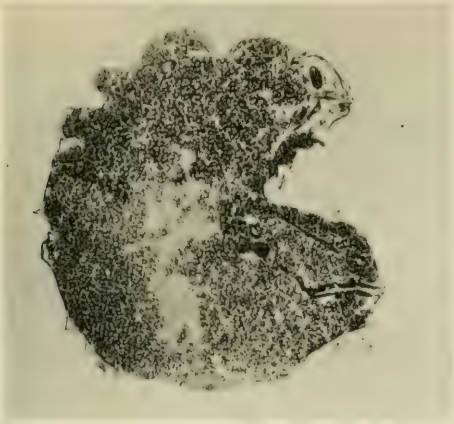

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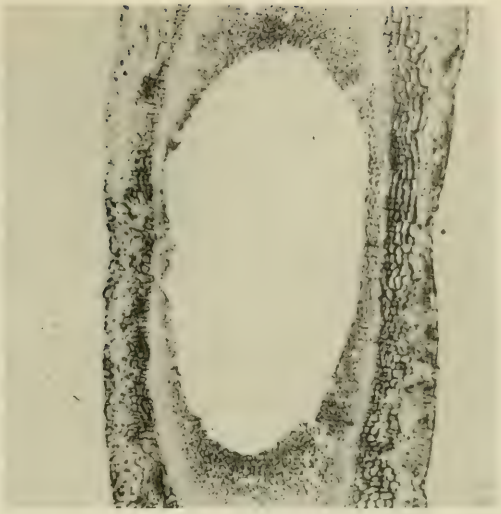

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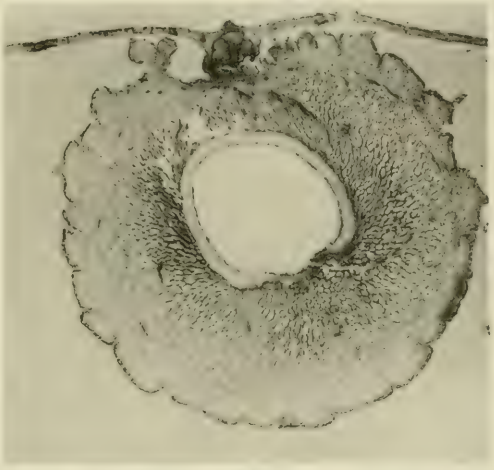

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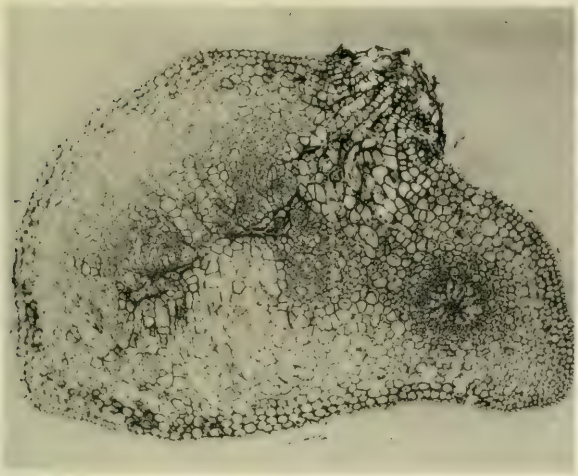

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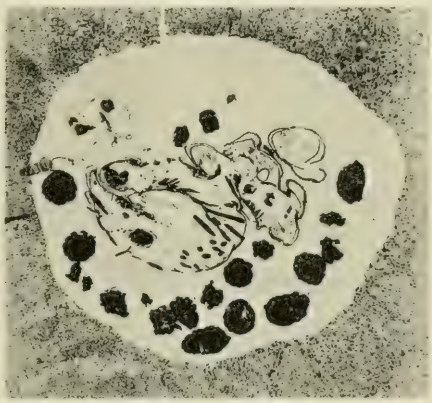

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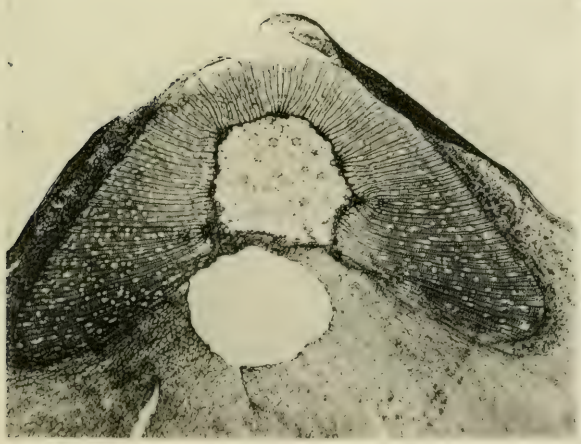

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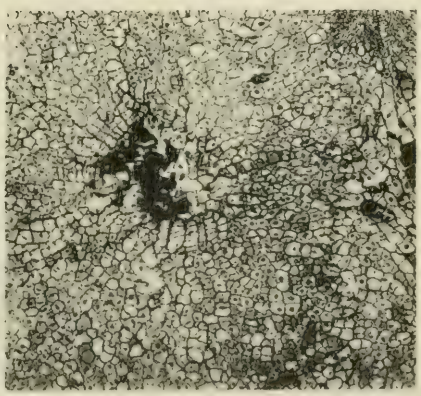

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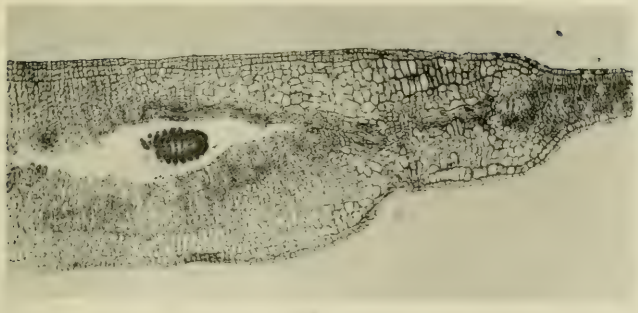

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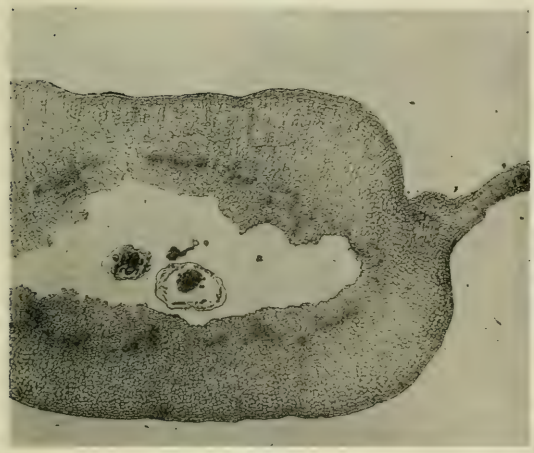

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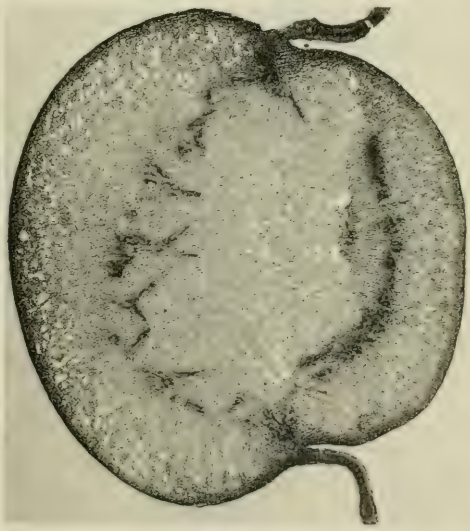

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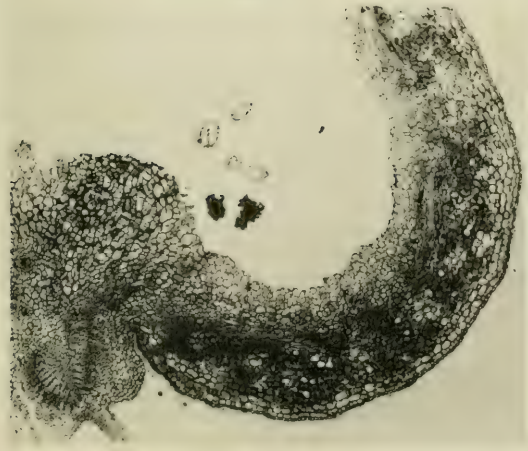

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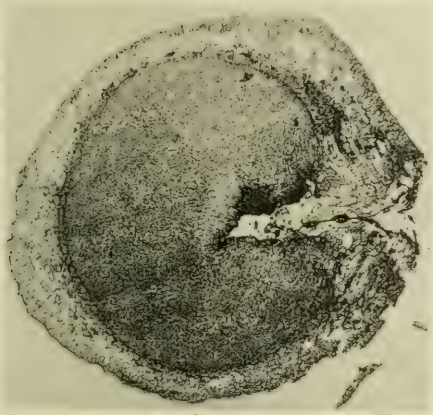

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UNIVERSITY OF TORONTO STUDIES

BIOLOGICAL SERIES

No. 14: EGG MATURATION, CHROMOSOMES, AND SPERMATOGENESIS IN CYCLOPS, BY ROBERT CHAMBERS

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# EGG MATURATION, CHROMOSOMES AND SPERMATOGENESIS IN CYCLOPS.

BY

ROBERT CHAMBERS, M.A., PH.D.

BIOLOGICAL SERIES No. 14.

ERRATA.

Page 16, line 16, for "Fig. 13" read "Figs. 13 and 20".

Page 28, third line from last, for "chromosome" read "chromatin".

Page 30, sixth line from last, for "Jen. Zeitschr., 37" read "Jen. Zeitschr., N. F., 30".

# EGG MATURATION, CHROMOSOMES AND SPERMATOGENESIS IN CYCLOPS.*

## I.—Introduction.

Most species of Cyclops show some degree of periodicity in their breeding habits. *Cyclops viridis* and its close relatives, *C. parcus*, *brevispinosus*, and *americanus*, with which this paper has most to do, may be found in sexual activity all the year round. They are, however, especially active during the spring months.

During copulation, the male, which is about one-third to one-half the size of the female, attaches a pair of spermatophores to the median ventral aperture of the seminal receptacle which lies in the first abdominal segment of the female (cf. Wolf '05). The peculiar ejaculatory bodies in the spermatophores then swell and drive the spermatozoa into the seminal receptacle.

Oogonial mitoses occur periodically so that there is always a number of cells in the same stage being gradually carried onward through the ovary. When the oocytes pass into the paired oviducts they grow very rapidly and, by distending the several branches of the oviducts, cause them to occupy the greater part of the interior of the cephalothorax. A gelatinous material fills the distal ends of the two oviducts, and as the eggs pass out this is pushed out ahead to form a large distensible sac in which the eggs come to lie (cf. Gruber '78). The eggs are fertilized as they roll in rapid succession out of the oviducts. Development is, therefore, comparatively uniform for all the eggs in both egg sacs.

A female may lay six to seven batches of eggs, all of which may be fertilized by the spermatozoa derived from one male. These batches, consisting of thirty to fifty eggs, are

* Recommended for publication in the University of Toronto Studies by Professor R. Ramsay Wright, Professor of Biology, University of Toronto.
Paper received May, 1912.

generally deposited once every two to four days. The eggs are laid usually in the early hours of the morning (cf. Haecker '97). Ova showing maturation divisions I secured between 2 and 5 a.m., after having isolated the night before a number of gravid females. These are easily distinguishable because the yolk-laden eggs in their interior makes them appear black in transmitted light.

Spermatogonial mitoses exhibit a certain degree of periodicity which is by no means so marked as in the oogonial divisions.

Males are usually found in abundance together with the females. Their span of life, however, seems to be shorter than that of the females, and periods occur when few or no males are to be found.

## II.—MATERIAL AND METHOD.

An easy method for collecting an abundance of material is the following: Scour the ditch or pond with a fine cheesecloth net; invert the net in a jar of water and shake so as to release the catch into the water; pour the water through a coarse sieve so as to get rid of leaves and larger undesired objects. Then pour the water into a funnel plugged with absorbent cotton. When the water has drained through, pick out the plug of cotton with a pair of forceps, turn it over and dip rapidly into the fixing-fluid. Most if not all the Cyclops will at once liberate themselves from the cotton and soon fall to the bottom of the fluid.

As fixing-fluids I have used sublimate alcohol in various proportions both hot and cold, picro-acetic alcohol (McMurrich), picro formol (Bouin), Zenkerformol, Gilson's mercuro-nitric mixture, vom Rath's picro-aceto-osmic, Flemming's strong solution, Meves' modification of Flemming, and Carnoy's fluid (improved formula: glacial acetic, 1; absolute alcohol, 1; chloroform, 1; the mixture saturated with corrosive sublimate).

For the early stages in the ovary and for spermatogenesis, I found nothing so good as Flemming's strong solution. For oviduct eggs (showing late diakinesis figures) warm sublimate

alcohol (100 c.c. 70% alc., 5 grms. corros. subl., 0.2 grms. NaCl.; cf. Braun, '09), picroformol, and Carnoy's improved fluid gave very good figures. For ova in the egg sacs, showing maturation and early segmentation stages, I found Carnoy's improved fluid to be the best.

Material in warm (40°C.) sublimate alcohol was left for about 20 to 30 minutes, then removed to 70% iodized alcohol. In Carnoy the objects were allowed to remain about 3 to 4 minutes when they sank. They were then at once washed in 70% iodized alcohol. Sections were made from 3 to 10$\mu$ in thickness. The nucleus of an ovum of *C. parcus* in the late diakinesis stage is, on the average, about 15$\mu$ in diameter.

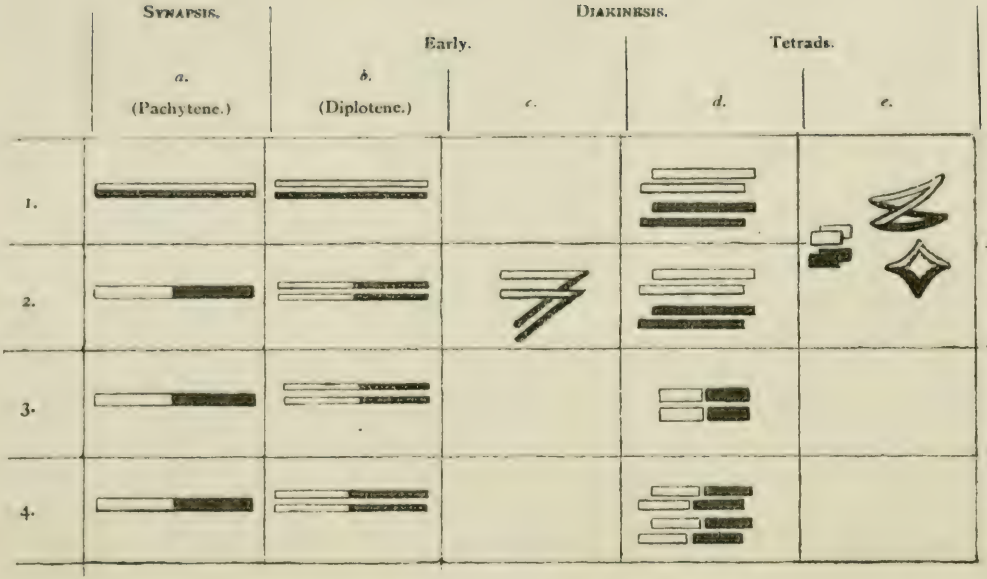
As staining reagents, Heidenhain's iron haematoxylin was found to be the best for the oogonial stages and for spermatogenesis. Iron haematoxylin, Delafield's haematoxylin, and Babe's safranin followed by light green were all used to advantage for staining late oocytes and the maturation and segmentation stages.

Investigation for this paper was begun in the University of Toronto, carried on at Woods Hole and completed in the Biological Laboratory of Columbia University. I wish to acknowledge my indebtedness to Professor F. R. Lillie for his kind courtesy in giving me a table at Woods Hole during the summer of 1911, and to Professor E. B. Wilson and Professor T. H. Morgan for the privilege of working in the Columbia Biological Laboratory and for their highly esteemed counsel.

## III.—THE CYCLOPS "TETRAD".

Four principal methods have been described to explain the formation of tetrads preparatory to the maturation divisions. These I have attempted to show in Text-figure 1.

*Text-fig. 1, Row 1.*—The parasynaptic pachytene filament (*a*) splits to form two parallel filaments (*b*) presumably the same filaments that went into parasynapsis, except that during synapsis they may have interchanged material. Each filament then splits again longitudinally but in a plane




| Advocators. |
|---|
| Grégoire, '04, '07. Strasburgher, '05. Schreiner, '04, '06. Winiwarter et Sainmont, '00. Janssens, '01. |
| Montgomery, '03. Farmer and Moore, '03, '05. McClung, '05. |
| Goldschmidt, (Dicrocoelium), '08. Dublin, (Pedicellina), '05. Ishikawa, (Diaptomus), '93. Rückert, (Cyclops), '94. |
| Braun and Matschek, (Copepoda), '09, '10. Tretjakoff, (Ascaris), '04. Jörgensen, (Sycon), '10. |

Text-figure 1.—Different interpretations for the formation of tetrads: 1, through parasynapsis; 2, 3, and 4, through telosynapsis. In 1, 2, and 3 one of the maturation divisions is reductional. In 4 both maturation divisions are equational, the cross-suture taking no part.

at right angles to that of the first split (*d*). This tetrad may take on a variety of shapes according to the greater or less divergence along one or the other of the splits and also according to the varying thickening and shortening of the four rods, forming rings, crosses, etc. (*e*).

*Row 2.*—The telosynaptic pachytene filament (*a*) splits lengthwise to form two parallel filaments (*b*). These fold on themselves (*c*), along the line of union of the original telosynaptic filaments. The filaments then break at the bend to produce a tetrad (*d*) superficially resembling that of (1) and exhibiting the same variety of shapes (*e*).

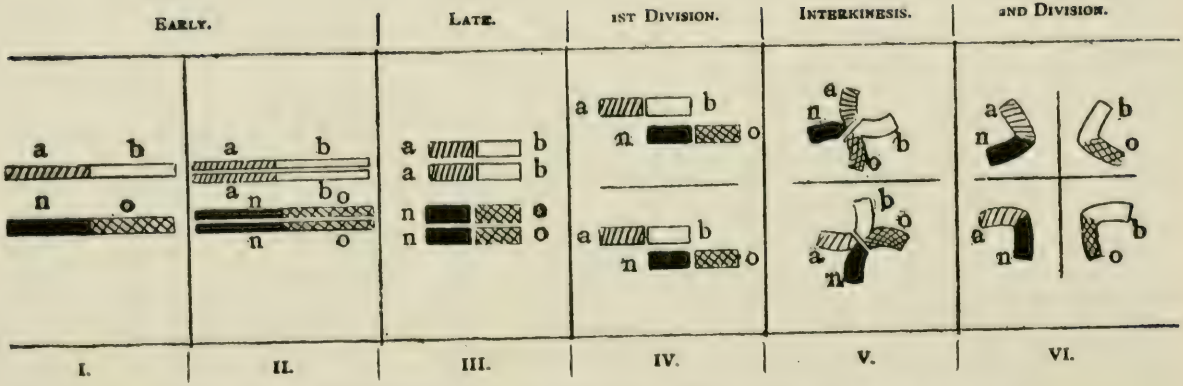
*Row 3.*—The telosynaptic pachytene filament (*a*) splits as in (2). No folding, however, occurs. The filaments break at the point of synaptic union to form a simple tetrad (*d*). One maturation division is along the longitudinal split, the other along the cross-suture.

*Row 4.*—The telosynaptic pachytene filament (*a*) splits twice longitudinally. The resulting filaments then break along the line of synaptic union to produce a *ditetrad.* The transverse break, or cross-suture (*Querkerbe*), takes no part in the maturation divisions. It is interpreted by Haecker, Matschek, ('10), and Jörgensen ('10) as the point of union, in pairs, of somatic chromosomes in the early germ-tract cells. They thus assume that the chromosomes throughout the germ-tract exist in the haploid number.

For *Cyclops strenuus*, Rückert ('94) described the synapsis spireme as consisting of a single series of chromosomes joined end to end. After splitting longitudinally the spireme breaks up into rods half the somatic chromosomes in number. These longitudinally split rods (Text-fig. 1, row 3) exhibit a cross-suture which passes through their middle and indicates their bivalent nature. The first maturation division divides these tetrads along their longitudinal split and is equatorial, the second passes through the cross-suture and is reductional.

Haecker ('95) gave the same interpretation to the tetrads in Canthocamptus, a genus closely related to Cyclops.

For *Cyclops brevicornis*, however, he ('95b, '02) gave a different interpretation. This species, according to him,




Text-figure 2.—Haecker's interpretation of the method of synapsis and maturation in *Cyclops brevicornis*.

I. A bivalent chromosome *a b* pairs with another *n o*. II. Each splits lengthwise. III. A cross-break (*Querkerbe*) appears, rendering apparent the bivalency of each chromosome. IV. In the first maturation division the longitudinal halves of each chromosome separate. V. During interkinesis the chromosomes fuse in pairs so as to form cross-like structures. VI. The second maturation division passes through the original cross-suture so that each definite chromosome in the mature egg is both grandpaternal and grandmaternal in origin (synmixis).

possesses twenty-four somatic chromosomes. In the germ-tract the chromosomes are twelve in number, each consisting of two chromosomes joined end to end. In the oocyte these twelve bivalent chromosomes arrange themselves into pairs (Text-fig. 2, I). Each one of a pair then splits lengthwise (II) and also breaks across the line of the original telosynaptic union (III). Each pair, therefore, is formed into a tiletrad or two tetrads lying parallel to one another. During the metaphase of the first maturation division these tetrads are so arranged, six in each of two parallel planes, that every tetrad in one plane lies parallel to, and directly opposite, another tetrad in the other plane (III). He assumed that the six tetrads in the one plane are of paternal, and the other six of maternal, origin. In the first maturation spindle the longitudinal halves of each tetrad are carried to separate poles (IV), the division being homœotypic. During interkinesis, the two dyads of the formerly opposite pairs become contiguous and fuse at their middle points to form double V figures (V). The plane of division in the second maturation spindle, as in other copepods, passes through the cross-suture of the original tetrads, but because of the fusion of the dyads into double V figures, the chromosomes which go to the poles of the spindle are still bivalent, but consist of new sets of halves, e.g., *a n*, *b o*, etc. (VI). A synmixis of both grandpaternal and grandmaternal chromatin elements is thus produced within the pronucleus.

