THE ORIGIN AND GROWTH OF THE GONADS IN HYDROIDS

ORIGIN AND GROWTH OF THE GERM CELLS OF TUBULARIA CROCEA WITH SPECIAL REFERENCE TO THE GERM-PLASM THEORY

by

Chien-Kang Liu

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the require-ment for the degree of Doctor of Philosophy in Zoology

McGill University, Montreal

October, 1947

ACKNOWLEDGEMENT

The author wishes to express his sincere thanks to Dr. N. J. Berrill, Strathcona Professor of Zoology, for the suggestion of this problem and his valuable advices in supervising this investigation. He also wishes to thank Dr. Dashu Nie, University of Pennsylvania, for his interest and discussion of various aspects of this work. To Mrs. Ruth Schachter, the author owes his appreciation for the rendering of the German passage quoted in this paper, and to Mr. J. R. Shaver, University of Pennsylvania, for the ribonuclease solution which he put at the author's disposal. Acknowledgement is also due to Mr. C. P. Hodge, Miss Hannah T. Broomell and Mr. J. W. Pollock for the skillfulness they showed in preparing the photomicrographs, and to Miss Helen Kielland and Miss Sue Williams for the neat presentation of this thesis.

TABLE OF CONTENTS

A. HISTORICAL REVIEW OF THE ORIGIN OF GERM CELLS IN HYDROZOA

1. ORIGIN IN SPACE

The members of the class Hydrozoa are the only coelenterates that are really diploblastic (Hyman '40). The general body plan, polyp and medusa alike, consists of an outer epidermis and an inner gastrodermis, separated from each other by a thin non-cellular mesolamella. The epidermis is derived from the embryonic ectoderm, while the gastrodermis is formed of the endoderm. Whether the sexual products originate from any particular layer, or whether there exists a sexual polarity between the two layers, has long attracted the attention of zoologists since the enunciation of von Baer's germ-layer theory. Many papers have dealt with the topographical origin of the germ cells in this group of animal, the results of most of which are summarized below in chronological order.

Dujardin (1845) found the hydroid Stauridium budding off small medusae which he called Cladonema. The latter have, in the endoderm of the manubrium, about a dozen of round reddish eggs which upon fertilization give rise to young Stauridium. The genital products are therefore regarded as endodermal in origin.

Gosse (1853) in the medusa Turris neglecta describes the eggs as taking origin in the endoderm of the manubrium. The fertilized eggs give rise to the ciliated planulae which develop into polyps bearing the characteristics of the genus Clava.

Huxley (1859) in his monograph on Calycophoridae and Physophoridae, believed that it is the wall of the hydrosoma, more particularly the ectoderm, that forms the spermatozoa or ova.

Agassiz (1860) observes the development of gonophore of Tubularia couthouyi, Parypha crocea and Thamocnidia spectabilis, and considers the entocodon as the place where germ cells develop. The entocodon is taken to be ectodermal.

Keferstein and Ehlers (1861) studied the siphonophores of Messina, and concluded the genital products to be ectodermal in origin.

Haeckel (1864) working on trachyline medusa Geryonides, stated that the egg as well as the spermatozoan originates from the endoderm.

Allman (1871) in his monograph on gymnoblastic hydroids gave his opinion that it is difficult to determine the actual origin of the generative elements, because in most cases they are seen filling the space between ectoderm and endoderm. From some favorable observations, however, he was convinced that the true origin of the ova and spermatozoa is to be found in the endoderm.

Schulze (1871) found that in Cordylophora lacustris the eggs, as soon as they are recognizable, are located outside of the membrane that separates the two layers. The eggs are therefore derived from the ectoderm.

Kleinenberg (1872) described in detail the formation of testis and ovary of Hydra at the expense of its interstitial tissue, which he considered to be merely the deeper part of the ectoderm.

Van Beneden (1874) was surprised at the diversity of opinion on this subject. In his research on germ cell origin of Hydractinia echinata, he discovered that the eggs develop exclusively from the epithelial cells of the endoderm, while the testes are formed at the expense of the ectoderm which has been invaginated to form the entocodon. The endoderm and the ectoderm are therefore sexually polarized. Van Beneden indeed extends

this conclusion to the germ layers of the "gastrulae": "The endoderm and the ectoderm have, on the sexual point of view, an opposed significance". The ectoderm is the male layer, the endoderm the female layer, and fertilization is to the effect of reassembling the chemical elements of opposed polarity as carried respectively by sperm and egg.

Grobben (1875) reported the eggs to arise from the ectoderm of the manubrium in Podocoryne carnea.

Allman (1875) considered the eggs of Myriothela phrygia to be formed from the endoderm, yet did not deny the possibility of their origin from the ectoderm.

Koch (1876), working on the female medusa buds of Coryne fruticosa, Tubularia larynx and the male medusa buds of Hydractinia echinata, T. larynx, T. Couthouyi and Parypha crocea, completely agrees with van Beneden's view on the sexual specificity of the two body layers.

Korotneff (1876), based on his histological study of Hydra, concluded that both male and female germ cells arise from the intermediary cells which may exist on both sides of the mesoglea. Consequently it is immaterial whether the sexual elements find themselves in the ectoderm or in the endoderm.

Ciamician (1878) reported that in Tubularia mesembryanthemum, eggs as well as sperm develop from the ectoderm. In Eudendrium ramosum, however, the egg is from ectoderm while sperm is from endoderm. Only in Hydractinia is Beneden's hypothesis verified.

Bergh (1879) in his study on the development of Gonothyræa loveni, supported van Beneden's view without even making a detailed study of the male gonophore.

Merejkowsky (1880) found the eggs of Eucope always developed beneath the mesolamella, hence endodermal in origin. Another reason for believing this is that a gradual transition exists between ordinary endodermal cells and the young eggs.

Korotneff (1880) working on Myriothela phrygia, concluded with "absolute certainty" that the genital products are of endodermal origin.

Fraipont (1880) confirmed van Beneden's idea in Campanularia angulata and C. flexuosa. Having found all transitional stages from ordinary endoderm cell to eggs in the entire endoderm he came to the conclusion that the ability to form egg is not limited to the gonophore region, but rather is possessed by the endoderm as a whole.

Goette (1880) described a new species of marine hydroid which does not form a definite sex organ of any kind. The eggs of this new species, Hydrella ovipara, are formed at the expense of endodermal cells of the stem, remaining there until maturity, when they are liberated.

Weismann (1880 a) agreed with Ciamician concerning the germ cell origin of Tubularia mesembryanthemum. As to that of Eudendrium ramosum, he considered what Ciamician stated was only a half-truth, because the eggs likewise take origin in the endoderm. His observation on Gonothyraea loveni confirmed Bergh's result, and study on Sertularella polyzonias, Plumularia setacea and Aglaophenia pluma enabled him to state that in all of them both male and female cells are formed from endoderm. He concluded by saying there are only three possible combinations with reference to sexuality and germ layers: (a) both sex cells originate from ectoderm, (b) both originate from endoderm, and (c) sperm from ectoderm and egg from endoderm.

In a second paper, Weismann (1880 b) considered the presence of egg cells in the stem and branches of the polyp hitherto sporadically reported by other workers to be much more widespread in occurrence and to have definite significance. He recognized in the hydroids two types of germ cell formation: (a) the blastogonic formation; the sex cells take origin in the sexual bud (sporosac, gonophore, or medusoid), and (b) the coenogonic formation; the oocytes first appear in stem or branches of the colony. In the case of coenogonic formation, the sex cells, arising from the transformation of either ectodermal or endodermal cells