This assumption of synmixis during interkinesis, based merely on the fact that the tetrads of *C. brevicornis* are peculiarly X-shaped, is elaborated by Haecker in order to account for the presence in the male or female pronucleus of both grandpaternal and grandmaternal chromatin elements. The assumption is made necessary because of Haecker's claim that gonomery obtains in the germ-tract nucleus,

Unfortunately for both Rückert and Haecker, recent investigations have placed the Cyclops tetrad in a very different light.

Braun ('09) in a study of some sixteen Cyclops species described paired chromosome rods which are longitudinally split and cross-sutured (Text-fig. 1, row 4). He also showed

that the peculiarly X-shaped tetrads of *C. brevicornis* are not produced during interkinesis but exist already in the oviduct egg and are due to the divergence at their ends of the longitudinal halves of the tetrads. Compare this with my own observation, Pl. 1, Fig. 4. The first maturation division separates the two parallel sets of tetrads; the second separates the diverging longitudinal halves of the tetrads.

Matschek ('10) made an extensive study of tetrads in species belonging to six families of the Copepoda. He agrees with Haecker and Rückert in regard to the origin of the tetrads from an incompletely segmented longitudinal spireme. He confirmed Braun's discovery, however, that Rückert's tetrads are really ditetrads or octads (Text-fig. 1, row 4). The oogonial chromosomes being twelve in number, there are six ditetrads in the oviduct egg. The primary longitudinal split is much broader than the secondary, so that the ditetrad gives the appearance of two tetrads lying parallel to each other. The two maturation divisions divide the ditetrad along the two longitudinal splits. The cross-suture being interpreted as the place of conjunction of two chromosomes and taking no part in the maturation divisions, Braun and Matschek consider both maturation divisions equational.

Lerat ('05) discountenanced the existence of the cross-suture in Cyclops. He worked on a variety of *Cyclops strenuus* found in ditches, the same species studied by Rückert. According to Lerat's description leptotene filaments in the young oocyte conjugate in pairs by parasynapsis to form pachytene filaments. These subsequently split along the line of conjugation (cf. the split spireme of Haecker, Rückert and Matschek). As the filaments shorten and thicken, they form paired chromosomes. These show no signs whatever of a cross-suture. Lerat did not go beyond the metaphase of the first maturation division.

It is interesting to note here that Braun mentions the case of a winter form of *C. strenuus*, living in ponds which dry up in the summer. In this form the chromosomes are long and U-shaped and the cross-suture is barely noticeable. Other individuals of the same species, living in lakes or in small ditches throughout the summer, possess chromosomes

with a very distinct cross-suture. Lerat may have studied only the winter form. The apparent contradiction in the statements of Lerat and Braun concerning the cross-suture may also be due to the fact that the two used different killing and staining fluids. Lerat used Gilson's mixture and Heidenhain's iron haematoxylin. Braun used sublimate alcohol and Delafield's haematoxylin.

Miss Krimmel ('10) is the latest to describe tetrads in Copepoda. She made a study of the generative cells during the late embryonic development of *Diaptomus coeruleus*. In her preparations she finds cross-sutured chromosomes both in oogonial and in somatic cells. The chromosome number in the germ-tract she claims to be thirty-two. In the somatic cells, however, she finds the number to vary anywhere from sixteen to thirty-two. Her paper is a preliminary report. We may defer criticism therefore, until her complete paper is published.

Tetrads whose cross-suture takes no part in either of the two maturation divisions have been described in few forms outside the Copepoda.

Tretjakoff ('04a) in the egg maturation of *Ascaris megalocephala bivalens* described two chromosome groups each consisting of two parallel chromosomes, each of which is longitudinally split. A transverse suture often appears in the middle of these split chromosomes which later on disappears without taking part in the maturation divisions.

In a later paper ('04b) Tretjakoff described the shape of the prophasic spermatocyte chromosomes of Ascaris that may well account also for the transverse suture he saw in the prophasic egg chromosomes. The chromosomes first appear as ribbon-like bodies with thickened ends. Later their middle region becomes so narrow as to consist of a mere thread connecting the two ends. Tretjakoff suggested that the middle region of the chromosomes consists of trophochromatin, and the two ends of idiochromatin. During the maturation stages the trophochromatin disappears, thus giving the chromosomes the appearance of being broken in the middle.

Boveri ('04) and Montgomery ('04), however, and more recently Griggs ('06), mention no such suture in the Ascaris

forms studied by them. This and the general appearance of Tretjakoff's figures make one rather sceptical of its normal occurrence in Ascaris.

Marcus ('06) in *Ascaris canis* described a cross-suture giving the tetrads the appearance of ditetrads. Edwards ('11), however, in a closely related species, *Ascaris felis*, found nothing of the sort.

A very recent paper which describes a condition similar to that of the Cyclops tetrad as explained by Haecker is one by Blanckertz ('11). Blanckertz describes eight "chromosomes" in the first maturation prophase of *Ascaris megalocephala univalens*. The eight fuse end to end, in pairs, to form the four elements of the Ascaris tetrad. Each element of the tetrad is thus bivalent in the sense of Haecker's bivalent Copepod chromosomes.

In Sponges, Jörgensen ('09) describes in a Sycon eight tetrads which appear in the first spermatocyte equatorial plate. During metaphase I the tetrads split into dyads which in the anaphase appear again as tetrads. In metaphase II these divide again into dyads. The spermatid chromosomes thus appear to be bivalent.

Tetrads have been described by Buchner ('09) as being found also in the oogonial cells of Gryllus.

The artificial production of such structures in somatic cells and in the egg (Haecker, '00; Schiller, '08; Della Valle, '09) through the action of the strychnine and other poisons should render us cautious in accepting statements as to their normal occurrence in cells, at least where chromosomes are known to be diploid in number.

My own observations have been limited to *Cyclops americanus*, *C. parcus*, and *C. brevispinosus*.

Double chromatin filaments in the reduced chromosome number come out of the synizesis stage and persist as such throughout the growth period of the ovum. The two elements of the double filament, which are at first close together, separate more and more as they contract to form short paired rods. During the late prophase of the first maturation division these paired rods are scattered throughout the nucleus (Pl. 1, Fig. 4.)

When the nuclear wall breaks down, the paired rods are drawn into the biserial arrangement (Figs. 5-10).

From a careful study of a large number of sections of this stage in *C. americanus*, *parcus* and *brevispinosus*, I am convinced that the so-called *Querkerbe*, or cross-suture, is not an actual break but rather a clear area of the chromosome due to the faint staining power of that region. The chromosome rod is somewhat larger at its two ends than along its middle. If we assume that this narrower, more faintly staining region is easily broken through when effected by killing reagents, we may account for the presence of a cross-suture in so many recorded instances.

Fig. 9 gives the side view of several chromosome pairs from different oviduct eggs in the same individual. All were found on the same slide so as to insure as far as possible uniformity in fixation and staining action.

That the cross-suture, or rather the clear area, frequently does not lie in the middle of the chromosome rod has been commented upon by Rückert himself ('94, p. 308). An instance of this is to be seen in Fig. 9a. It is noteworthy that the clear area is always in the same region for the two rods of the same pair. Fig. 9b shows one rod completely broken into two portions, probably an artifact, for its mate is intact. Fig. 9c shows the clear area in the middle. Fig. 9d exhibits a condition very frequent in my preparations when the rods show no sign whatever of a clear area.

During all stages except that of the biserial arrangement the chromosomes are U-shaped. Here only do the two arms of the chromosome stand out to form a more or less rigid rod. May not the same force that holds them in this way cause a massing of the chromatin substance toward the two ends? This would leave an achromatic substance in evidence at the middle.

The suggestion that a chromosome consists of two substances, an achromatic framework or substratum and a chromatic substance, is in accordance with the view of Bonnevie ('11) for Allium and Amphiuma, that a chromosome consists of an achromatic core around which is coiled a chromatic spiral thread.

## IV.—MATURATION OF THE OVUM.

Just before the eggs pass out of the oviduct a second nuclear membrane differentiates, enclosing a much smaller area than the former nuclear membrane of the germinal vesicle (Pl. 1, Fig. 10).

Upon fertilization, as the eggs pass out of the oviduct, this secondary nucleus approaches the periphery of the egg. No typical metaphase figure is ever formed, the chromosome pairs in the biserial arrangement passing directly into anaphase I (Figs. 11, 12, 13).

As Matschek has already observed, the first polar body is formed within two to three minutes after the egg is laid. Very little, if any, cytoplasm is given off with the polar body. As the nucleus moves to the periphery of the yolk-laden egg, it leaves a path of cytoplasm behind it (Fig. 11). When it reaches the periphery it protrudes, pushing out in front of it a membrane (Fig. 13). During the late anaphase the chromosomes assume their original U-shape, and from now on no sign whatever of the clear area in their middle can be seen. The nuclear membrane remains intact until a constriction at its middle occurs which finally cuts off the polar body.

Matschek's figures are remarkable because of the peculiar distinctness with which the "cross-suture" is depicted during maturation divisions. By examining my objects with the powers used by Matschek (Zeiss Imm. 1/12, Comp. Oc. 12), I found that I could readily deceive myself as to the presence of a cross-suture by not taking into account the U-shape of the chromosome, for the middle portion of the chromosome is left out of focus at the time that the two ends are in view.

That side of the nuclear membrane from which the polar body is constricted off shows a break for some time (Pl. 2, Fig. 14). It is soon repaired, however, to form a closed nucleus in which the split chromosomes arrange themselves for the second division by turning 90° on their axes. The split halves now are drawn asunder by spindle fibres which are distinctly visible (in contrast to those of the first maturation division) (Figs. 17, 18, 19). The metaphase figures

are similar to those of the oogonial chromosomes, the spindle fibres being attached medially or subterminally.

Fig. 20 shows the second polar body about to be constricted off. The chromosomes are somewhat massed together in the telophase. The nuclear membrane is still intact.

In Fig. 21, the polar body has been given off and the female pronucleus has receded into the egg, surrounded by a very indistinct membrane. The chromosomes are losing their definiteness of outline and will soon form the reticulum of the female pronucleus.

Fig. 22 is a polar view in *C. parcus* of the male and female pronuclei lying in the first segmentation spindle. The three chromosomes of one of the pronuclei, presumably the female pronucleus, are already definitely formed and are beginning to show signs of splitting.

Haecker and his pupils, also Rückert, and Ishikawa, have already described the remarkable autonomy of the male and female pronuclei during the earlier segmentation processes. The autonomy goes so far that one may often observe two almost complete spindles side by side each with its proper chromosomes.

## V.—CHROMOSOME NUMBER IN CYCLOPS.

### (*a*)—CHROMOSOMES IN THE GERM-TRACT.

There is no doubt that the chromosomes in the germ-tract cells are unreduced in number. Krimmel ('10) has shown this to occur in Diaptomus. In *Cyclops americanus* I have been able to make out ten U-shaped chromosomes in several tissue cells. That the chromosomes occur in the same number in the oogonia and spermatogonia of the same form may be seen from Fig. 1 and Figs. 26, 28, 29.

The conclusions of Krimmel and myself are contrary to the statements of vom Rath ('95) who observed thirty-two elements in the mitoses of the alimentary canal cells of *Anomalocera patersonii*, a marine Copepod, and sixteen

elements in the mitoses of the oogonia; and Matschek ('10) who figures an oogonial anaphase in *Cyclops fuscus* showing seven chromosomes, whereas in the biserial arrangement seven pairs are to be found.

Figs. 2 and 3 show the six oogonial chromosomes in *C. parcus*. The chromosomes are usually U-shaped and apparently very plastic in nature. During the oogonial metaphase they split longitudinally and in the anaphase the slender halves shorten to form thick, semi-curved chromosomes of about half the length of the mother chromosomes. In none of my preparations is there any figure approaching that of a tetrad such as Krimmel depicts in the Diaptomus germ-tract cells.

### (*b*)—CHROMOSOME COUNTS IN DIFFERENT SPECIES.

Braun ('09) and Matschek ('10) have ascertained the chromosome counts for sixteen of the European species of Cyclops. Braun made a study of the relation between the chromosome number and the external specific characters of the species. Taking the condition of the fifth rudimentary foot and the number of antennal segments as criteria, he found in general that those which show least signs of rudimentation possess the greatest number of chromosomes. He made the following conclusions: (1) that the highest developed forms (e.g. many marine Cyclopidae) possess the greatest number, and those which are most highly specialized possess the smallest number, of chromosomes; and (2) that closely related species possess equal or nearly equal chromosome counts and, therefore, that the chromosome number may be used in the determination of species relationship.

As far as I have been able to make out, the chromosome counts for American species fit well into Braun's phylogenetic scheme. On the other hand, his statement that closely related species possess equal or nearly equal chromosome counts is quite untenable, at least for our American forms. The following table gives the species with their diploid chromosome counts as I have found them:

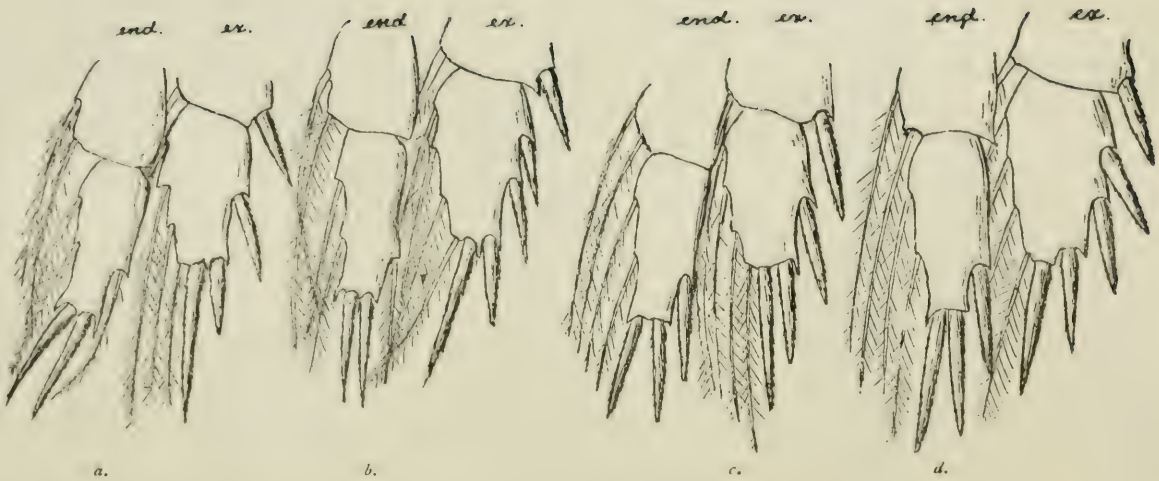
| | |
|---|---|
| *Cyclops fuscus* | 14 |
| " *albidus* | 14 |
| " *bicuspidatus* | 18 |
| " *viridis* | 12 |
| " " var. *parcus* | 6 |
| " " " *americanus* | 10 |
| " " " *brevispinosus* | 4 |
| " *modestus* | 8 |

*C. fuscus*, *albidus*, *bicuspidatus*, and *viridis* (cf. Chambers, '12) are morphologically identical with their European representatives. Their chromosome numbers are also identical with those found by Braun and Matschek. The other species mentioned in the table appear to have no European representatives. In the latest revision of the North American species of Cyclops, Marsh ('10) classifies *C. americanus*, *parcus*, and *brevispinosus* as American varieties of the European *C. viridis* Jurine. *C. viridis* (typ. sp.) has been described only by me as being found in American waters.

In their external features *C. americanus*, *parcus*, and *brevispinosus* are barely distinguishable, the only main difference being the number of spines on the terminal segments of the swimming feet (Text-fig. 3). It has been suggested (Byrnes, '10) that *parcus* and *americanus* are two phases in the life-history of the same form. I have discussed this matter elsewhere ('12). *Americanus* and *parcus* breed true for generations. Slight variations in the number of spines of the swimming feet among individuals of the same culture occasionally occur, but the chromosome number always remains constant.

*Cyclops brevispinosus* differs from *americanus* and *parcus* in frequently becoming sexually mature before the swimming feet attain the number of spines characteristic for that variety. We may therefore have a parcus-like form (Text-fig. 3, *c*) or an americanus-like form (Text-fig. 3, *d*) except for the presence of a spine on the outer side of the terminal segment of the endopodite of the fourth swimming foot and the presence in the cells of four chromosomes.*

* The caudal styles of *C. brevispinosus* are slightly thicker and shorter than those of *C. parcus* and *C. americanus*. All three are abundant in ditches and pools, although not associated in the same pool. The three appear equally infested with a unicellular green alga which often covers them completely.




Text-figure 3.—Terminal segments of the endopodite and exopodite of the 4th swimming feet in

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| (*a*) *C. parcus* | 2 | spines on the endopoditic segment, | | 3 | spines on the exopoditic. | | |
| (*b*) *C. americanus* | 2 | " | " | " | 4 | " | " |
| (*c*) *C. brevispinosus* (*parcus* form) | 3 | " | " | " | 3 | " | " |
| (*d*) *C.* " (*americanus* form) | 3 | " | " | " | 4 | " | " |

*C. viridis* (typ. sp.), averaging 2.2 mm. in length, is the largest form in the *viridis* group (excluding occasional giant forms of all the varieties). It has twelve chromosomes. *C. brevispinosus* with four chromosomes comes next, averaging 1.6 mm. in length. *C. americanus* with ten chromosomes, and *C. parcus* with six chromosomes, come last and are barely distinguishable from each other in size.

The size of the chromosomes varies greatly in the three varieties, *C. brevispinosus* possessing by far the largest, and *C. americanus* the smallest, chromosomes. The proportions for the different forms are such that we could readily assume a relationship between the average sizes and the amount of their chromatin content.

An explanation of the discrepancy of chromosome number in closely related forms is offered by Wilson ('09) in the following words:—

"It seems to me a natural view that the nucleus consists of many different materials or substances that segregate in a particular pattern; that different chromosomes need not, however, represent a complete separation of different substances but are in many cases, perhaps in all, compound bodies; and that the particular form of segregation may readily change from species to species. Marked or even extreme changes might have taken place in the number and size relations of the chromosomes that would involve little or no change in the essential quality of the nuclear substance, and the apparent anomaly presented by differences in the chromosome groups of nearly related forms would disappear."

## VI.—CHROMOSOME SIZE-RELATIONS AND ARRANGEMENT IN *Cyclops parcus*.

In *C. parcus* the six somatic chromosomes occur in three sizes, there being a pair for each size.

Pl. 1, Fig. 2 shows the oogonial chromosomes on the point of being arranged in the equatorial plate. In spite of the fact that they are somewhat bent, one may readily pick out the pairs, two long, two medium-sized, and two short chromosomes. We may see here as Wilson ('06) has already pointed

out for Anasa and other Hemiptera that the chromosomes of a pair do not necessarily lie together in the nucleus, an assumption held by many botanists.

The constancy with which the chromosomes retain their relative size during the different stages of maturation may be seen from a comparison of Figs. 2, 6, 15, and 22.

During the biserial arrangement the chromosomes of a pair lie parallel to each other. An exception is the case of one of the pairs in an individual taken from a culture of *C. parcus*. This individual possessed an abnormal number of spines on the terminal segment of the external rami of the swimming feet, the number for the four feet being, 3, 4, 4, 3, respectively, instead of 2, 3, 3, 3, the characteristic number for *parcus*. The chromosomes of the oviduct eggs were found to be in the biserial arrangement, and the smallest pair showed a deviation constant for all the eggs, some fifty or eighty, in the oviducts. The two chromosome rods instead of lying parallel to each other, as was the case for the other pairs in this individual, lay almost at right angles to each other (Fig. 7). It is remarkable that this abnormal arrangement should be so constant for that individual.

## VII.—SPERMATOGENESIS IN *Cyclops americanus*.

### 1.—LITERATURE.

Ishikawa ('93) described the spermatogenesis of a Diaptomus sp. He gives eight to be the somatic chromosome number. After the loosening of the synaptic clump he makes out, rather doubtfully, eight filaments. These shorten to form the definite chromosomes of the spermatocyte of the first order. He describes no pairing of the chromosomes. The first maturation division is equatorial and the second is reductional, four of the eight chromosomes going to one pole and four to the other. His evidence, however, is very doubtful.

Haecker has published no account of spermatogenesis in Copepoda except a brief mention in his paper of 1902. There he figures a longitudinal section of a young Heterocope testis

to illustrate his contention that the paternal and maternal elements of the nucleus in the grown cell keep more or less independent of each other. He figures numerous nuclei to show their dual nature as evidenced by their bilobed appearance and the possession of double nuclei. In two instances two independent spiremes are shown in one prophase nucleus. In the spermatid his figures show double nucleoli very prominently. In two of the spermatocyte nuclei he figures distinct tetrads.

Lerat ('05) gave an account of the spermatogenesis in *Cyclops strenuus*. Although he was unable to count the spermatogonial chromosomes, he assumed them to be unreduced in number. He claims that reduction takes place through parasynapsis as the chromatin filaments come out of the contraction stage. His studies went no farther than the anaphase of the first maturation division, but in that stage he figures the daughter chromosomes split lengthwise preparatory to the second maturation division. He found no sign whatever of tetrads.

In *Cyclops americanus* the testis is single and median, lying immediately under the dorsal wall of the thorax. From its anterior end two *vasa deferentia* rise, and after winding several times, one on each side of the alimentary canal, pass back to open, one on each side of the first abdominal segment.

### 2.—THE KEIMPOLSTER.

In old individuals a cup-shaped depression containing a disorganized mass is to be observed at the blind end of the testis (Fig. 24). This depression indicates the location of the Keimpolster, or primitive germ-cell group, from which the testis is derived.

In immature individuals the Keimpolster is a rapidly proliferating mass of cells (Fig. 23).

In young sexually mature individuals it appears as a syncitium containing a number of deeply chromatic nuclei rather irregularly disposed but chiefly arranged along the periphery. The nuclei are small, barely two-thirds the size of the largest spermatogonial nuclei. Heavy strands of chromatic material cause them to acquire a dense stain.

The absence of mitotic figures in the Keimpolster of all sexually mature individuals, and the fact that the Keimpolster is separated from the testis proper by a sharply defined boundary, renders likely the supposition that, after producing a number of spermatogonia, it becomes inert and soon disorganizes, the growth of the testis henceforth being due entirely to spermatogonial mitoses.

This Keimpolster corresponds to that described by Haecker in Canthocomptus and is, according to him ('95a), to Amma ('10), and to Krimmel ('10), the direct descendant of the germ-cells differentiated as early as in the first cleavage of the egg.

Lerat ('05) was unable to find a typical Keimpolster in *C. strenuus*, He describes an apical cell from which he assumed the spermatogonial cells were derived. It is much more probable that this "apical cell" is merely one of the spermatogonial cells and that he failed to find the true Keimpolster as it may have been already disorganized in the individuals studied by him.

### 3.—MULTIPLICATION ZONE.

The region following the Keimpolster consists of a large number of proliferating spermatogonia forming a mass of closely appressed cells. Lerat figures this region as a syncitium. My preparations, however, give clear evidence of definite cell boundaries (Fig. 24). The resting nucleus (Fig. 25) possesses an irregularly blotched chromatic reticulum. Division figures are periodically frequent (Fig. 27). Definite spindle fibres are plainly visible. The chromosomes in the equatorial plate are diploid in number and are more or less U-shaped (Figs. 28, 29).

The size of the cells varies greatly, owing partly to difference in time of growth and partly to the number of spermatogonial divisions that the cells have passed through, the nuclei and cells near the blind end of the testis (Figs. 25, 28) being considerably larger than those about to pass into the synizesis stage (Figs. 29, 30).

### 4.—SYNIZESIS AND SYNAPSIS ZONE.

The term synapsis is generally used indifferently by European writers for the massing of the chromatin filaments in a nucleus and their conjugation. The term synizesis, first proposed by McClung ('05), is much more applicable to the massing of the chromatin, while the term synapsis ought to be restricted to the conjugation of the filaments.

In Cyclops there is a decided synizesis stage. The chromosomes of the last spermatogonial division do not pass directly into the synizetic filaments, there being an appreciable zone of resting nuclei next to the synizesis region. Figs. 30 to 33 represent a number of contiguous nuclei in which one may see the gradation between the irregular network of the resting nucleus and the entangled mass of fine threads in the synizesis nucleus.

A distinct sub-spherical nucleolus is noticeable at this stage. It is always situated at one side of the synizetic mass. No bouquet-like orientation of loops can be distinguished but the threads are clearly leptotene filaments. The nucleolus never attains the great size and irregular shape seen in Lerat's figures. It is somewhat rounded in outline, rather small, and never shows the intimate connection with the chromatin filaments as figured by Lerat. Lerat's figures give one the impression of incomplete extraction of the haematoxylin stain.

The nuclei of the cells undergoing synizesis are never larger than the small last spermatogonial nuclei. Gates' interpretation ('09) that synizesis figures may be due to the growth of the nucleus unaccompanied by growth of the chromatin content, cannot apply, therefore, to the case of Cyclops.

No positive result was reached as to the likelihood of para- or telo-synapsis taking place during this stage. There seems to be no doubt, however, that synizetic nuclei containing leptotene filaments exist together with synizetic nuclei containing pachytene filaments. The two types of filaments are easily distinguishable, there being no intergradations such as Matschek claims to be the case in the

oogenesis of Cyclops. Nuclei with pachytene filaments (Fig. 34) are most numerous in the region farthest from the spermatogonial zone.

### 5.—EARLY AND LATE DIAKINESIS.

As the synizetic coil begins to loosen, the pachytene filaments give the appearance of being lumpy along their lengths (Fig. 34). Numerous short splits longitudinally arranged on the filaments soon appear (Fig. 35). The coil finally resolves itself into five long filaments each consisting of two filaments tightly twisted about each other (Fig. 36). The spirals untwist as these filaments thicken (Figs. 37, 38, 39) until the five paired definite chromosomes of the spermatocyte of the first order are formed (Fig. 40).

There is a slight growth of the cells during the synizesis and early diakinesis stages. In the oocyte there appears to be some connection between cell growth, which is enormous, and the simultaneous increase in size of the nucleolus which is very great. In the spermatocyte, on the other hand, where growth is comparatively slight, no appreciable increase in size of the nucleolus is to be observed.

The two elements of the bivalent chromosomes are distinctly elongate dumb-bell-shaped. In one individual I found several spermatocytes of the first order which contained four bivalent chromosomes and two single elements lying at some distance from one another (Fig. 41). Undoubtedly the two single elements are halves of the fifth bivalent chromosome which have accidentally broken apart.

### 6.—MATURATION.

The spindle in Division I is an ordinary one with conical poles and numerous fibres (Fig. 42) and with no resemblance to that in the maturation of the ovum. Insertion of the fibres is either subterminal or median. In metaphase the two halves of the bivalent chromosome usually break away first at one end (Figs. 42, 43). The split in the chromosomes for Division II appears during Anaphase I (Fig. 44). In telophase (Fig. 45) the chromosomes become somewhat massed

together but their distinctness is never lost during interkinesis. During this time the split in each chromosome becomes very prominent (Fig. 46), each half appearing distinctly dumb-bell-shaped. In Division II the chromosome halves are drawn away from each other much as in Division I (Figs. 47 and 48). The spermatocytes of the second order (Fig. 49) contain five slender dumb-bell-shaped chromosomes.

### 7.—SPERMIOGENESIS.

The more or less rigid dumb-bell-shaped chromosomes become U-shaped (Fig. 50). They then lose their distinctness of outline through the appearance of irregular projections over their surface (Fig. 51). These projections grow and develop in such a way that a hollow sphere of a reticular chromatin mass is formed (Fig. 52), similar to that described by Montgomery ('12) in the spermiogenesis of the Peripatus. The sphere is then drawn out into the form of a spindle (Figs. 53, 54). As the spindle lengthens, it becomes compressed from side to side. At the same time it increases somewhat in size, and the small amount of cytoplasm originally about the sphere appears to be sloughed off.

The spermatozoon in the *vas deferens* (Fig. 55) is a slender, faintly staining, finely reticular mass with a slight spiral curve and long tapering ends. In cross section it appears narrow ovate (Fig. 55a). In the seminal receptacle of the female the spermatozoa are often curled in the form of a corkscrew.

## VIII.—SUMMARY.

### EGG MATURATION IN *Cyclops americanus, parcus and brevispinosus.*

1. The oogonial and spermatogonial chromosomes are diploid in number.

2. The tendency for the chromosomes, both of the oocyte and of the spermatocyte, to assume a characteristic U-shape seems to be subordinated during the prophase of the first maturation division to a force which causes them to assume a more or less rigid rod-shape, somewhat swollen

at the ends. In the oocyte this massing of chromatin at the ends leaves a clear area in the middle of the chromosomes. Such a clear area does not appear in the spermatocyte chromosomes.

3. Both egg-maturation spindles are entirely within a nuclear membrane. The spindle fibres, attached subterminally or medially to the chromosomes, appear most distinctly in the second maturation spindle. The spindle poles are very broad so that the fibres appear to run almost parallel to one another.

4. The four American "varieties" of *Cyclops viridis* exhibit a constant difference in chromosome number. *C. viridis* (typ. sp.) has twelve, var. *americanus* has ten, var. *parcus* has six, and var. *brevispinosus* has four chromosomes.

5. The six chromosomes of *C. parcus* are in three sizes, there being a pair for each size. The chromosomes of a pair do not necessarily lie together in the spermatogonial or oogonial nucleus.

### SPERMATOGENESIS IN *Cyclops americanus*.

6. In the mature Cyclops a Keimpolster distinct from the adult testis may exist.

7. Nuclei in synizesis are smaller, if anything, than the last spermatogonial nuclei. In the testis synizesis is accompanied with only a very slight growth.

8. The nucleus in synizesis resolves itself into five pachytene filaments, from each of which develop two filaments, spirally coiled about one another. The five double filaments uncoil and become the five paired chromosomes of the spermatocyte nucleus.

9. The single elements of the double spermatocyte chromosomes are elongate, dumb-bell-shaped, similar to those of the oocyte.

10. The spermatid chromosomes resolve into a hollow sphere of a reticular chromosome mass. The ripe spermatozoon consists of the spermatid nucleus drawn out into a slightly spiral spindle-shaped body, with fine tapering ends.

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## EXPLANATION OF PLATES.

All the figures, except Figs. 23 and 24, were drawn with a Zeiss 1.5 mm. oil-immersion objective and a Zeiss No. 12 compensation ocular. For Fig. 23, ocular No. 8 was used; and for Fig. 24, ocular No. 6. The drawings were made with a Zeiss camera lucida at the level of the base of the microscope, and the reproductions are of the size of the originals.

## PLATE 1.

*Cyclops americanus.*

FIG. 1. Oogonial equatorial plate, showing 10 chromosomes. (Fixation, Flemming; stain, Heidenhain's iron haematoxylin).

*Cyclops parcus.*

2. Oogonial equatorial plate, showing 6 chromosomes. (Fl.; H.H.)

3. Polar view of oogonial anaphase daughter chromosomes. (Fl.; H.H.)

*Cyclops Sp.*

4. Nucleus of oviduct egg, late diakinesis showing 5 pairs of chromosomes. (Carnoy; H.H.)

*Cyclops americanus.*

5. Biserial arrangement of chromosomes in oviduct egg. A partial side view showing only 4 of the 5 paired chromosomes.

*Cyclops parcus.*

6. Biserial arrangement. Polar view showing 3 paired chromosomes. (Sublimate alcohol (Braun); H.H.)

7. The same, showing smallest chromosome pair abnormally arranged. (Sbl. alc.; Delafield's haematoxylin.)

8. The same. End view of chromosomes.

9. The same. Lateral view of several chromosome pairs to show clear area when present is not always in middle of the chromosome rod. In 9b one rod appears broken in the middle, the other remaining intact. (Sbl. alc.; Delaf. H.)

*Cyclops brevispinosus.*

10. Biserial arrangement. The egg is lying in the latter end of the oviduct and is compressed from side to side, giving the cytoplasmic area about the chromosomes a spindle shape. There are two pairs of chromosomes. (Carnoy; Delaf. H.)
11. Anaphase I in egg immediately after being laid. (Carnoy; Delaf. H.)

*Cyclops parcus.*

12. Anaphase I. (Sbl. alc.; H.H.)
13. Anaphase I. (Sbl. alc.; H.H.)

PLATE 2.

*Cyclops parcus.*

14. First polar body given off. The chromosomes in the egg are turning on their axes preparatory to the next division. (Sbl. alc.; H.H.)
15. Polar view in telophase I. (Picroformol; H.H.)

*Cyclops brevispinosus.*

16. Polar view in telophase I. (Carnoy; H.H.)

*Cyclops parcus.*

17. Metaphase II. (Picroformol; H.H.)

*Cyclops americanus.*

18. Metaphase II. Only 4 of the 5 chromosomes are visible in the section of the egg nucleus. (Carnoy; H.H.)
19. Chromosomes in metaphase II.
20. Telophase II. (Carnoy; safranin.)
21. Second polar body given off. Female pronucleus retreating into egg. (Carnoy; H.H.)

*Cyclops parcus.*

22. Polar view of contiguous male and female pronuclei preparing for the first segmentation spindle. In one pronucleus the chromosomes are already splitting. (Carnoy; H.H.)

PLATE 3.

SPERMATOGENESIS IN *Cyclops americanus*.
(Fixation, strong Flemming; stain, Heidenhain's iron haematoxylin).

*a. Keimpolster.*

23. Young Cyclops sp.? Keimpolster lying immediately under dorsal wall of cephalothorax.
24. Disintegrating Keimpolster at tip of adult testis.

*b. Multiplication Zone.*

25. Resting spermatogonium.
26. Spermatogonial prophase, showing 10 chromosomes.
27. Spermatogonial metaphase.
28. Spermatogonial monaster showing 10 chromosomes.
29. Spermatogonial monaster taken from the end of the testis farthest from the Keimpolster.

*c. Synizesis and Synapsis Zone.*

30. Resting spermatogonium.
31. Network of spermatogonium forming into filaments and being drawn from nuclear wall.
32. Early synizesis figure.
33. Later synizesis figure.
34. Pachytene stage. Filaments lumpy along their lengths.
35. Diplotene stage.

*d. Early Diakinesis.*

36. Five double chromatin filaments. The two filaments to the right extend out of the plane of the section and are therefore only partially shown.

37–38. The double chromatin filaments untwisting.

*e. Late Diakinesis.*

39. Two double filaments entirely untwisted. One is much contracted and thickened.
40. Definitely formed 5 double chromosomes of the spermatocyte of the first order.
41. The same. Abnormal in that the single elements of one of the pairs are separate.

*f. Maturation I.*

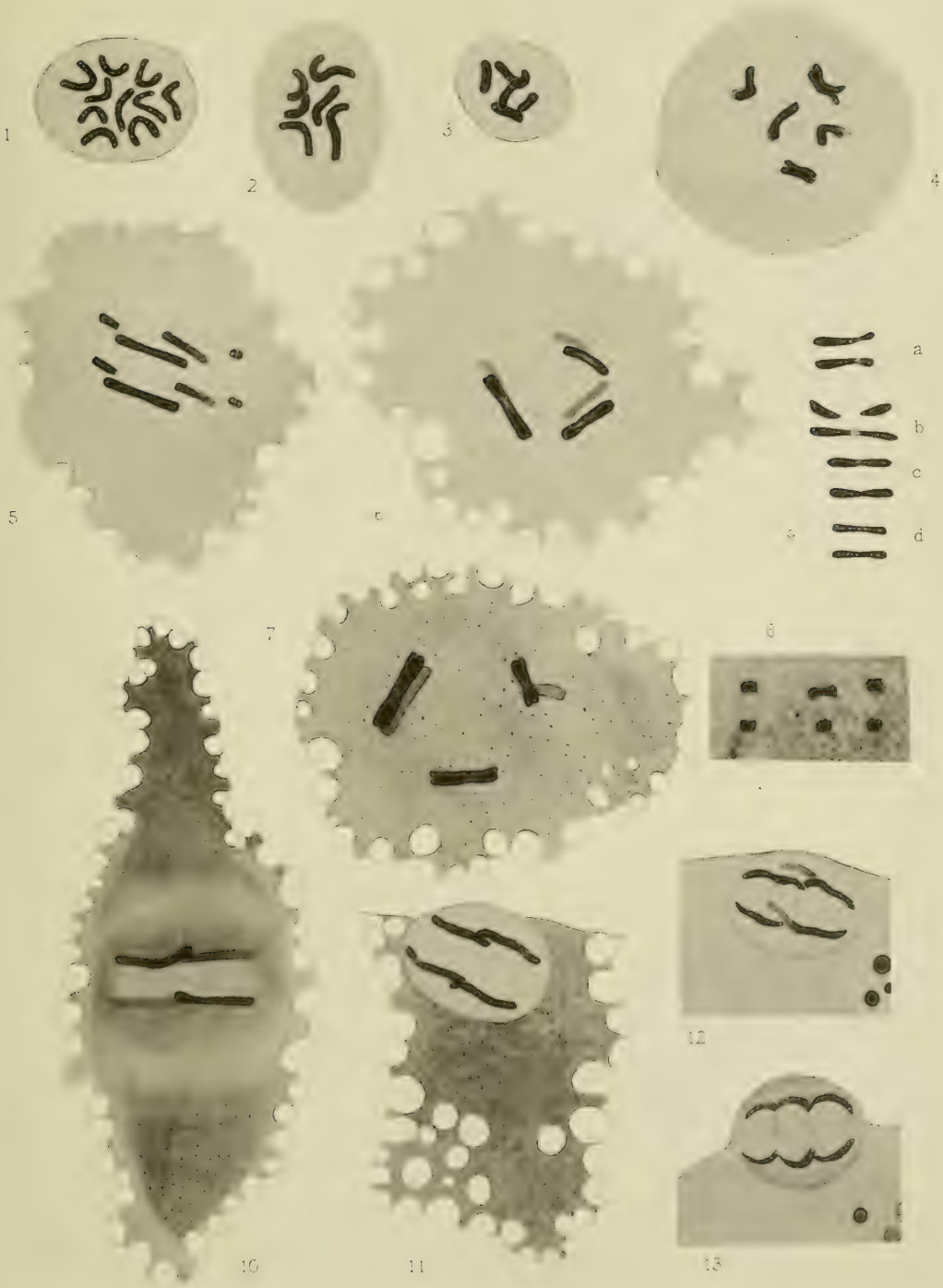
42. Anaphase I. Lateral view.
43. Chromosomes of metaphase I.
44. Late anaphase I. Polar view.
45. Telophase I.

*g. Maturation II.*

46. Spermatocyte of the second order.
47. Metaphase II.
48. Telophase II.

*h. Spermiogenesis.*

49. One spermatid with 5 chromosomes.
50. The same. Later stage.
51-54. Chromosomes being transformed into a hollow ball of chromatin network which becomes drawn out into a spindle form.
55. Mature spermatozoon.
55a. Transverse section of a mature spermatozoon.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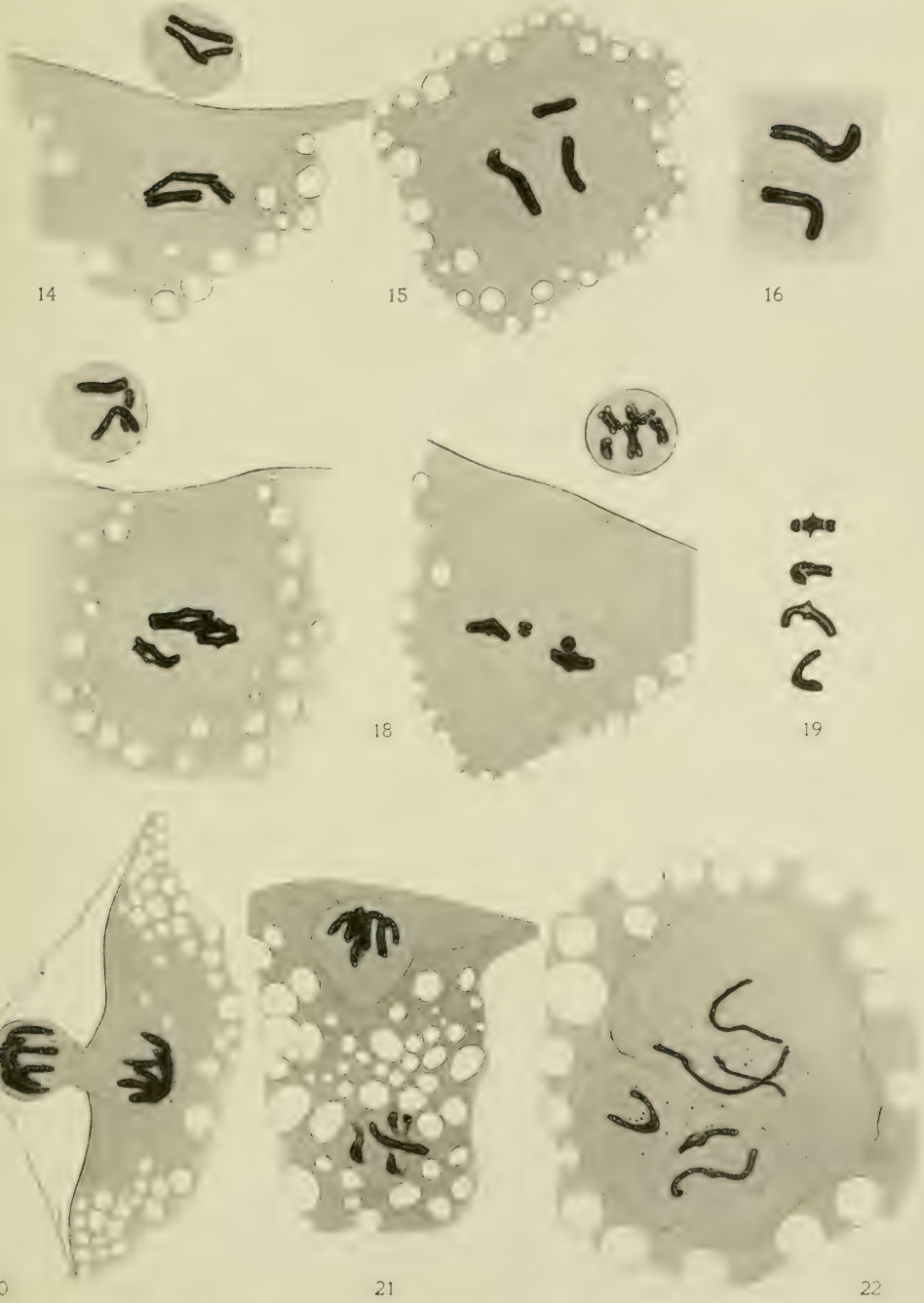
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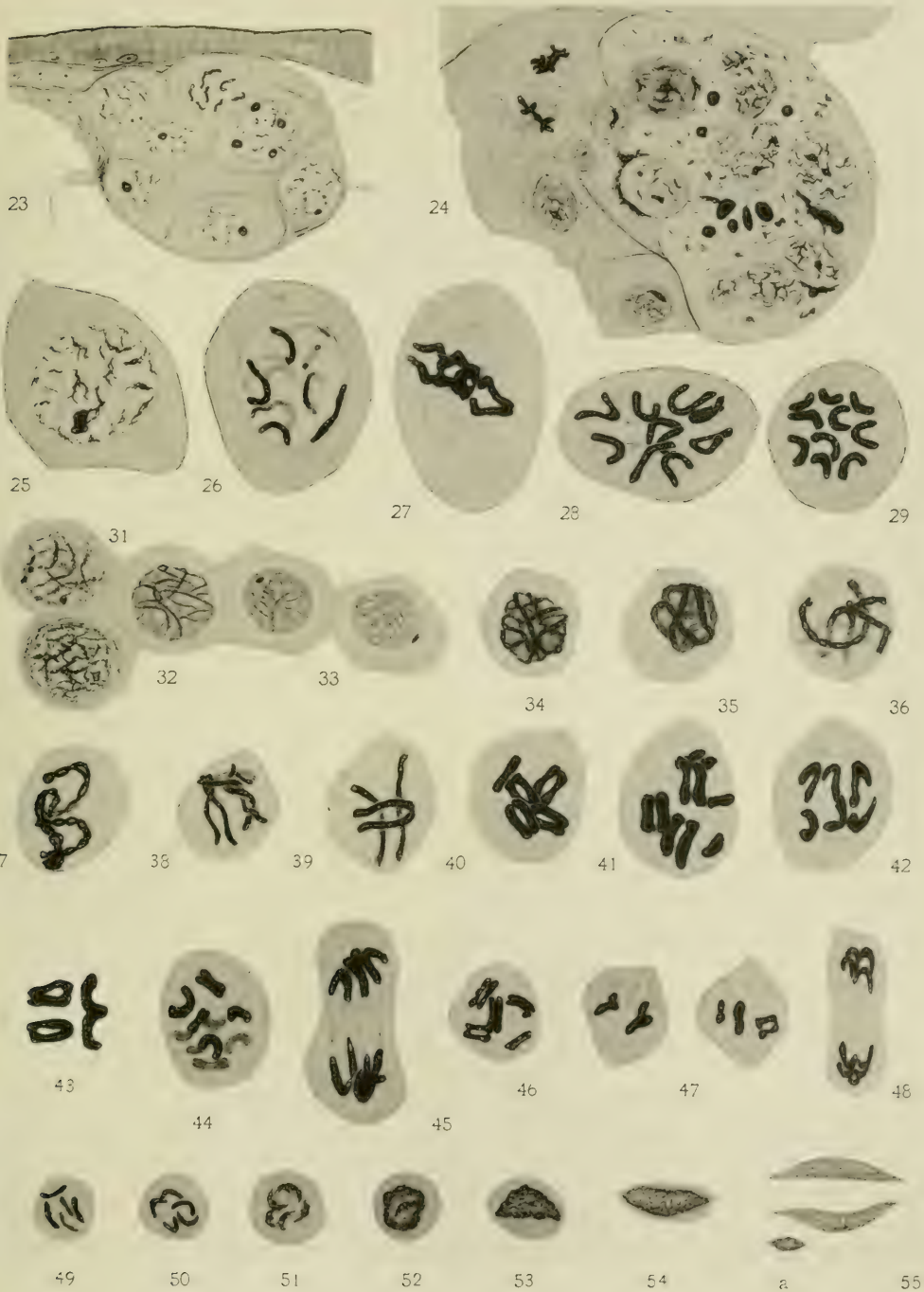
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UNIVERSITY OF TORONTO STUDIES

BIOLOGICAL SERIES

No. 15: A NEW CESTODE FROM *AMIA CALVA* L, BY A. R. COOPER

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# A NEW CESTODE FROM *AMIA CALVA* L.

BY A. R. COOPER, M.A.,

(*Read 26th October, 1914*)

A few years ago, Professor R. R. Wright drew the writer's attention to a Bothriocephalid which, during the course of his earlier helminthological researches, he had found in *Amia calva* L. and believed to be entirely new. Later specimens of the same genus, and perhaps, too, of the same species, were procured from the same host taken in the vicinity of the Lake Biological Station on Georgian Bay; and, since a preliminary examination showed that the worm had apparently not yet been described, it was thought advisable to make it the subject of a more or less thorough investigation, and to publish the results.

The writer wishes to herewith express his indebtedness to Professor B. A. Bensley for valuable assistance and advice in connection with the preparation of this paper, and to Professor H. B. Ward, of the University of Illinois, for opinions on a preliminary description and for material from his private collection.

The following paper is concerned only with the morphology of the worm, a consideration of its systematic position having been dealt with in a second paper published in the Transactions of the Royal Society of Canada (Series III, Vol. VIII, 1914, pp. 1-5).

## MATERIAL.

Apart from a few examples kindly sent to the writer by Dr. Ward, the material consists of worms ranging in length from a few millimeters to about ten centimeters, taken from the duodenum of three or four specimens of *Amia calva*, L. These were all fixed in Alcoholic-acetic-sublimate*, and stained in bulk for transparency-preparations with Meyer's Acid Carmine and in sections with Heidenhain's Iron-Haematoxylin and Orange G or Mallory's stain, the latter to bring out basement membranes in particular.

## GENERAL APPEARANCE.

When removed from the anterior end of the intestine of the host to normal saline solution the cestodes are quite active, undergoing changes in length and breadth particularly in the middle and posterior portions of the strobila; those in the scolex and most anterior proglottides are less

*The Taenioid Cestodes of North American Birds, by B. H. Ransom; Proc. U.S. Nat. Mus., Bulletin 69, 1909.

extensive. When greatly extended they appear somewhat thread-like to the unaided eye, when contracted, during life or after preservation, if no care has been taken to stretch the specimens, somewhat like a string of fine beads, which characteristic has been incorporated in the specific name. This is due anteriorly to the thickened hinder ends of the foremost joints, while farther back it is caused by the uteri being greatly distended with eggs.

The largest specimens examined were two, 110 and 96 mm. in length, containing respectively 59 and 55 proglottides. From uncleared material the number of the latter is obtained by merely counting the joints forward and depending on the distension of the uteri—the male and female genital openings are very minute—in the hinder end of the strobila to indicate the sets of reproductive organs, there being no other in evidence of proglottidation in this region.

The scolex is quite small, simple externally, and with the unaided eye can scarcely be distinguished from the first joints. It is shaped roughly like a rectangular solid, hollowed out laterally to form simple depressions and dorso-ventrally the shallow bothria or organs of attachment. The summit is somewhat prolonged as a low pyramidally shaped disc, quite comparable to that ("Scheitelplatte") found in the members of the subfamily Triaenophorinae Luehe, 1899. Furthermore, although to all outward appearances this structure is unarmed, certain modifications of the cuticle on the edges, as well as on those of the foremost proglottides, to be described below, strongly remind one of the minute hooks with which *Ancistrocephalus microcephalus* (Rud.) is provided. The opposite end of the scolex is modified to form two pairs of auricular appendages closely resembling internally as well as externally those of the foremost joints (Fig. 1). The following measurements of scolex will be of use for future diagnoses of species:—

| | |
|---|---|
| Width, at base of terminal disc..... | 0.20 — 0.40 mm. |
| Width, at posterior end of bothria..... | 0.17 — 0.25 mm. |
| Width, at tips of appendages..... | 0.24 — 0.38 mm. |
| Length, including appendages..... | 0.38 — 0.48 mm. |

A neck is absent, proglottidation beginning immediately behind the scolex (Figs. 1, 5 and 6). Here the joints are short and crowded closely together even in relaxed states of the strobila. The appendages are united to form a sort of ring into which the narrow anterior end of the next joint fits, leaving recesses between these two parts, which pass forward a little farther laterally and dorsoventrally than at the ends of the diagonal diameters (Fig. 35). In many preserved specimens these

appendages with those of the terminal disc stand out as thin leaf-like structures, concaved anteriorly, thus suggesting their probable use as accessory organs of attachment ("Stützorgane") to the wall of the host's intestine. The bothria, although provided with a well-developed musculature (vide infra), would seem to be incompetent to securely fasten the worm; possibly the appendages of the scolex and foremost proglottides may combine to act as temporary suckers, as suggested by several authors. Unfortunately no observations on the methods of attachment were made on the living animals.

On passing backwards, the joints are seen to elongate considerably, especially in all parts ahead of the ring of appendages which remain relatively more constant in size. A transverse section through the former is oblong in shape, while one through the latter is more broadly elliptical to circular in outline. This part of the strobila is the most mobile, elongation often reaching the degree mentioned above in which the appearances are quite like a knotted thread. Fig. 2 shows different degrees of contraction in a portion of the chain, but it can be seen that the middle joint is naturally somewhat shorter than the other two. In many chains this region is subject to considerable variation. It was observed that now and then one of the longest proglottides was provided with one or two additional pairs of appendages, generally abortive and situated anteriorly some distance apart. In a few cases staining and clearing brought out a distinct division of the parenchyma, especially posteriorly, into what seems to be the beginnings of a division of the longer proglottis into several smaller ones. Furthermore in one strobila an undivided region was intercalated between two jointed regions, the the second of which was followed by the normal posterior end. Young scolices are shown in Figs. 5 and 6. (In this connection note evidence given below under the excretory system that the latter are incomplete). Although the foregoing facts point to possibly occasional augmentation in the number of proglottides in this region in adult worms, the usual appearances are as described below.

Beginning at the 15th to 17th, the proglottides enlarge somewhat abruptly until the size shown in Fig. 3 is reached. The dotted ovals here represent the gravid uteri which give rise to the distended appearances of the posterior two-fifths, or nearly, of the joints. There is also some increase in width anteriorly. On the other hand the auricular appendages gradually diminish in size, until after the 23rd or 24th joint they are not to be seen, the strobila then resembling a ribbon swollen at regular intervals, as mentioned above. For some distance farther the remains of the constrictions of the anterior ends of the joints are seen in slight approaches of the lateral borders, while still farther back a tendency

for material cleared in oil of cedar, which is very brittle, to break immediately behind the ovary is the only other indication, apart from the reproductive organs, of proglottidation. This tendency, however, so far as could be determined is not based on any differentiation of the parenchymatous tissues internally at this level but more probably on mere differences of support in the latter, the ovary rendering the parts immediately ahead more resistant to strain. The following are some relative measurements of a typical strobila (Fig. 3):—

| Proglottis. | Length. | Greatest Width. |
|---|---|---|
| 20 | 1.85 mm. | 0.48 mm. |
| 22 | 2.37 mm. | 0.48 mm. |
| 24 | 2.03 mm. | 0.58 mm. |

What is apparently the end-proglottis is rounded posteriorly (Fig. 12) and provided with a functioning set of genital organs. The endings of the excretory vessels in this joint, however, seem to point to some part of the strobila (perhaps, also, of the plerocercoid) being lost at an early stage (vide infra).

## CUTICULA.

The cuticle, a well-developed structure excepting in the oldest portions of the strobila where it is often much torn or even missing over small areas, is from 3 to 4$\mu$ in thickness. It is divisible into two principal layers in each of which other layers can be distinguished. The outer of these, about two-thirds as thick as the inner, does not stain as well as the latter owing to the fact that it is made up of alternating dark and lighter areas arranged so as to give a striated appearance. The darker lines seem to be composed of minute granules while the lighter are more homogeneous (Fig. 7). Bounding this layer peripherally there is to be seen in many sections an extremely narrow clear line, followed by a sort of external limiting membrane, while in others, especially those through young strobilas, only very minute teeth which seem to be continuations of the darker lines are visible. The inner layer of the cuticula takes stains much more readily than the outer and is quite homogeneous with the highest magnifications. The line separating the two, however, is slightly darker than even the inner, which is perhaps due either to larger granules than those in the dark lines of the outer layer or a greater number packed more closely together. Bounding the inner layer on the inside there is a well-developed basement-membrane, brought out best by Mallory's stain. This is often separated from the homogeneous layer by a clear line as indicated in the figure. Then again just outside the basement-membrane the former is slightly granular in some quite thin sections. The cuticle is traversed at short intervals by the minute

excretory canals forming the foramina secundaria which appear in tangential sections as circular openings in a homogeneous matrix. Since these course through the cuticle quite obliquely, they give the latter the appearance of being pierced with holes at different levels. Two of them are shown in Fig. 7, one having reached the outside while the other has not yet passed the basement-membrane.

In many cases a splitting of the outer layer of the cuticle into processes takes place evidently along the lighter striations. It is quite conceivable that the cuticular processes, if not "cilia", described for many Bothriocephalids may arise in this manner in young scolices.

The cuticle covering the scolex is, on the whole, somewhat thinner than that on the posterior proglottides. This statement is also applicable to that on the inside of the auricular appendages of the scolex and foremost joints. The other modification of the cuticle, referred to above, is best seen in young scolices where the minute spines have not been worn away. It will be seen, by reference to Fig. 8, that the latter are developed as a thickening in the outer layer followed by a breaking up of the material into stout spine-like processes. These minute spines are restricted to a very narrow line running along the edge of the auricle, and are all directed towards the inner concave surface of the latter, that is, towards the central longitudinal axis of the worm. They gradually disappear with the appendages posteriorly. Since these spines appear in great numbers, and, since the appendages are provided with well-developed sets of muscles (v.i.), obviously arranged to activate them, they must be of actual service to the worm in obtaining a hold on the smooth mucous lining of the host's intestine.

### Subcuticula.

The subcuticular cells (Fig. 7) are not clearly defined as to boundaries but are fused together to form a syncitium the extent of which is indicated chiefly by the nuclei. There are, however, condensations of protoplasm around the latter in ripe proglottides, giving the appearance of columnar cells which have been described for many Bothriocephalids. These may even be more or less distinct towards the centre of the proglottis, yet they are directly continuous with processes from the cells of the parenchymatous tissue beneath, the whole forming in many places a meshwork of protoplasmic strand surrounding vacuoles, as shown in the left of the figure. The nuclei are comparatively large structures with well-defined walls, non-uniform in thickness, and clear contents, excepting for the deeply-staining "nucleoli". The thickness of the subcuticula varies in different regions, especially since its inner boundaries are rather indefinite, averaging about 25$\mu$. Numerous processes proceed towards

the cuticula, beyond the basement-membrane of which they could not be traced. The space between the latter and the circular cuticular muscles stains less deeply, since there seems to be a condensation of protoplasm into strands which traverse it. In some places, in sections of young strobilas, these processes appeared to be more or less grouped opposite the columnar condensations in the syncitium, mentioned above. The subcuticula is poorly developed in the scolex.

### PARENCHYMA.

The parenchyma is divisible into the usual parts, a medulla ("Markschicht"), at the centre of the strobila, surrounded by a cortex ("Rindenschicht"), extending to the subcuticula, the two being separated by the longitudinal muscles. This division into two parts is based rather on the arrangement of the nuclei, since the cytoplasm forms a very open reticulum, excepting immediately around the nuclei (Fig. 35), in which cell-boundaries cannot be seen. In the anterior proglottides most of the nuclei, each from 4 to 5$\mu$ in diameter, are restricted to two regions, those of the medulla close around the excretory vessels and nerve strands, those of the cortex, much more numerous than the inner lot, close to the subcuticula and among the outer transverse muscles. The myoblastic nuclei of the transverse (inner especially) and dorso-ventral muscles are easily confused at first sight with the nuclei of the parenchyma, but on closer examination they are found to be somewhat smaller and to contain more chromatin granules, a distinct nucleolus being difficult to locate. The nuclei of the parenchyma, on the other hand, are slightly smaller than those of the subcuticula. In Fig. 7 the two smallest nuclei farthest from the cuticle doubtless belong to the peripheral region of the cortex.

In young strobilas the parenchymatous reticulum is very vacuolated, being indicated mostly by granules at the intersections of fine protoplasmic strands, while in mature proglottides it is evidently used up for the growth of the reproductive organs which fill up almost the whole space within the subcuticula.

At the summit of the terminal disc of the scolex very many nuclei are crowded around the small shallow depression to be found there in many specimens, but they show no evidence of having any special function. Probably they have been pushed out of the immediate neighbourhood by the growth of the powerful muscles situated there (v.i.).

In all of the material studied there appeared to be no traces of chalk bodies in the parenchyma, not even spaces such as can be seen in plerocercoids of the genus *Proteocephalus*, which might have accommodated them before they were dissolved out by the acetic acid in the fixing fluid.

## Musculature.

The musculature consists of two series of fibres, namely, the muscles of the parenchyma, coursing in three different directions, and those of the cuticle, which are closely related through what will be described below as the outer longitudinal group of the former. Since a careful study of the muscles was made, they will be dealt with somewhat in detail, beginning with the simplest histologically, the dorso-ventral and coronal fibres. Those of the scolex will be described separately.

In his researches on *Bothridium pithonis* Blain. Roboz ('82) was unable to find the myoblastic nuclei of the longitudinal muscles, which, he says, are pointed at both ends, but observed a longitudinal fibrillar striation. Zernecke ('95), working on several species, makes the following statements concerning the individual muscle-fibres: "Hier finden wir denn auch die von Salensky für die Muskeln von *Amphilina* beschriebene Differenzirung der Fasern in eine centrale (Mark-) und eine periphere (Rinden-) Schicht. Letztere umgiebt den centralen Theil als ein breiter Ring und ist von diesem durch die intensive Farbung zu unterscheiden. Sie ist von homogener Structur und starker lichtbrechend als das Centrum. Letzteres erscheint im Querschnitt als eine dunklere, feinkörnige plasmatische Markmasse"; further, "Hier (at the level of the myoblastic nucleus) ist der Zusammenhang der Marksubstanz mit der Zelle zu sehen. Die Rindenschicht bildet hier nicht mehr ein geschlossenes Rohr um die Markmasse, sondern offnet sich an einer Seite, so dass eine Rinne entsteht, durch welche das Plasma der Bildungszelle mit dem Mark communicirt." Essentially the same conditions were found in the musculature of this form, excepting that the peripheral layers of the individual fibres of all of the different groups are characterized by being broken up into a varying number of fibrils (Figs. 10a, b, c, and 9a) which diverge at the ends, excepting in the case of the longitudinal fibres of the parenchyma. An example from the coronal series (Fig. 9a) shows how the fibrils are related to the nucleus and cytoplasm. Although in most of the fibres of the longitudinal muscles the latter are situated close to the fibrils, as shown in Fig. 9d, others, Fig. 9b and c, are widely separated from them, the connection being scarcely visible in many cases. The two figures given are of the most distinct examples that were seen. The fibrils themselves are very easy to follow in every part of the strobila. In cross-sections of the external longitudinal fibres at certain levels, a large area of highly-staining material at one side of the fibre (Fig. 10a and b) was considered to be the remains of the nucleus, since no other trace of it was found. This and the fact that the myoblastic nuclei of the dorso-ventral fibres between the bothria were somewhat degenerate and quite closely related to the

fibrils (Fig. 10c) points to a specialization among many sets of fibres in the direction of the complete loss of the nuclei, the presence of which is made the basis of a classification of the muscles of cestodes by Braun ('94–'00).

The coronal or transverse series of muscles is arranged as two thin sheets of fibres lying immediately within the longitudinal muscles of the parenchyma, thus assisting the latter in forming the boundary between the medullary and cortical parenchyma. In transverse sections the fibres of these two layers diverge laterally so that the innermost cross or interdigitate before they become attached to the cuticle. In the posterior end of the anterior proglottis each layer sends many fibres to the auricular appendages of the same surface of the worm (Fig. 35), which curve slightly posteriorly to form part of the radiating fibres of the latter. While the dorsal and ventral bands are continuous from joint to joint throughout the anterior portion of the strobila, there is a decided augmentation in the number of fibres in the posterior portion of the proglottis opposite the auricles into which they pass. Farther back they diminish in number with the reduction in the size of the appendages, relatively more quickly in the forward part of the joint, until in the unsegmented hinder end only a few straggling fibres appear, in the interproglottidal regions, between the testes and the vitelline glands.

In addition to these another series of transverse fibres, more circularly arranged, appears in the anterior end of the strobila, especially well-developed in the first three or four joints. They are divided into eight groups, two for each surface, and are situated immediately beneath the subcuticula. Each group consists of parallel fibres arising along the whole of the edge of the proglottis ahead of the appendages and passing obliquely and posteriorly into the opposite appendage of the same surface. Thus there is a decussation in the mid-line, giving rise to rather complicated appearances in cross-sections. The two pairs of lateral groups which can be best seen in sagittal sections, are related to each other in exactly the same way; they are, however, not quite so extensive, as may be expected from the ligulate habit of the worm, the dorso-ventral diameter even in these foremost joints being considerably less than the transverse. Beginning at the base of the appendages, that is, the anterior edge of the ring (vide supra) to which they are attached, small groups of these coronal muscles are cut off from the inner groups at the ends of the diagonal diameters of the joint by the external longitudinal fibres of the parenchyma to pass back into the appendages and supply them with a circular musculature (Fig. 35). All of the oblique fibres gradually disappear with the auricles posteriorly, so that they are developed evidently

for the movements of the auricles alone. From their arrangement they doubtless serve, in conjunction with other fibres to be described below, to extend the appendages away from the body as the leaf-like structures mentioned above.

The dorso-ventral or sagittal muscles are divided into six groups by the three excretory vessels and the two nerve strands which in the foremost joints occupy most of the medulla and are situated so close together in many sections that only individual fibres appear between them. The fibres themselves are more numerous, like the coronal muscles, anterior to the junction of two proglottides where the four most lateral groups, i.e., those between the nerve strands and the lateral vessels and those outside of the nerve strands pass from auricle to auricle on each side of the worm (Fig. 35). In the forward end of the joint more fibres are situated between the vessels and fewer laterally. The middle lot could not be traced beyond the subcuticula, while the lateral groups, on the other hand, can be easily followed to the cuticula of the auricular ring and appendages, in which latter they, along with the coronal fibres mentioned above, constitute the transversely radiating group. Farther back they dwindle down gradually until in gravid proglottides only a few coiled fibres appear between the testes and vitelline follicles or alongside the cirrus-pouch and uterine-cavity. The individual fibre closely resembles that of the coronal series, shown in Fig. 9a, excepting that it is shorter.

The longitudinal muscles of the parenchyma are divisible into two series, an inner and an outer, of which the latter appears only in the anterior end of the strobila. In transverse sections through the middle of the foremost joints they are arranged in small groups, with no constant number of fibres in each, in two concavo-convex bands between the medullary and cortical parenchyma, that is, about half way from the centre of the section to the periphery excepting laterally where they are situated relatively farther out. Here the thin edges come together immediately outside of the nerve strands. Throughout their course transversely they are penetrated by the sagittal muscles. As one nears the very short region between successive proglottides, in following through a transverse series, some of the fibres (more correctly fibrils, from the above view of the constitution of the fibre) decrease in diameter and number, especially laterally, and become more loosely arranged, as they diverge from one another. Immediately ahead or behind, as the case may be, they again appear as above. On the other hand a great many pass from joint to joint uninterrupted. From this fraying out of the fibres between successive joints it was concluded that the lengths of some of them, at least, did not exceed that of the proglottis: in the mature,

unsegmented portion of the strobila the question of the length is a very difficult one to decide upon. Perhaps only the longer kind is to be found here, since, as above stated, no indication of proglottidation, apart from the separate sets of reproductive organs, was detected. There is thus a certain amount of interruption in the course of the longitudinal muscles corresponding to the division into proglottides as pointed out early by Leuckart for *Taenia saginata* Goeze (Braun '00). Furthermore, there is a slight contraction of the whole cylinder of fibres as the interproglottidal space is neared (Fig. 37), which is not to be seen after the auricles have disappeared.

The outer longitudinal muscles appear only in the anterior proglottides and scolex in connection with the appendages, for the movement of which they are obviously developed. In the anterior half of the proglottis they lie very close to the longitudinal cuticular muscles from which they can be distinguished only by their slightly larger size. As they pass the slight indentation which in coronal series marks the anterior end of the appendage, they are joined by other fibres attached to the anterior portion of the outer wall of the latter, so that when they are converging towards the center to pass into the next porglottis, they form a ring of fibres more prominent in cross-section than those of the inner longitudinal group (Fig. 35). Throughout their course they are also more prominent opposite the auricles than at the ends of the dorsoventral and transverse diameters. Just ahead of the sinus or pocket behind the auricle a few fibres are cut off from the main body to pass about half way along the inside of the appendage. The latter is further supplied with very many fibres belonging to the same group (Luehe '97) which pass between its outer and inner walls to the very tip (Fig. 37) and, by their contraction, obviously serve to protract the edge of the auricle and thus to allow the minute spines to catch in the mucous lining of the host's intestine. As the appendages diminish in size this series of muscles gradually becomes restricted to the hinder end of the proglottis and eventually disappears with the former.

Thus, so far as proglottidation in relation to the arrangement of the external longitudinal fibres is concerned, this species resembles *Ligula uniserialis* Rud. and strongly substantiates Luehe's generalization that: "wenn die Proglottiden eines Cestoden, wie dies in der Regel wenigstens bei jugendlichen Proglottiden der Fall ist, am Hinterende einen grösseren Querschnitt besitzen, als am Vorderende, dergestalt, dass die einzelne Proglottis mit einem seitlich abgeflachten Kegel verglichen werden kann und ein Langsschnitt durch mehrere Proglottiden eine der Schneide einer Säge ähnliche oberflächliche Begrenzung besitzt, so sind stets auch Muskelfasern vorhanden, welche an der Aussenflache

der Proglottis entspringen und sich z. T. am Hinterende derselben den Bündeln der ausseren Langsmuskeln beigesellen, z. T. an der freien Hinterflache der Proglottis inseriren. Diese Muskelfasern werden nur dort vermisst, wo entweder eine aussere Gliederung fehlt oder die einzelnen Glieder nicht jene, fur die jugendliche Proglottis charakteristische, regelmässige Form eines abgestumpften und seitlich abgeflachten Kegels besitzen."

The cuticular muscles are arranged in the unjointed portion of the strobila in the typical manner, while anteriorly they are modified somewhat in relation to the great development of the appendages. The outer cuticular fibres follow the cuticle closely (Fig. 7) even on the outside of the auricles and are diminished in number and size only opposite the bands of minute spines (Fig. 8). The longitudinal fibres, on the other hand, are largest and most numerous on the outside of the appendages to the tip of which they extend, while only a very few appear on the inside, being connected with those of the following proglottis after passing forward around the auricular pockets. This description applies also to the scolex in which, however, all of the cuticular musculature is not so well developed.

The musculature of the scolex is essentially the same as that of the anterior proglottides, but there are in addition two sets of fibres which do not appear in the latter. Furthermore, all of the muscles are better developed, that is, more numerous and larger, as one might expect in this portion of the strobila, specially differentiated for adhesion.

The coronal fibres are first seen about 70$\mu$ from the summit, after which they become more numerous, especially opposite the posterior boundaries of the longitudinal arcuate fibres (vide infra) then again opposite the appendages into which many of them pass as in the foremost joints. Their arrangement is shown in Fig. 34, a section through the middle of the scolex. The other series of coronal or transverse muscles in the scolex, the obliquely decussating group, are related to the auricles as are those in the proglottides immediately behind, excepting that they do not pass relatively so far forward at the edges or "walls" of the bothria (Fig. 36). A third series of transverse muscles, one of the two sets mentioned above, is composed of large fibres arranged concentrically towards the centre of the scolex from the edges of the bothrial walls (Fig. 34), which they protract, thus helping to deepen the bothria during attachment. They are situated in the middle third of the scolex, not extending beyond the limits of the bothrial depressions. These fibres interdigitate somewhat laterally and intermingle dorsoventrally with the attenuated edges of the sagital fibres. They are quite homologous with the four groups

of fibres figured by Zograf ('92) for the scolex of *Triaenophorus nodulosus* (Pall.) and observed by the writer in confirmatory sections.

The sagittal fibres are arranged in the posterior portion of the scolex in quite the same way as those of the foremost joints. As you follow them forward, however, the two middle groups, that is, those between the excretory vessels, which separate somewhat to accommodate them (Fig. 34), enlarge considerably to form the chief muscles of the bothria. Contraction of these in conjunction with that of the tangentially arranged transverse fibres will deepen the bothria and thus form an efficient sucking-apparatus. By their relaxation and the contraction of the coronal fibres the bothria will, on the other hand, be loosened from the substratum. Anteriorly the dorso-ventral fibres gradually diminish in number and size until none appear in the first 70$\mu$ of the scolex from the summit.

The inner longitudinal muscles of the parenchyma do not pass to the tip as in many Bothriocephalids, but only about half way along the scolex, where they disappear.

The outer longitudinal fibres are arranged as in the foremost joints, but they are slightly more numerous. They extend forward as four groups each of which is situated near poorly developed cuticular muscles at the edge of the bothrial wall outside of the tangential groups (Fig. 34) ahead of which they do not appear, that is, they do not pass to the tip of the scolex.

The second group of muscles peculiar to the scolex only is to be seen in its anterior third. These are longitudinally arcuate fibres arranged concentrically around the edges of the terminal disc in four groups, one at each end of the diagonal diameters of the section through this region. Their function is obviously to protract the edge of the former with its bands of minute spines (Fig. 37).

The individual fibres of the transversely and longitudinally arcuate as well as those of the dorso-ventral bothrial muscles are comparatively short and spindle-shaped. Approximately their middle thirds take the strain much more readily (Figs. 34 and 37) than their ends which seem more muscular in composition and can be easily followed to the cuticle. This is due to the fact that it is in this middle portion that most of the cytoplasm and the nucleus are located (Fig. 10c).

The musculature of the end-proglottis bears out the above statement that an earlier portion of the strobila seems to be missing. The longitudinal muscles of the parenchyma dwindle down rapidly, the individual fibres diverging near the end-vesicle of the excretory system, while the cuticular fibres, excepting a few circular ones which pass farther on towards the latter, quickly disappear among the much altered subcuticular cells on the hinder border of the terminal joint (Fig. 12).

## Nervous System.

The nervous system consists of a nerve-ring situated immediately beneath the tip of the scolex and covering the median excretory vesicle (vide infra) like a cap, and the two chief strands passing back from it through the whole of the strobila. The former is a comparatively weakly developed structure (Fig. 11), elliptical in transverse section, with diameters of 60 and 40$\mu$. The chief nerve strands are 18$\mu$ in diameter in the scolex, in which they are situated between the middle and lateral thirds of the medullary parenchyma (Fig. 34), while in the anterior proglottides they are somewhat larger, excepting in the interproglottidal region. Here they narrow down suddenly to a diameter of 8$\mu$. In the posterior unsegmented portion of the strobila they are quite flattened laterally, opposite the gravid uterine sacs (Fig. 19), on the whole somewhat smaller than in the jointed region and situated in the medulla but quite close to the longitudinal muscles (Fig. 18). The nerve-ring gives off besides the two chief nerve strands, eight others, four being grouped around each of the former (Figs. 34 and 35). It was at first difficult to decide whether these were distinct strands or only the intersections of an extensive meshwork of nerves situated in the cortical parenchyma and thus comparable to the "plasmatische canal system" of Sommer and Landois ('72); however, with further search eight strands could be followed throughout the segmented portion of the strobila. The difficulty in following them is due to the fact that the nervous branches given off mostly centrally are quite as large as the strands themselves and that they anastomose freely with one another and with the chief strands which are, however, much more distinct. These collateral nervous tracts gradually disappear with the appendages posteriorly. Thus they are apparently developed in connection with the extra musculature of the latter. Since the Golgi method of impregnation was not used on any of the material for this study, the nerve-strands were seen to be made up of only a very fine fibrillar meshwork containing extremely minute granules and vacuoles.

## Excretory System.

There are three excretory vessels coursing throughout the strobila, a large median one evidently the morphological equivalent of the dorsal pair of many Bothriocephalids and two much smaller, ventro-laterally situated, all being located in the medullary parenchyma ("Markschicht") between the chief nerve strands (Figs. 34, 35 and 37). Immediately behind the nerve-ring the median vessel expands to form a somewhat spherical vesicle from 25 to 40 $\mu$ in diameter, into which the lateral

vessels open without any change of diameter. The junction of the median vessel and the vesicle is, on the other hand, not abrupt but gradual or funnel-shaped. In the scolex all three vessels take a comparatively straight course, gradually narrowing until, as they enter the first proglottis, they are, median, 30$\mu$, and lateral, 8$\mu$ in diameter; while in the anterior proglottides they take the form of irregular spirals, the coils of the lateral vessels following more or less those of the median, excepting as they pass the interproglottidal space where they narrow down and straighten out slightly. The comparatively small size and straighter course of the vessels in the scolex is doubtless due to the great development of the dorso-ventral bothrial muscles through which they pass.

Posteriorly their course is modified by the development of the reproductive ducts in the median line. This applies more particularly to the larger median vessel, since the other two, being situated ventro-laterally, are not much disturbed. Between the sets of reproductive ducts the median vessel lies in the median coronal plane, separating the testes into two lateral fields (Figs. 17 and 18), while the smaller vessels are situated below the testes but within the ring of vitelline follicles. As the former approaches the cirrus-sac it usually rises (it is somewhat depressed in Fig. 38) and passes dorsally to the right or left, along the uterus-sac and over the ovary and lateral portion of the generative space to the median line again. However, it frequently crosses from one side to the other dorsal to the anterior end of the developing uterus-sac or the space between it and the opening of the vagina, as shown in Fig. 17. But the greatest changes in the course of these vessels comes when the uterus becomes gorged with eggs. The smaller vessels then appear greatly flattened laterally, within the testes that appear in these sections, and not so distinctly towards the ventral surface (Fig. 19). No trace of the larger median vessel is to be seen along the middle of the uterus-sac excepting in younger stages where it is in the form of an almost obliterated tube situated dorso-laterally. Anteriorly and posteriorly, however, in several series this vessel apparently passed right into the uterine sac tangentially, the opening thus being closed with a flap-like valve. While this was very difficult to make out and was considered of only secondary importance, it was thought that perhaps the much distended condition of many of the uteri in the posterior end of the strobila, especially behind the region of closure of the temporary uterine opening (vide infra), might be due to fluid from the median vessel escaping into the uterine cavity by the absorption or rupture, during distension, of the two extremely thin walls between.

The relations of the excretory vessels in what has been called the end-proglottis are rather peculiar. The median vessel (Fig. 12) gradually

expands to form a vesicle, varying in diameter from 25 to 55 $\mu$ and is situated immediately within an invaginated portion of the cuticle into which it opens. The openings of the lateral vessels are very difficult to make out, since they seem to be quite closed in many cases. All stages between the condition shown in Fig. 12 and one (in small scolices, Fig. 6) in which all three vessels opened separately on the concave posterior surface of the strobila, were observed. Thus it would appear that this species bears out Leuckart's view that the relations between the posterior openings of the excretory system are developed after some part (in most cases an earlier proglottis) has been separated from the strobila. In fact Fig. 12 is quite suggestive in all of its parts of a simple contraction of the hinder end of the worm to form a cuticular invagination, all of the vessels formerly opening on the outside.

The flame-cell (Fig. 13) is quite typical in structure and closely resembles that of the genus *Proteocephalus* Weinland, which has been studied by the writer, in that the vestibulum in which the "ciliary flame" is located is provided with peculiar darkly-staining longitudinal thickenings which do not seem to be mentioned in the literature on the excretory system of the cestodes. Their significance is, of course, merely conjectural. The cell-body is usually not as distinct as that shown in the figure, since the cytoplasm is quite clear, but the nucleus and basal body, as well as the "flame", are very easily made out in sections. It was found impossible to trace with certainty the canaliculus from the flame-cell to any of the larger vessels or smaller canals mentioned below. The flame-cells, themselves, are few in number and arranged more or less radially close around the large vessels.

The structure of the latter is shown to a certain extent in Fig. 14. Although the wall is extremely thin, the following parts could be discerned with high magnifications: a thin cuticular layer, with a distinct basement membrane, lining the tube; outside of that a clear line in transverse sections and dotted in longitudinal sections, thus resembling a layer of extremely fine cuticular muscles; and farthest peripherally, a condensation of cytoplasm with nuclei slightly smaller than those of the parenchyma, but hard to distinguish from the myoblastic nuclei near at hand. The circular striations appear to be more protoplasmic than muscular in nature and in many places cannot be differentiated from the basement-membrane.

Foramina secundaria are to be found in the anterior proglottides, especially on the outside of the ring to which the appendages are united (Fig. 35). The openings, themselves, are very minute (vide supra, under the cuticula), but the course of the capillaries leading to them through the subcuticula and peripheral portions of the cortical parenchyma is

clearly defined by the contents being highly stained by counterstains such as Orange G. In spite of the readiness with which these capillaries can be followed through the subcuticula, it was found impossible to trace them far towards the centre of the strobila, much less to connect them with any of the main excretory vessels. In the cortical parenchyma, however, they seem to unite to form a quite compact plexus, the diameters of the tubes of which vary from 2 to 6$\mu$. In the foremost joints there are more foramina secundaria on the anterior portion of the proglottis than on the auricular ring; while very few are to be met with in the scolex.

## GENERATIVE ORGANS.

There is a more or less definite point in the strobila, at or about the 15th proglottis, ahead of which the genital organs do not seem to develop and behind which in older strobilas they appear very quickly. For instance, in one strobila 96mm. long and containing 55 joints, only the beginnings of the vitelline follicles are to be seen in the 14th joint; more and a few testes in proglottis 15; no appearance, in sections, of the generative ducts in the median line in the 14th; a mass of nuclei around the median excretory vessels (from transparent preparations) in 15; and an uterus full of eggs in 16! One must look then to the younger strobilas in which the proglottides are yet immature to see the earliest stages in the development of the reproductive organs, especially of the ducts. Here, of course, the stages are more gradual.

The genital ducts develop from a long, more or less cylindrical anlage surrounding the posterior half or two-thirds of the median excretory vessel, as shown in Figs. 15 and 16, which are from transparencies of proglottides 16 and 17, respectively, of a young strobila. Soon after the earliest traces of it can be seen in transparent preparations, the anterior end enlarges to become later the anlage of the cirrus-sac and entrance to the vagina, while the posterior end gives rise to the ovaries and organs of the "generative space", including the "uterine tube". From the middle part arises the "uterine sac", vagina and vas deferens.

All of the ducts seem to develop lumina almost simultaneously, but the vagina and cirrus do not pierce the ventral wall of the proglottis until somewhat later. Even the uterine sac approaches the ventral surface at its posterior end in the early stages. During the necessarily brief study of the development of the genital ducts the writer was able to corroborate, in general, the finds of Young ('13) and Schaefer ('13) as to the manner of formation of the lumen and epithelium from the syncitial anlage. Further remarks on the possible fate of the epithelial nuclei during the formation of the cuticle in the distal portions of the ducts will be met with below in connection with the more detailed description of the

cirrus and vagina. The fact, however, that the epithelium of the genital ducts of this species seems to be almost entirely a syncitium, even in the mature proglottides, should have special emphasis at this point.

The cirrus and vagina open very close together (actually from 0.02 to 0.07 mm. apart) on the ventral surface, about two-fifths of the length of the proglottis from its anterior end, in that part of the strobila where the auricles define the extent of the joints and relatively much farther forward posteriorly where proglottidation is absent (Fig. 17). This latter is partially due to the developing uterus-sac pushing them farther forward. There is no genital sinus, although in some states of contraction a more or less well-defined depression into which the two ducts appear to open, much resembles one,—in fact in some proglottides the cirrus and vagina open into each other on the ventral surface. The opening of the uterus is to be found on the ventral surface also, just ahead of the posterior end of the uterus-sac.

All of the reproductive system is accommodated in the medullary parenchyma, and, excepting testes and vitelline glands which are situated peripherally, the latter immediately within the longitudinal muscles of the parenchyma, all parts are much elongated antero-posteriorly, an adaptation apparently to the narrowness of the strobila. The limbs of the ovary are even squeezed together, making the whole organ horseshoe-shaped.

The "generative space", that is, the space enclosed by the limbs of the ovary, is filled with the proximal ducts of the female system (Fig. 27).

## Male System

In young proglottides, in which the uterus-sac is short and narrow, the t e s t e s are from 55 to 70$\mu$ in diameter and almost spherical in shape (Fig. 18), while in those in which the uterus is gravid they are ellipsoidal and from 70 to 115$\mu$ long. Opposite the distended uterine cavity and near the reproductive ducts behind and forward they are more or less flattened—in the former position, greatly flattened (Fig. 19).

Like the vitelline glands the testes are continuous from one proglottis to the next. They are separated into two lateral fields by the medially situated genital ducts but come together in front and behind these, in the interproglottidal regions, to form a layer, also divided by the median excretory vessel (Fig. 18). This layer, which is situated in the medullary parenchyma, is made up of as many as six testes, in cross-section, three on each side and all in the medial coronal plane of the body. No more than one testes at a time is seen on each side of the section through the gravid uterus-sac (Fig. 19). In mature joints it is difficult to say how many testes there are, but from the posterior edge of the ovary of one set of genital organs to that of the ovary of the next there are about 40 in each lateral field, or about 80 in all (Fig. 17).

Each testis is surrounded by a very thin membrane which is directly continuous with the wall of the vas efferens (Fig. 20), a point which is rather difficult to make out since the testes are packed closely together and the vasa efferentia anastomose freely between them. Numerous, even about ten, developing cytophores in various stages may be seen in the younger testes.

The anastomoses of the v a s a  e f f e r e n t i a are best seen in the vicinity of the posterior end of the vas deferens (Figs. 17, 21 and 22) and not so well, among the testes laterally and in the interproglottidal regions. Thus it is conceivable that sperms developed in testes situated in the regions between the sets of genital ducts may find their way to the vas deferens of the same proglottis or to that of the proglottis ahead or behind, as the case may be. This would be facilitated by the rupturing of the delicate walls of the testes, which alone separate them in ripe joints, to form larger and more accessible channels for the sperms. Many instances of such ruptures were seen in the serial sections studied. Sommer and Landois ('72) found that the testes in the anterior part of the proglottis of *Dibothriocephalus latus* (Linn.) passed their sperms to the vas deferens of the joint ahead, but these relations were not found in this species in spite of the otherwise general resemblance between the arrangement of the genital ducts of the two. The vasa efferentia, themselves, vary considerably in diameter and possess very thin walls in which scattered and flattened nuclei are situated, as observed by Lonnberg ('91) in *Bothriocephalus rugosus* (Batsch), (Figs. 20 and 21).

Just ahead of the uterine opening the vasa efferentia unite to form a rather indefinite s p e r m  r e s e r v o i r, directly continuous with the posterior end of the vas deferens (Figs. 17, 21 and 22) and thus resembling the similar structure of many Bothriocephalids. Its walls are intermediate, as to the structure of the epithelium, between those of the vasa efferentia and those of the vas deferens (Fig. 21). The anterior boundary of the sperm-cistern is marked by the position of the foremost of one to three separate vasa efferentia which join the vas deferens on that side towards which the latter is directed in development (vide infra); rarely do vasa efferentia empty into the vas deferens ahead of this short region.

While it was found impossible to determine the lengths of the s p e r m a t o z o a in sections, it was noticed that their anterior ends were differentiated as quite long and narrow cylindrical heads, slightly larger in diamenter than the rest of the sperm, evidently pointed at their anterior ends and graduated less abruptly towards the tail. These heads stain very densely with Heidenhain's Iron-Haematoxylin and are consequently quite easy to pick out, while the other parts are scarcely discernable in the masses to be seen in various portions of the male ducts.

The vas deferens passes forward from the sperm-reservoir almost in the median line and dorsal to the uterus-sac, taking many irregular coils in its course (Fig. 17). In older proglottides, however, owing to the relatively enormous distension of the latter, it is pushed to one side until all parts, excepting those close to the vesicula seminalis, may eventually become obliterated. It seems to be crowded more often to the right, doubtless because of its position in younger stages; at any rate, the anastomotic reservoir formed at its posterior end by the vasa efferentia lies more often to the left. Fig. 17 is an exception to this, as it is a dorsal view.

In ripe joints before it is pushed aside by the developing uterine cavity, the vas deferens is tubular in shape, from 11 to 14 $\mu$ in diameter at its anterior end where it joins the vesicula seminalis, 17 to 25 $\mu$ at its middle and 22 to 35 $\mu$ at its posterior expansion, the latter being the diameter of the sperm-cistern. Later when it becomes gorged with sperms and the walls are, in consequence, thinner, the diameter varies from 40 to 55$\mu$.

The wall of the vas deferens consists of a low epithelium in which, as in the sperm-reservoir, no cell boundaries can be made out, supported by a poorly-developed basement-membrane (Figs. 21, 23a and b). It is thus a syncitium. In older proglottides, where the vas deferens contains sperms, the epithelium is flattened out so that the nuclei appear here and there along the duct as thickenings in an otherwise thin membrane. In young, and, as yet, non-functioning vasa deferentia nuclei from the outer layer of the anlagen remain close to the basement-membrane, especially towards the vesicula seminalis, to form the myoblasts of scattered and fine circular muscles (Fig. 23).

The vesicula seminalis, which is morphologically an expansion of the vas deferens, is situated close to the dorsal body-wall, immediately behind the cirrus-pouch (Fig. 17). It is ovate to spherical in shape in mature proglottides, before it is flattened against the latter by the gravid uterus-sac, with the more pointed end directed anteriorly, while in younger (but ripe) joints it graduates less abruptly posteriorly, that is, it is more broadly spindle-shaped. The wall has the same structure as that of the vas deferens, excepting that the syncitial epithelium is so much thinned out, especially when the organ is filled with sperms, that the nuclei, which appear singly or in groups of two or three and surrounded by small amounts of clear cytoplasm, seem to be applied to the inside of the basement-membrane itself. Outside of the latter there are to be seen numerous fine muscle-fibres, with their myoblastic nuclei, coursing in general longitudinal and circular directions. These are similar in structure to those surrounding the vas deferens. On

account of the extremely small size of these fibres it was found impossible to determine whether they are arranged in one or more layers. The following are the averages of the measurements of four vesiculae seminales:—

| Length. | Width. | Depth. |
|---|---|---|
| 0.140 mm. | 0.092 mm. | 0.090 mm. |

The vas-deferens narrows very abruptly again to a diameter of 15$\mu$ as it enters the postero-dorsal portion of the cirrus-sac (Fig. 17) to become the d u c t u s e j a c u l a t o r i u s. This portion of the duct takes three of four turns in the dorsal third of the cirrus-pouch and then passes on as an enlargement, the s e c o n d v e s i c u l a s e m i n a l i s, occupying approximately the middle third of the pouch (Fig. 38). While the walls of the proximal portion of the ductus ejaculatorius quite closely resemble in structure those of the vas deferens behind the larger or posterior seminal vesicle, those of the distal vesicula seminalis are very thin, showing few nuclei closely applied to the basement-membrane. The diameter of the duct at this point is about 38$\mu$. As the junction between the ductus ejaculatorius and the inner vesicula seminalis is approached the epithelium becomes broken up into numerous processes which, however, did not appear to be true cilia. As a matter of fact cilia do not seem to present in any part of the male reproductive ducts.

The third division of the vas deferens within the cirrus-pouch, the c i r r u s p r o p e r, usually commences at the posterior pole of the latter, courses forward and then backward again to pierce the wall of the pouch and open on the ventral surface of the proglottis at the point shown in Fig. 17. The diameter of the cirrus at the bend in its course (Fig. 38, c) is about 20$\mu$; it enlarges gradually to 30$\mu$ before opening to the outside.

This region of the male duct can be evaginated, presumably, as in most cestodes, to form a copulatory organ, yet in all the material at hand not a single case of everted cirrus was observed. Consequently, nothing can be offered, in regard to its function, apart from the suggestion that from the frequent approximation of the male and female genital-openings, noted above, self-fertilization may possibly occur in this species. The structure of the cirrus would at least indicate that after eversion it might become quite an efficient organ. Its wall (Fig. 24) is composed of an inner lining of cuticle thrown into folds of varying heights, supported by a basement membrane which can be distinguished as such only in young stages. Outside of the latter appear two sets of circular muscles (Fig. 24, cm), separated by a comparatively clear protoplasmic area which is traversed by the longitudinal and the retractor fibres (rmp) and numerous

processes from parenchymatous cells lying farther out. The circular muscles increase in number at the opening of the cirrus and form a distinct sphincter. In that portion of the cuticle next the lumen, that is, towards the functional outer surface of the organ, there are to be seen numerous highly-staining granules which seem to be the bases of fine bristle-like processes extending into the lumen. While the granules show very plainly in sections, the processes themselves are difficult to make out clearly in many cases. They are, however, probably homologous with the spines, hooks, etc., described for the cirri of other species.

Fig. 25 shows a somewhat younger stage in the development of the cirrus than that shown in Fig. 24, and is of interest in connection with the problem of the formation of the cuticle. Considerable attention was paid to detail in this figure in order to bring out the following points. It will be seen that four or five nuclei lie close to the cuticle, in fact against the basement-membrane, while others farther out appear to be connected with the cuticle, or at least with the syncitium of protoplasm immediately outside of it, by fine strands. Many of these peripherally situated nuclei belong to the myoblasts of the circular muscle-fibres, as indicated by the letters "cmc", and some of them to the few longitudinal fibres, but they, especially the former, are fairly easy to distinguish from the majority of the number which have the central protoplasmic connections. Young ('13) and Schaefer ('13), working with different species of cestodes, came to quite opposite conclusions regarding the fate of the epithelial nuclei during the formation of the cuticle in the distal portions of the vas deferens and of the vagina. Young asserts that the nuclei disintegrate in situ as the cuticle is being formed, while Schaefer observed what is doubtless the migration of the nuclei into the surrounding cytoplasm. The writer does not pretend to have gone into the matter at all exhaustively, but from the few observations he has made on the material studied it would appear that this species falls into line with Schaefer's discoveries. At any rate, no conclusive evidence of nuclei having disintegrated in situ in either the cuticle of the cirrus or that of the vagina was met with, but appearances like that shown in Fig. 25, where the original syncitial nuclei seem to have migrated some distance from the developing cuticle, retaining their protoplasmic connections and possibly functioning in the formation of that layer by secretion, are very common. In later stages, evidently when the cuticle is completely formed, these connecting strands fuse with the general mass of parenchymatous cytoplasm surrounding the cirrus and its retractor muscles, giving the appearances shown in Fig. 24. More will be given below in this connection under the vagina which, on account of its comparatively greater length, is better adapted to show the stages in the development of the cuticle.

The cirrus-sac is situated about midway between the dorsal and ventral surfaces of the proglottis, immediately ahead of the vesicula seminalis (Figs. 17 and 38). In shape it is spheroidal, being flattened laterally and somewhat protracted ventro-posteriorly where it follows the cirrus to the latter's opening, as the following measurements indicate: longitudinal diameter, 0.16 to 0.21 mm.; transverse diameter, 0.14 to 0.16 mm.; vertical diameter, 0.18 to 0.20 mm.

The wall of the cirrus-pouch, although quite thin, is wholly muscular and composed of two sets of fibers which can be better distinguished as such in younger proglottides than in older or gravid joints where they course irregularly and obliquely. Of these two sets the inner are circularly disposed while the outer are arranged longitudinally, thus corresponding to the description, by Sommer and Landois, of the parts in *Dibothriocephalus latus*. The fibers in the postero-dorsal portion of the wall intermingle with those of the vesicula seminalis; postero-ventrally they converge towards the opening of the cirrus, around which, with the dorsoventral parenchymal fibers of the immediate neighbourhood, they attach to the cuticle of the ventral surface. A very few fibers, on the other hand, difficult to distinguish from these dorsoventral parenchymal muscles, pass from the dorsal wall of the cirrus to the dorsal body-wall. Thus retraction of the cirrus, if, indeed, it is ever everted, would appear to be brought about by the mere elasticity of its tissues and of those surrounding it.

The dorsal half to two-thirds of the space within the cirrus-sac, which accommodates the ductus ejaculatorius and its expansion, the second vesicula seminalis, is filled with numerous parenchymal cells grouped irregularly around the duct outside of the fine longitudinal muscular fibers following the course of the latter. The myoblastic nuclei of these are visible as spindle-shaped, highly-staining bodies, closely applied to the fibers themselves. The ventral half to one-third of the space, on the other hand, appears much more compact in sections and transparent preparations, since it is in this region that the large retractor fibers of the cirrus are located. The latter are arranged in groups (Fig. 24) and attached evidently to the cuticle centrally, while they intermingle peripherally with the fibers composing the wall of the sac. The myoblastic nuclei are related to these fibres as in the case of the longitudinal muscles of the parenchyma, that is, one nucleus is associated with three or four fibrils. In addition to the circular fibers situated immediately outside of the cuticle of the cirrus proper, there are other finer ones to be seen for some distance beyond the cytoplasmic area, above-mentioned, intermingling with the large retractor fibers (Fig. 24).

### Female System.

The v a g i n a opens on the ventral surface of the proglottis immediately behind the opening of the cirrus and from 0.02 to 0.07 mm., from it (Fig. 17). While in most cases the aperture is circular in outline and from 20 to 30$\mu$ in diameter, it is occasionally found in preserved material to be transversely elongated, more especially when it approximates the male opening (vide supra). The first portion of the vagina is in the form of a somewhat elongated vesicle, 56 $\mu$ in transverse diameter and situated beneath the vesicula seminalis; it is quite comparable, in shape at least, to the "Scheideneingang" of Sommer and Landois. After being slightly deflected dorsally, as in *D. latus*, the duct then passes back along the ventral side of the uterus-sac, on either side of the median line, or crosses from one side to the other at different levels ahead of the uterus-opening—in young proglottides ahead, necessarily, of the limb of the uterus directed towards the latter. In either case it turns to the median line again close to the posterior wall of the uterus-sac, and then passes over the ovarian isthmus and into the "generative space" where it expands, as it courses ventrally again, to form a receptaculum seminis.

The structure of the vagina is quite comparable, on the whole, to that of the vas deferens. Posteriorly it is lined with a syncitial epithelium, supported by an indistinct basement membrane which is relatively somewhat thinner than that of the vas deferens of the same proglottis, excepting in the region of the receptaculum seminis (vide infra). This is doubtless due to the fact that during the period of differentiation of the two tubes from the middle and narrower portion of the common anlage of the genital ducts, the vagina is somewhat in advance of the vas deferens, that is, it develops a lumen slightly previous to the formation of one in the latter, and then, evidently keeps in advance of it during subsequent growth and distension. From a point opposite the anterior end of the uterus-sac to its opening the vagina is lined with a cuticle which in many cases is lacerated and torn, especially at the surface next to the lumen. In this region, at the proper stage, that is, about the time when only a few eggs appear in the uterus-sac, what was considered by the writer to be the transformation of the epithelium into the cuticle can be observed much more clearly than in the case of the cirrus where only a comparatively short length of duct develops a cuticle. This seems to be brought about almost wholly by the sinking of the nuclei into the surrounding mass of cells derived from the outer layers of the anlage and lying outside of the basal membrane and circular muscles, and by the subsequent alteration of the epithelial substance to form the homogeneous cuticle. Very few nuclei in their passage through the membrane were seen, since no lengthy study of this subject was undertaken and since, as suggested

by Schaefer the process takes place, in all probability, quite rapidly, thus rendering the finding of the nuclei in all of the stages a matter of some difficulty in a comparatively small number of series. Three figures are given, however, to illustrate what was observed by the writer in this connection. Fig. 39 is a photograph of a coronal section through the first portion of the vagina, the entrance to the vagina being shown at "v". The latter is seen to be surrounded by a number of radiating cylindrical cells with rounded peripheral ends towards the parenchyma, somewhat resembling the cells of the subcuticular layer. They are much more numerous around the enlarged portion of the vagina than around the duct farther back. At "x" one of these elongated cells, with the nucleus situated at its extremity, is attached to the cuticle tangentially and in such an intimate manner as to lead one to think that it still functions, possibly, in the formation of the latter. Again at "x" and "y", Fig. 40, two nuclei with the surrounding cytoplasm appear to have passed through the basement-membrane but to have gone only a short distance beyond it. A similar case is shown in Fig. 41 at "y", while at at the point marked "x" a nucleus half way through the basement-membrane is to be seen. As the nuclei pass through the latter they are surrounded in many cases by clear areas, possibly cytoplasm quite thin in consistency, as noted by Schaefer in *Bothridium pithonis* Blain. Thus it appears—to the writer, at least—that, in the transformation of the epithelium of the distal portions of the vagina and vas deferens into the cuticle, the nuclei of the former pass into the surrounding parenchymatous tissue, and may there function in the formation of the latter. While the above evidence is scarcely to be considered as conclusive, it is given in the hopes that it will be at least suggestive to the reader in connection with the question of the formation of the cuticle in cestodes, which is again occupying the attention of helminthologists.

The musculature of the vagina is composed of circular fibers only, as in *Cyathocephalus truncatus* (Pallas), which are situated immediately outside of the basement-membrane. Very few of them surround the greater part of the canal, including its anterior enlargement, but a comparatively large number are developed in the short region between the latter and the opening to form a powerful sphincter, 30 to 40 $\mu$ in length.

From a point opposite the posterior end of the uterus-sac and ventral to the uterine tube ("Uteringang") the vagina gradually enlarges as it passes dorsally over the ovarian isthmus to form the r e c e p t a c u l u m s e m i n i s . The posterior, rounded end of the latter is situated within the generative space dorsal to the oocapt, with its longitudinal axis almost vertical (Fig. 27). The diameter of the tube at this point varies from

30 to 45 $\mu$, depending on the amount of its distension with sperms. The receptaculum seminis is lined with a direct continuation of the syncitial epithelium of the vagina, in which, however, some tendency to form cell-boundaries appears, especially in the earliest stages. No valve-like modifications of the wall, as described by some authors for this part of the vagina of other species, were seen; there is simply a gradual enlargement of the duct up to the sudden constriction about to be mentioned. Furthermore, although the epithelium of the vagina and receptaculum seminis shows in many cases fine processes of different sizes, directed towards the lumen, these were not considered to be cilia, since, in the same regions of the other proglottides of the same chain, the epithelium was quite smooth and bounded by a more or less distinct membrane. There are few circular muscle-fibers surrounding the receptaculum seminis until a point is reached, immediately ahead of the constriction which bounds it proximally. Here they are greatly augmented and directly continuous with a well-developed musculature which surrounds the beginning of the spermaduct (Fig. 26). This musculature is evidently developed for the purpose of passing along, by swallowing movements, only a few sperms at a time, as indicated in the drawing which shows a string of sperms connecting the mass in the center of the receptaculum with the spermaduct. The latter in all such cases is filled with spermatozoa.

Immediately behind and ventral to the receptaculum seminis the vagina narrows down abruptly to form the s p e r m a d u c t . While its first portion, as indicated in Fig. 26, is very small, being only from 5 to 10 $\mu$ in diameter, it soon enlarges to almost twice that diameter, the size which obtains throughout the rest of its course. On account of the intense staining powers of the surrounding musculature it is very difficult to ascertain the nature of the wall at this level; however, it is composed of a very thin epithelium in which no nuclei were seen. On the other hand, certain nuclei situated outside of the basement-membrane and connected with it by cytoplasmic strands, on the whole reminding one of the radiating cells surrounding the cirrus and the entrance to the vagina, may possibly have been located within the epithelium at an early stage in development. Some of them are obviously the myoblastic nuclei of the circular muscles. To determine the exact origin of these nuclei it would be necessary to make a special study of the development of the ducts of the generative space, since the musculature of the spermaduct arises very early, even before some of the other ducts in the immediate neighbourhood are completely differentiated. The circular fibers diminish in number throughout the remainder of the duct, but are much more numerous than the few longitudinal fibers, arranged somewhat spirally outside of them.

The o v a r y is an annular or closed horseshoe-shaped organ, situated ventrally at the posterior end of the middle field of the proglottis (Fig. 17). Although in most cases it appears to be completely closed posteriorly, it is in reality made up of two limbs,—they can be distinguished as such in the very young stages of development—connected anteriorly by an isthmus, on the ventral side of which is situated the oocapt. The limbs themselves are generally enlarged anteriorly, so that, on the whole, they somewhat resemble those of *D. latus*, which are, however, widely separated behind (Sommer and Landois). The organ is surrounded by a very thin wall and is divided by a continuation of the same into a number of irregular, tubular compartments which accommodate the ova. Scattered throughout these partitions and the outer capsule itself, very small, flattened nuclei, from 1 to 2$\mu$ in diameter, are to be seen. This description applies to all of the ovary, excepting that portion of the isthmus lying quite near the oocapt. Thus, from the fact that the isthmus—with the contained ova—is solid, it would appear that the views of Sommer and Landois and not those of Leuckart, who considered the isthmus or "bridge" to be a mere duct-like portion of the organ for the passage of the ova, are applicable to this form.

The largest ova (Fig. 28a), which appear in the ventral part of the isthmus and are thus ready to be passed on for fertilization by the oocapt, vary in longitudinal diameter, since the cytoplasmic outline is somewhat irregularly oval, from 10 to 12$\mu$. The greater part of the comparatively large nucleus, which is about 7$\mu$ in diameter, stains much less deeply with Heidenhain's Iron-Haematoxylin than does the surrounding protoplasm; the "nucleolus", on the other hand, comes out extremely dark blue. With Mallory's stain, however, it appears orange, which colour is seen in no other part of the body. Consequently, the nucleolus seems, from its staining powers, to be a definitely functioning body and not a mere aggregation of nucleoplasmic particles. Yet such aggregations, quite as large as the nucleolus itself, are to be seen in other parts of the nucleus; so that from this and the further fact that the outline of the nucleolus is very often irregular, it is a matter of conjecture as to what is the true nature of the body in question. In the cytoplasm of many ova small clear areas, often provided with darkly staining bodies resembling nucleoli, are to be seen (Fig. 28b and c). Some of these may be nuclei forming in the protoplasm *de novo* (after Young's views), but others so closely resemble small free ova as to lead one to think that they may be abortive ova which have come into intimate contact with the cytoplasm of the normal ova and been subsequently absorbed by them, stages in the process of which absorption are probably represented in Fig. 28c.

The oviduct begins on the ventral surface of the ovarian isthmus with the oocapt which is a broad funnel-shaped or hemispherical structure directed ventrally in the median line (Fig. 27). The diameter of the latter, using the outer limits of the circular musculature as the boundary, since the organ is very gradually continuous with the wall of the isthmus, varies from 15 to 25$\mu$. It is lined with a cuticle-like substance, which shows no nuclei for a short distance, and is surrounded by a system of circular muscles, arranged and extended quite like those of the spermaduct and posterior end of the receptaculum seminis. Furthermore, the resemblance in structure is the more exact from the fact that the constricted portion of the duct, which immediately follows the muscular funnel, is surrounded by radially arranged nuclei, many of which belong, of course, to the myoblasts of the circular fibers. The constricted part has a diameter of from 8 to 9$\mu$; after which the oviduct gradually enlarges to 15$\mu$, as it courses to the right or left and posteriorly until it meets the spermaduct almost in the median line of the proglottis. The wall is made up of a ciliated epithelium, in which are to be seen one layer of nuclei but no distinct cell-boundaries in the somewhat vacuolated cytoplasm, supported by a well-developed basement-membrane.

A short distance from its union with the spermaduct the oviduct is joined by the short duct from the yolk-reservoir. Just behind this point there is a slight constriction, around which the circular muscles are augmented in number to form a small sphincter while they are accompanied by a few longitudinal fibers.

Two vitelline ducts, each about 6$\mu$ in diameter, collect yolk from the lateral fields of vitelline follicles and pass towards the median line to unite either within or outside of the generative space ventral to the ovary. Union within the latter is the usual arrangement, in which case each duct is accommodated in the groove situated on the ventral surface of the ovary between the oocapt and the anterior end of the limb on each side. Each of these ducts may receive material from a few follicles on the opposite side of the proglottis, but, in general, it collects from the same side to which it is directed. Their walls are composed of a thin epithelium, showing small flattened nuclei distributed at wide intervals, on the whole resembling those of the vasa efferentia. Their courses are easily followed by observing the, in many places, greatly extended yolk-cells on their way to the yolk-reservoir (Fig. 29b.). On the other hand, the arrangement and structure of the smallest ducts in immediate connection with the yolk-follicles were not determined to the writer's satisfaction, since the latter are packed so closely together; but from various appearances they seem to anastomose.

The common duct, which is quite short (Fig. 27), is slightly larger than the collecting ducts, and its epithelium contains relatively more nuclei. It is furthermore provided with cilia, directed towards the yolk-reservoir.

After passing for a short distance dorsally and towards either side of the proglottis, depending on the arrangement of all of the ducts in the generative space, this common yolk-duct expands into the vitelline reservoir, an ellipsoidal or somewhat spherical sac varying from 25 to 55$\mu$ in diameter according to the amount of yolk it contains. Even when yolk is absent, however, it is larger than the common duct and shows very few cilia; thus it seems to be a true reservoir, in that it is possibly differentiated early in development, and not a mere temporarily functional dilatation. The epithelium is naturally considerably distended and flattened by the contained yolk. The reservoir unites with the oviduct through a short length of common vitelline duct whose structure is identical with that of the above-mentioned portion.

The vitelline follicles, like the testes, are situated in the medullary parenchyma, that is, within the longitudinal muscles and consequently within the nerve-strands (vide supra), thus resembling, as to situation, those of the genera, *Ancistrocephalus* Montic., and *Anoncho-cephalus* Luehe, (Luehe, '02). There they form a continuous cylinder from one proglottis to the next, enclosing the excretory ducts and reproductive organs, including the testes, but broken ventrally and dorsally by middle fields corresponding in extent to the region occupied by the generative ducts, that is, from the anterior end of the cirrus-pouch to the posterior end of the ovary (Figs. 17, 18 and 19). Posteriorly they crowd the ovarian limbs very closely, a few even passing above and below their hinder ends. Thus, in extent, the vitelline follicles are comparable to those of *Cyathocephalus* (Kraemer '92), *Schistocephalus* (Kiessling '82), and *Bothriocephalus dendriticus* Nitzsch and *B. ditremus* Crep. (Matz '92), excepting that in the three latter forms the dorsal middle-fields are occupied by them, while in the first genus neither dorsal nor ventral middle-fields are left free of follicles. In *D. latus*, on the other hand, both fields accommodate no vitelline follicles (S. and L. '72).

The follicles themselves are usually spherical to ellipsoidal in shape; but in ripe joints, where they are very closely packed together, the outline is somewhat polyhedric. Furthermore, they vary greatly in size, the smallest being only about 8$\mu$ in diameter, while the largest, which are more numerous, are even 50$\mu$. The yolk-cells also vary in size, being from 5 to 15$\mu$ in length, obviously owing to their relative states of maturity. This is shown in Fig. 29a, which also gives some idea of their variety of outline. The latter, however, seems to be the result of the accommodation of a number of semi-fluid bodies within a fairly tense

membrane—the yolk-cells within the follicular wall. That the yolk-cells are semi-fluid in consistency cannot be doubted when one observes them in their passage through the vitelline ducts, as noted above in connection with the description of the latter, and as shown in Fig. 29b, where the nucleus with its surrounding clear area is distending the wall of the duct. The nucleus and, for that matter, the whole cell in many cases, resembles that of the ova; in fact, it is often quite difficult to decide which is the ovum in the egg-complexes to be found in the uterine tube. In most follicles the smaller cells are arranged around the wall more or less like an epithelium, as described by Sommer and Landois for *D. latus*, while the larger ones are to be found in the middle. The wall itself is a very thin membrane in which no definite nuclei were seen, although small flattened nuclei situated between the yolk-cells and close to the wall may belong to it. Perhaps the most noteworthy peculiarity of the yolk-cell is the large almost clear area to be seen in the cytoplasm, often surrounding the nucleus (Fig. 29), which is doubtless the fluid yolk which will later be absorbed by the developing egg.

A short distance from the point where the oviduct receives the common vitelline duct are located the s h e l l - g l a n d s . Here the oviduct expands slightly—to a diameter of 20$\mu$. In most of the series examined the shell-glands formed a sort of vacuolated meshwork, in which, although there were to be seen well-developed nuclei, 4 to 5$\mu$ in diameter, it was extremely difficult to distinguish individual glands. However, in one series, where quite a length of oviduct was cut longitudinally, two or three club-shaped unicellular glands could be made out (Fig. 30). Their connections with the former were in the form of darkly-staining bars traversing the epithelium between lighter areas of about the same widths. Furthermore, numerous thread-like processes situated in the lumen, of the oviduct and directed towards the uterine tube corresponded with these dark bands, at least in position, since they were divided into groups, each group being opposite a dark band, as shown in the figure. While the outlines of the glands are quite difficult to discern, their connections with the oviduct are readily seen in sections through the region in almost any plane, tangential sections, for instance, showing dark circular spots on a much lighter background. Again, in younger proglottides treated with Mallory's stain, the glands and the otherwise dark bands appeared much lighter than the epithelium, which fact further supports the view that they are related anatomically. Thus, from the foregoing description, it appears that the processes in the lumen probably constitute the material secreted by the glands, and that this material is passed along from the bodies of the cells through the narrow necks which act as ductlets, suggestions which are strengthened by the facts that the so-called

secretion is to be seen mostly opposite the glands and that the ductlets have very thin but distinct walls.

The uterine tube ("Uteringang") is usually considered to commence immediately beyond the shell-glands, but in this species its first portion so closely resembles the oviduct posterior to the latter that the writer is inclined to place the region of demarcation somewhat farther ahead. The circular muscles are better and more uniformly developed, but what appears to be a decided augmentation in the number of cilia is probably a continuation of the threads of material secreted by the shell-glands. While the shell-gland region of the tube is directed dorsally, anteriorly and generally to the right of the proglottis, the beginning of the uterine tube makes a sharp turn and then passes backward again or expands immediately into what might be called the second division of the uterus, (Fig. 27). But this is not the second division of the uterus according to Braun ('00), since he does not seem to recognize in the "Uteringang", or uterine duct, two divisions, differing histologically; his second division is the uterus-sac, or "Uterushöhle". In this species it is in the form of a tube from 25 to 55$\mu$ in diameter, the walls of which are very thin and composed of a greatly extended epithelium in which quite flattened nuclei appear at irregular intervals. Commencing in the dorsal portion of the generative space, it courses forward in the median line above the ovarian isthmus as a somewhat flattened spiral, and in ripe proglottides often narrows down appreciably before entering the uterus-sac tangentially. It is usually filled with young eggs, each composed of many yolk-cells surrounding the "egg" (fertilized ovum) or a small number of cells resulting from the first divisions, all enveloped by a thin shell. In development the uterine duct is quite similar to the other ducts, possessing in the earliest stages a syncitial epithelium developed from an axial strand of cells surrounded by another layer of several cells in thickness, between which the basement membrane appears.

The uterus-sac ("Uterushöhle") arises in the same way from the middle piece of the elongated anlage (Figs. 15 and 16), but is from the outset distinctly separate from the uterine tube, the latter opening into it dorsally and slightly ahead of its posterior end. Beneath this opening the uterus-sac sends a diverticulum ventrally to the point where the aperture will later appear, while the remainder of the organ is directed forward in the median line some distance from the ventral surface, at first as a narrow tube, later as an elongated sac (Fig. 10). At this stage the wall of the uterus resembles, in general, that of the generative ducts. It is composed of a syncitial epithelium with scattered nuclei, a well-defined basement membrane, and outside of the latter a thin layer of parenchymatous cells. All of these parts are thinned out considerably

with growth, so that eventually, in gravid joints, the wall appears as a very thin membrane, showing practically no structure (Fig. 19). In slightly younger stages than that shown in Fig. 17 the lumen is not so uniform in outline, since its anterior half is divided into shallow evaginations, somewhat comparable to those seen in the uteri of the species of the order *Tetraphyllidea*. These soon become obliterated, however, the inside of the wall in gravid conditions begin quite smooth.

The opening of the uterus-sac, situated, as shown in Fig. 10, towards its posterior end, seems to function for a short time only, since in the longest strobilas no trace of it was found near the end of the chain. In the middle region, on the other hand, it appears as a very narrow slit, about 0.1 mm. in length, in only a few proglottides. Furthermore the uteri in which these openings are to be seen are generally almost free of eggs, as if the openings had been used for the dispersal of the eggs in the usual manner among the Bothriocephalids, while those behind the region in question are tensely filled. These facts would lead one to think that in most proglottides the eggs are freed by the rupture of the uterus and the body-wall, as in the higher cestodes, beginning with the *Tetraphyllidea*, while the uterine aperture either functions for a short time only or in proglottides, probably more or less constant in number and location. Fig. 31a is a view of the opening drawn from a transparent preparation in the uterus of which were comparatively few eggs. It is seen that the slit is surrounded by a clear area beyond which there is a more deeply-staining region. The latter is in reality made up of radially arranged nuclei which are related to the clear area in a manner better shown in coronal sections of stages prior to the breaking through of the slit (Fig. 31b). Here they are seen to be connected with the dark line, where the slit will appear later, by fine striations which continue farther out into the surrounding cytoplasm. Whether these radiating nuclei form a glandular organ around the aperture or give evidence of a migration from the clear area which remains as a cuticular rim, is difficult to say; but, from the close resemblance to the structure of the cirrus and of the entrance to the vagina, the writer is inclined to the latter view.

Development of the fertilized ovum, which begins, as mentioned above, in the uterine duct proceeds in the uterus-sac, eggs, bearing oncospheres, being obtainable from proglottides situated towards the posterior end of the strobila.

The e g g of this species is an ellipsoidal structure, from 60 to 70$\mu$ in length and from 40 to 43 $\mu$ in breadth. The shell is uncolored and perfectly transparent, so that the contents can be observed quite easily (Fig. 32). It is lined by a very delicate membrane which, however, can be seen only when it is, in some cases, separated from the former (Fig. 33).

The embryo is composed of two portions, an inner, the oncosphere, and an outer, the mantle or so-called ectodern, well supplied with cilia. The movements of the latter can be seen even when the embryo is within the shell, especially if a little pressure be applied to the cover-glass. In this case they vibrate so vigorously that the whole embryo is driven to the larger end of the egg, and numerous, supposedly vitelline granules, are kept continually in motion, and, at the same time, arranged in two groups, one close to the mantle and, to all appearances, among the bases of the cilia, and the other in the smaller posterior end of the egg. The shell is provided at its anterior end with a well-defined operculum, the raising of which, evidently due to the pressure from within, permits the escape of the embryo. This, however, is a somewhat difficult matter on account of the size of the latter, as can be seen from Fig. 33. As fast as the cilia are freed they proceed to vibrate strongly in the surrounding saline solution, and as soon as the embryo has escaped from the shell it swims away quickly, taking either straight courses or moving about erratically in irregular curves. It was also noticed that the cilia are all directed posteriorly from what might be called the apex of the body, both within and without the shell,—posteriorly, since this apex is anterior, not only from its direction during motion, but from its being situated at the end of the oncosphere opposite to that which accommodates the hooks. While the mantle is comparatively constant in size, its diameter being about 45$\mu$, the oncosphere varies from 30 to 35 $\mu$ in length. Practically no structure was observed in the substance of the mantle itself. The oncosphere, on the other hand, shows the usual three pairs of hooks, a pair of flame-cells and a few spherical bodies of doubtful significance (Fig. 32). The movements of the hooks and of the body of the oncosphere are quite typical. They take place even when the embryo is yet within the shell, but, as has been verified in many preparations, only when the whole egg has been stimulated by pressure, in which case they are quite irregular and necessarily considerably restricted. They are perhaps a little freer when the embryo, including the ciliated mantle in situ, is liberated. At rest the three pairs of hooks are arranged, as shown in Fig. 32, in the form of a tetrahedron, the apex of which is situated at the center of the oncosphere while the base is directed posteriorly. From this position the peripheral ends of the hooks approach each other until they are quite close together, while the central ends diverge towards the bounding membrane of the oncosphere. This causes a slight retraction of the tip of the latter. Then follows a comparatively vigorous separation of the hooks, to the extent that their outer ends are about 180° apart while the inner are close together. At the same time the individual hook protrudes from the surface of the

oncosphere up to the small process, situated a short distance from its tip (Fig. 32). This process and the slightly swollen central end of the hook seem to act as bases of attachment for what appears to be a well-developed musculature actuating them. The hooks again approach and the whole cycle is completed. In the most vigorous specimens these movements take place at the rate of about three per minute. As might be expected, the slightly smaller anterior end is much affected by the movements of the other end; however, it exhibits movements of its own, consisting of small waves of contraction commencing at the inner ends of the hooks and passing forward, thus in a direction opposite to those seen in the plerocercoid and in the young strobila.

Concerning the life-history of this species nothing can be offered at the present. It was only noticed, as mentioned at the outset, that plerocercoids a few millimeters in length were found in the intestine of the host along with the largest strobila taken. The food of *Amia calva* consists, however, evidently entirely of small fish, mostly minnows, and it is possible that one or more species of these are the intermediate hosts.

## Summary.

The form of the body of this worm is peculiar in that proglottidation is expressed externally only in the anterior end of the strobila, beginning immediately behind the scolex. Here the proglottis is provided at its hinder end with four ear-like appendages directed posteriorly, which, in conjunction with their fellows of the neighbouring joints, may act as important accessory organs of attachment, perhaps by forming temporary suckers or using certain rows of spines, arranged around their edges, to obtain a hold on the mucous membrane of the host's intestine. Posteriorly these appendages disappear, leaving no indication of proglottides apart from the sets of reproductive organs which follow each other at regular intervals in the usual manner.

The scolex differs little internally as well as externally from the foremost joints, the two bothria or suckers being comparatively feebly developed.

The musculature is particularly well expressed in the jointed region of the strobila which is consequently the most mobile. All of the usual groups of muscles to be seen in Bothriocephalids are present, the external longitudinal fibers being quite distinct from the inner or longitudinal muscles of the parenchyma but confined to the anterior end only of the strobila, while the outer transverse series is divided into two sets on each surface of the proglottis, the fibers of which are directed postero-laterally and thus made to decussate in the mid-line. The individual fibers of nearly all of the groups of muscles are characterized by having their

cortical or contractile layers divided up into a number of fibrils, which, however, still retain their connections with the protoplasmic substance of the myoblasts.

The nervous system consists of two chief strands, situated laterally in the medullary parenchyma ("Markschicht") and united beneath the tip of the scolex to form a very small ganglionic ring. Connected with these are eight collateral strands, four located around each chief strand, which appear in the jointed portion of the strobila only.

The excretory system is composed of one large median vessel,—the equivalent of the usual dorsal pair—and two smaller, situated laterally and ventrally. All of these unite in the scolex to form a median vesicle accommodated in the hollow behind the nerve-ring. Foramina secundaria and flame-cells are fairly numerous, but their connections are difficult to trace.

The genital organs are simple, on the whole resembling those of *Dibothriocephalus latus* (Linn.). The genital apertures are all situated on the ventral surface in the median line, that of the vagina close behind the cirrus-opening towards the anterior end of the proglottis, that of the uterus much farther back and evidently a temporary aperture only. There is no distinct genital atrium or cloaca.

The testes are all in one plane and separated into two lateral fields by the median excretory vessel. Opposite the genital ducts both testes and vitelline glands separate dorsally and ventrally to leave clear "middle fields". The vas-deferens, which courses in the median line dorsal to the uterus-sac, is provided, at its posterior end near the middle of the proglottis, with a sperm-reservoir, and with a large almost spherical seminal vesicle situated immediately behind the cirrus-pouch. The latter is spheroidal in shape, simple in structure, and contains the continuation of the vas-deferens, divided into three regions, an ejaculatory duct, a second seminal vesicle and the cirrus. The cirrus is lined with cuticle in which there are small stout spines and around which there is a series of well developed circular muscles.

The vagina, the entrance to which is also lined with cuticle and supplied with a sphincter muscle, courses ventrally and expands within the "generative space" to form a seminal receptacle, sharply separated from the very small and short continuation, the spermaduct which unites with the oviduct in the usual way. The ovary and shell-glands are median and respectively ventral and dorsal. The yolk-glands are composed of numerous follicles, arranged cylindrically around the testes,—both within the longitudinal muscles of the parenchyma. There is a large yolk-reservior, situated in the generative space. The uterus is divided into two distinct portions from the earliest appearances in the

common genital anlage, a much-coiled, proximal, thin-walled tube, the uterine tube ("Uteringang"), and a capacious uterus-sac ("Uterushöhle") which, when gravid, occupies almost the whole of the central portion of the proglottis. The eggs are provided with opercula.

All of the genital ducts are lined with an epithelium which, on account of cell-boundaries being almost entirely absent, is of the nature of a syncitium. In certain regions, namely, in the cirrus and in the entrance to the vagina, this syncitial epithelium becomes transformed into cuticle with an accompanying migration of its nuclei through the basement-membrane and into the surrounding parenchymal cytoplasm.

From the foregoing description it is to be seen that, although this species is in most respects a typical Bothriocephalid, its characters are such as to render the placing of it in the existing classification of the families and genera of the order, Pseudophyllidea, a matter of considerable difficulty. However, since this subject is dealt with in another paper, as mentioned at the outset, it will be sufficient to state here that, so far as the writer has been able to ascertain, this is a new species of cestode which must also be accommodated in a new genus. Consequently, the following names are proposed: Genus, *Haplobothrium* (ἁπλοῦς, simple; βοθρίον, a small hollow or trench); species, *globuliforme*, (*globulus*, a bead; *forma*, shape or form), the significance of which specific name has been referred to above.

The type-specimen of this species is included in the writer's private collection, while a co-type has been donated to Dr. H. B. Ward of the University of Illinois.

Biological Department,
University of Toronto,
*August, 1914.*

## EXPLANATION OF FIGURES.

All of the figures are camera-lucida drawings, excepting Figs. 34 to 41, inclusive, which are photomicrographs of sections or portions of sections.

*c*, cirrus.
*ccm*, circular cuticular muscles.
*cm*, coronal muscles.
*cmc*, circular muscles of cirrus.
*cn*, collateral nerves.
*cu*, cuticula.
*cub*, cuticula of cirrus.
*D*, dorsal.
*g*, ganglionic ring.
*ivs*, second vesicula seminalis.
*lev*, lateral excretory vessel.
*lmp*, longitudinal muscles of parenchyma.
*mev*, median excretory vessel.
*n*, nucleus.
*olm*, outer longitudinal muscles.
*pc*, cells of parenchyma.
*rmp*, retractor muscles of cirrus.
*rs*, receptaculum seminis.
*rvd*, right vitelline duct.
*sc*, cells of subcuticula.
*sg*, shell-gland.
*sr*, sperm-reservoir.
*t*, testes.
*us*, uterus-sac.
*ut*, uterine tube.
*V*, ventral.
*v*, vagina.
*ve*, vas efferens.
*vf*, vitelline follicles.
*ns*, nerve strand.

## PLATE V.

Fig. 1. Scolex and first three proglottides, × 32.
Fig. 2. 12th, 13th and 14th proglottides, × 8
Fig. 3. Proglottides 20 to 25, inclusive, coronal view, × 8.
Fig. 4. Same, laternal view, showing disappearance of auricular appendages, × 8.
Fig. 5. Young "scolex," showing beginning of proglottidation, × 16.
Fig. 6. Smallest plerocercoid observed, × 16.
Fig. 7. Longitudinal section through the cuticle and subcuticle: *cu'*, outer and *cu''*, inner layers of the cuticle; *fs*, foramen secundarium of the excretory system; *bm*, basement membrane; *lcm*, longitudinal cuticular muscles, × 1600.
Fig. 8. Longitudinal section through the tip of an appendage, showing the minute spines, × 1500.
Fig. 9. Relations between myoblasts and muscle-fibrils: a, coronal fiber; b, c and d, myoblasts of longitudinal muscles of the parenchyma, × 1500.
Fig. 10. Transverse sections of muscle-fibers: a and b, from the external longitudinal series of the parenchyma; c, from the dorso-ventral group, actuating the bothria; *f*, fibrils, × 1500.

Fig. 11. Reconstruction of the nervous system in the scolex and anterior proglottides, × 40.
Fig. 12. Coronal section of the "end-proglottis", showing the relations of excretory vessels; *cv*, dilatation of the median excretory vessel, × 150.
Fig. 13. Flame-cell: *bb*, basal body; *cf*, cilliary flame, × 300.
Fig. 14. Transverse section of a lateral excretory vessel in the posterior end of the scolex: *bm*, basement membrane; *dvm*, dorso-ventral muscles, × 1100.
Fig. 15. Proglottis 16 of a strobila, showing very early stage in the development of the reproductive organs: *agd*, anlage of the genital ducts, × 37.
Fig. 16. 17th proglottis of same strobila, × 37. Figs. 15 and 16 are drawn from oil-of-cedar transparencies.
Fig. 17. Transparent preparation of a mature proglottis,—testes and vitelline follicles not complete: *vs*, vesicula seminalis; *vd*, vas deferens; *cp*, cirrus-pouch; *co*, cirrus-opening; *vo*, aperture of vagina; *uo*, opening of uterus; *ov*, ovary; × 60.
Fig. 18. Transverse section through the interproglottidal region of the unjointed portion of the strobila, × 130.
Fig. 19. Same through the middle of the proglottis, only two eggs shown in the gravid uterus-sac, × 130.
Fig. 20. A single testis with its vas efferens: *cyt*, cytophore, × 365.
Fig. 21. Sperm-reservoir at the posterior end of the vas deferens, × 365.
Fig. 22. Anastomos of vasa efferentia near the sperm-reservoir: *m*, median line of the proglottis, × 200.

## PLATE VI.

Fig. 23. Cross-sections of vas deferens: *ev*, syncitial epithelium; *mcm*, myoblasts of circular muscles, × 1000.
Fig. 24. Cross-section of cirrus, × 500.
Fig. 25. Cross-section of younger cirrus, × 500.
Fig. 26. Longitudinal section of receptaculum seminis and first portion of spermaduct: *s*, sperms; *sd*, spermaduct, × 235.
Fig. 27. Genital ducts in the generative space, posterior view of a reconstruction: *cvd*, common vitelline duct; *od*, oviduct; *sd*, spermaduct; *oc*, oocapt; *yr*, yolk-reservoir, × 60.
Fig. 28. Ova, showing accessory cells in connection with two: *ac*, accessory cells; *cy*, cytoplasm, × 1000.
Fig. 29. Individual yolk-cells: a, from follicles; b, from a collecting yolk-duct, × 1000.

Fig. 30. Longitudinal section of the shell glands; *eod*, epithelium of the oviduct, × 1000.

Fig. 31. The uterus-opening: a, from a transparency; b, from a coronal section before the formation of the aperture, × 100.

Fig. 32. The egg, showing contained embryo, × 500.

Fig. 33. Another, showing the escape of the oncosphere surrounded by the ciliated mantle, × 500.

## PLATE VII.

Fig. 34. Photograph of a transverse section through the middle of the scolex: *ots*, outer transverse muscles; *b*, bothrium; *dvb*, dorso-ventral muscles of the bothria.

Fig. 35. Transverse section through the posterior end of one of the foremost proglottides: *app.* auricular appendage; *ldvm*, lateral dorsoventral muscles.

Fig. 36. Coronal section, slightly aside from the median line, through the scolex and first two proglottides: *ofs*, oblique fibres of the scolex; *cs*, cuticular spinules.

Fig. 37. Similar section, in the median coronal plane: *aev*, anterior excretory vesicle; *laf*, longitudinally arcuate fibers of the scolex; *mb*, bothrial muscles.

Fig. 38. Portion of a transverse section through the cirrus-pouch: *de*, ductus ejaculatorius; *wcp*, wall of the cirrus-pouch.

Fig. 39. Coronal sections through the vagina and its entrance, showing a migrating nucleus at *x*.

Fig. 40. Portion of a longitudinal section through the vagina, showing two nuclei at *x* and *y* leaving the epithelium.

Fig. 41. Another portion of the vagina, showing two nuclei passing through the basement membrane. Lettering as in the last figure.

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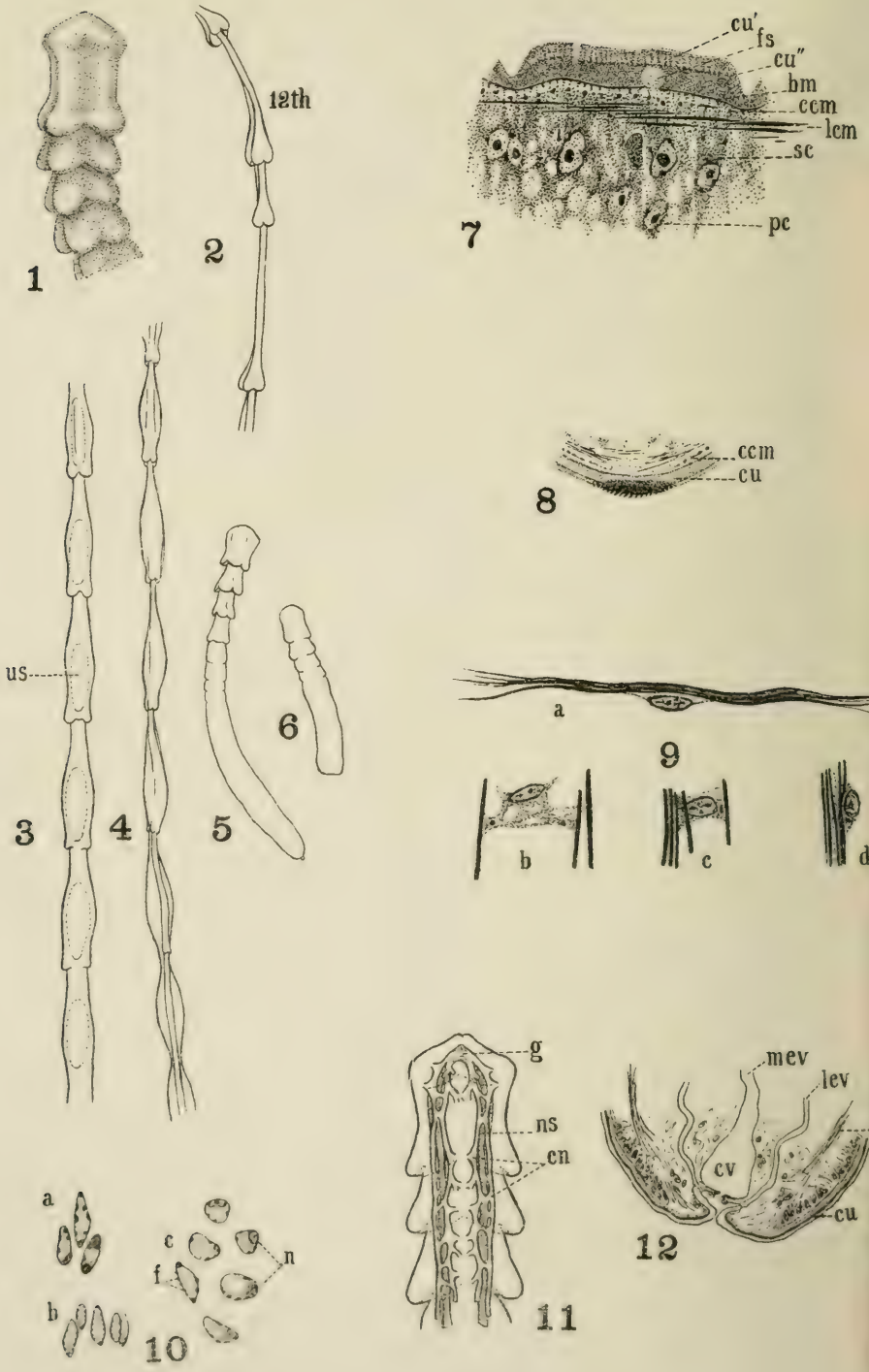


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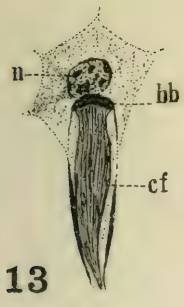


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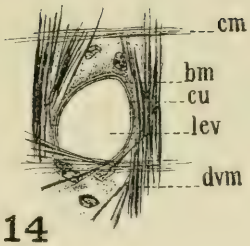


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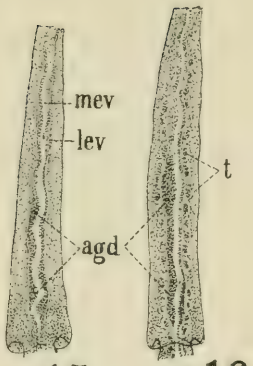


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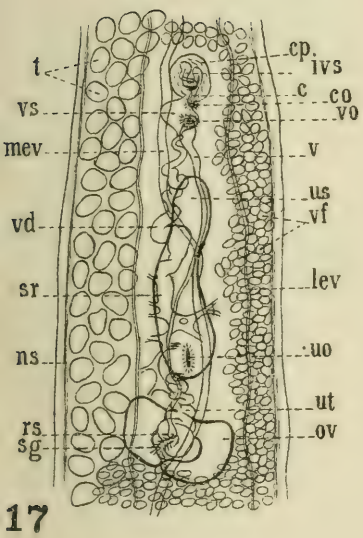


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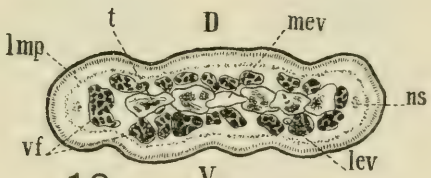


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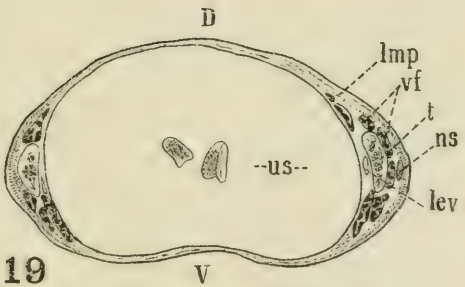


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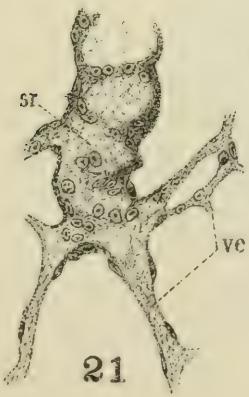


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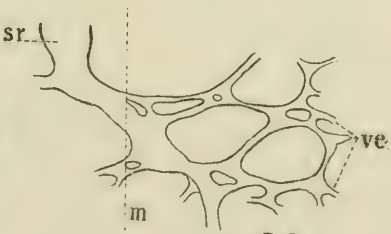


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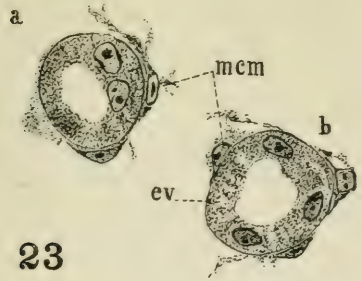


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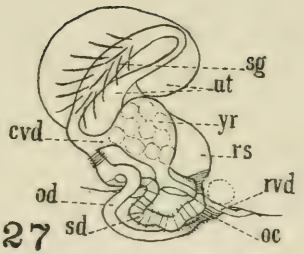


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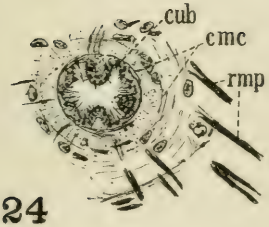


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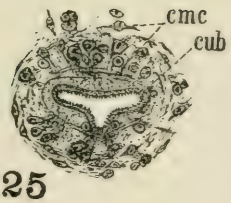


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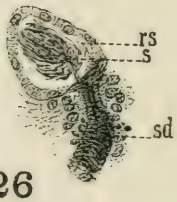


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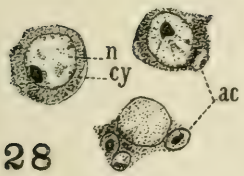


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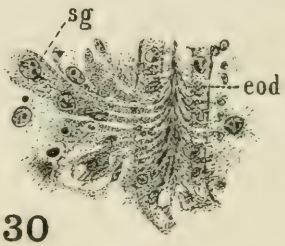


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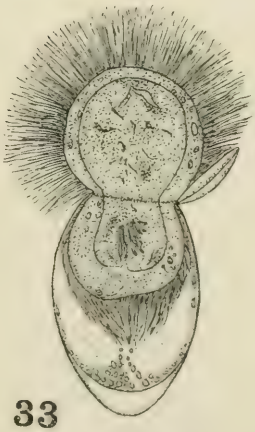

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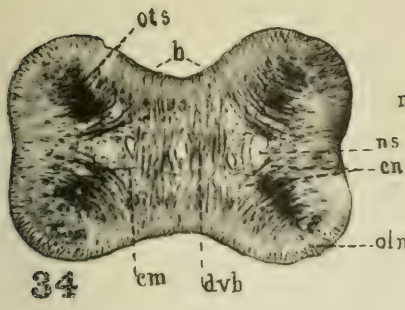


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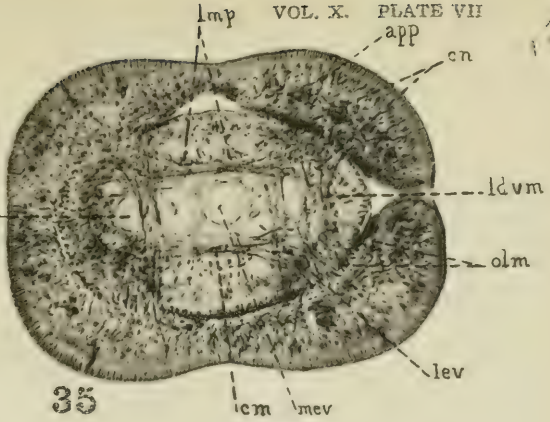


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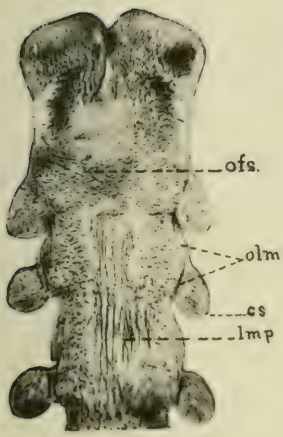


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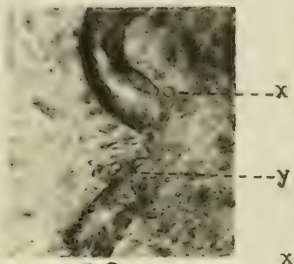


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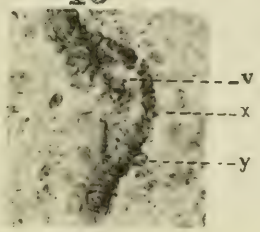


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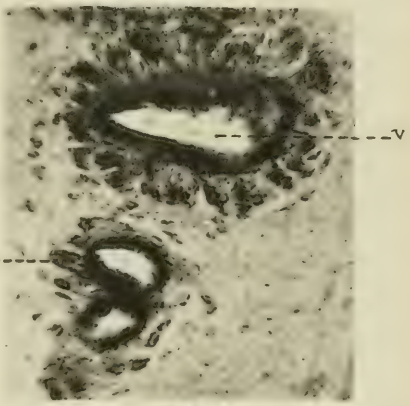


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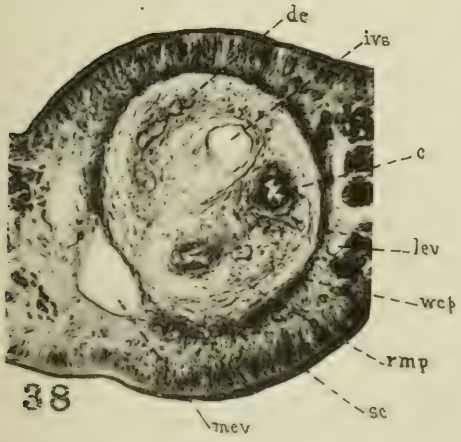


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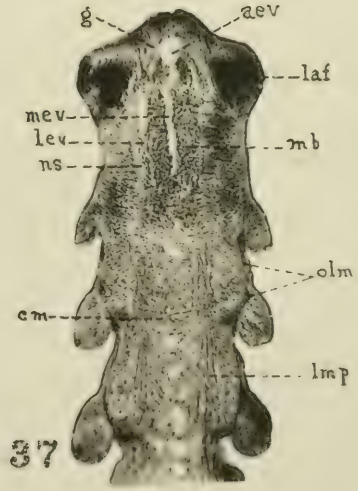


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UNIVERSITY OF TORONTO STUDIES

BIOLOGICAL SERIES

No. 16: THE EGG-LAYING HABITS OF *PLETHODON CINEREUS*, BY W. H. PIERSOL

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# THE EGG-LAYING HABITS OF *PLETHODON CINEREUS*

BY W H. PIERSOL, B.A., M.B.

(*Read 15th November, 1913.*)

TWO accounts of the natural history of this, our commonest salamander, have appeared, one by Miss M. E. Cochran (1911) and one by the writer (1909). Both agree in their descriptions of the eggs, but neither gives any information as to the mode of deposition. The writer has sought to determine this by observations made on *Plethodon* both in its natural habitat and in a terrarium. The following is an account of the more important observations together with comment and inference. Fertilization is internal; this had been predicted in the earlier paper (1909) and has since been confirmed by the fact that a female isolated in a terrarium for four days laid eggs that developed naturally.

*Case I.* On one occasion the actual extrusion of the eggs was observed. The female had been placed when captured in a small glass jar along with fragments of the log in which she was found; and the jar with others containing eggs was carried back to the laboratory in a small bag. Chiefly for the sake of the eggs which are very delicate the bag was guarded from shocks as far as possible, then for another hour it stood unopened. On removing the jar from the bag it was seen that the egg laying had just begun, fortunately in such a position that all its details could be observed. The lips of the cloaca are pressed against the surface from which the eggs will eventually hang and a small quantity of mucus is extruded and adheres firmly to it. This much had been completed before observation began so nothing can be said as to the interval that then elapses before the first egg is laid. The extrusion of each egg occupies about twenty seconds and an interval of five to ten minutes occurs before the next appears. The first three eggs were laid in contact with the mucus above mentioned; the fourth, and last, adhered to them in turn through the stickiness of the egg-envelopes. As the female did not move during the entire process, all the eggs were laid at the same point, each egg as it came, crowding the preceding ones aside, thus making sure of being in contact with them. For over an hour after the last egg was laid the female did not change her position; during the next hour she left the eggs a few minutes, then returned and coiled herself about them.

The extrusion of the egg causes it to become elongated; the greater axis may be almost twice the less. In the case above noted the spherical form was assumed within a few minutes; in other cases the elongation has taken more than an hour to disappear. Exceptionally the elongated form may be retained for a considerable time. The most extreme case met with was an egg found among natural surroundings with the longest axis 5.25 m.m. and the shortest 2.75 m.m. In the same cluster was another elongated egg, its axes being 4.0 m.m. and 3.0 m.m. The three remaining eggs were spherical; all five were in the process of gastrulation. Another egg, quite similar to the one first mentioned was laid by a female in a terrarium; it kept pace in development with the remaining eggs of its cluster up to the 50-60 cell stage. In the first two cases the segmentation cavity had formed near one end of the long axis, in the third case near one end of a short axis. As the eggs were fixed at the stages mentioned it is impossible to say how the further development would have been affected.

This mode of egg-laying places *Plethodon* at the end of a progressive series, the most primitive member being Cryptobranchus, with eggs laid in a uniform rosary-like string as described by Reese (1904) and Smith (1906). Next, as suggested by Wilder (1913), would stand *Desmognathus;* in this genus most of the eggs have left the main string of the rosary and lie at the sides of it, each retaining connection with it, however, by a short stalk. The next step is represented by such a case as *Spelerpes* (Wilder, 1899) or *Antodax* (Ritter and Miller, 1899); here the disappearance of the main string leaves each egg to be attached separately to its support—usually a stone—by a short stalk. The disappearance of this stalk for each egg, except the first, produces the separate eggs of *Plethodon*. This economy of material is highly desirable in so small an animal. The position of *Antodax* in the series given above is not that usually occupied by the genus in a series that shows progressive modification of some primitive habit; in most respects *Antodax* has departed furthest from the primitive amphibian mode of life, and *Plethodon* can only offer suggestions as to the path along which *Antodax* has travelled to its present condition. In habits, however, as in morphology, it does not follow that the higher member of a series must in every point have progressed beyond the lower.

Other observations differing from the foregoing are as follows:

*Case II.* In examining a terrarium on one occasion there was uncovered a female that had evidently just completed the extrusion of the eggs. Two eggs, approximately spherical, were in contact and cohering slightly; four other eggs, each more or less elongated, were lying separated from each other by intervals of about one-quarter of an inch; none

of them were suspended. Evidently under the somewhat unnatural conditions the female had moved after the extrusion of each of the last five eggs. *Case III.* In picking apart a decaying log there were exposed on one occasion a female and four eggs. One of these lay by itself, markedly elongated; the other three were in contact, two of them somewhat elongated, the third apparently spherical. All four were lying on the floor of the cavity, which fortunately had been opened from the side. Examination of the female revealed the existence of four eggs in the posterior parts of the oviducts. Evidently the egg-laying process had been interrupted by the opening up of the nesting-chamber.

These last two cases have been selected from among a few of the same general character because they differ from the rest in that the eggs were not suspended. In opening up logs a few clusters have been found unattached. At first, in such cases, it was taken for granted that the opening up of the nesting-chamber had involved the loosening of the eggs. Since attention has been directed to the possibility of a cluster not having been attached, two such have been found under circumstances that would seem to preclude the idea of their having been torn from their attachment. In neither of these two cases could a stalk attached to the cluster be found. It would seem that occasionally the tendency to reduce the amount of material devoted to forming stalks for the eggs goes so far as to eliminate even the stalk of the first egg. No exact count has been kept of the number of such cases as compared with the normal, attached ones, but the impression left is that it is very small.

As might be concluded from *Case I*, an examination of the relation of the stalk to the eggs shows that it does not come from any one particular egg, but from a quantity of mucus that adheres to the outer envelope of certain of them; the impression given is that of a material poured onto the bunch, part of it being drawn out to form the stalk. As is the usual case among Urodeles the outer envelope of each egg is of a much more sticky mucus than the inner ones. Plethodon is peculiar in having this outer layer unusually thin, and in depositing a still more sticky mass of mucus before the egg-laying proper begins.

In most amphibia the impulse toward the deposition of the eggs, once these are ready for the act, is an imperative one. In some cases (*e.g.*, many frogs) the assistance of the male is needed, but generally speaking, when the proper time comes the spawn will be deposited even with conditions and surroundings that are far from natural. Both *Rana pipiens* and *Rana catesbiana* that have been kept over winter, without feeding, in a tank in the basement of the Biological Building of the University, have been known to spawn in spring and early summer respectively. (Such spawn has never developed, evidently has never

been fertilized.) In *Plethodon* the instinct is more delicately adjusted. This is shown in the marked preference for some particular log as a site for egg-laying. For instance, one small plot of woodland was found to contain *Plethodon* in abundance during the spring of 1913 and was visited on June 21st in the search for eggs. A dozen or more rotting logs yielded only males or sexually immature specimens; at last one log was found which, though apparently not differing from the others, yielded eleven females with eggs. A number of similar cases have been met with. The logs so greatly preferred are invariably conifers, but other factors must enter into the quest on for another coniferous log that seems quite similar may be close at hand yet be entirely destitute. Equally striking is the difficulty that has been experienced in getting females to lay eggs in a terrarium. The thin, almost translucent ventral wall of the abdomen allows the easy recognition of females containing eggs almost ready for deposition. If pieces of the logs in which the animals have been found are brought from the field and the pieces piled together in a terrarium so as to reconstruct roughly the log, there is no difficulty in keeping the animals alive and in good condition for long periods. They will feed readily on small insects, *e.g.*, aphids; but, like most amphibia, seem to suffer little from long deprivation. Three specimens overlooked in a small terrarium last spring lived until the end of September with no attention; at the end of that period their physical condition and vigor had suffered so little that they could not be recognised after being allowed to mingle with others brought in from the field. In spite of this apparent easy acceptance of life in a terrarium, the change usually is sufficient to inhibit the egg-laying reactions, and the eggs are retained and absorbed during the next five or six weeks. Exceptionally they will be laid as under natural conditions, but only when the female has been brought from the field not more than three or four days before the time for egg-laying. It is not a question of previous impregnation or its lack, for as far as examined, all mature females have been found to have the receptacles filled with sperm some time before the egg-laying season arrives.

The character of the season has some influence on the depth beneath the surface at which the eggs are laid; in damp seasons they will be for the most part but an inch below the surface, in dry seasons they will be four or five inches below. This refers to the character of the season up to the time of egg-laying, not after.

The retention of one egg in the ovary was mentioned in the earlier paper. Later experience has confirmed the observation. The egg is always much under-sized and occurs in about one third of the females accompanying clusters of eggs in early stages of development; it is then rapidly absorbed, and must have considerable value as a

supply of nourishment for the female during her wait by the eggs. Occasionally it will almost equal the remaining eggs in size and then will be laid along with them, producing a cluster with one markedly small egg. For example, in one cluster of seven eggs, six of them had a diameter of 3.75 m.m., the remaining one of 2.75 m.m. From a difference so marked as this there is a gradual transition to the state where all the eggs of the cluster are the same size; such are about one half of all cases. The writer has twice found similarly undersized eggs of *Amblystoma;* the numbers were small, nine and eleven in the two cases, and the eggs of but two-thirds the normal size. They developed normally, producing under-sized larvae which were perfect anatomically but defective in their feeding instincts. The one lot would not feed at all; the other would snap fitfully at *Cyclops*, etc., but would not eat enough to grow or even to maintain life. This was quite striking for both lots were the species *jeffersonianum* the larvæ of which are normally voracious feeders and easy to raise. In *Plethodon* the early development of the small egg is quite normal, its fate has never been followed past the time when the larva is well formed.

One female, kept in a terrarium with her eggs, swallowed two of them, and three hours later regurgitated them. The eggs were killed by the process, whether by digestive action or by the mechanical violence it is impossible to say, for they were in the process of gastrulation at the time. This is a most critical period for the egg, its delicacy is at the maximum and very slight disturbance will cause its death. The swallowing of their spawn has been noted for many amphibia, usually where, as above, something has happened to pervert the natural instincts. Smith (1907) however, describes it as normal for *Cryptobranchus;* in this case moreover when regurgitated the eggs frequently continue to develop.

Means taken to determine the mating habits have so far been fruitless. The single observation of Wilder (1913) on *Desmognathus* is probably a close approximation to the habits of *Plethodon* in this respect.

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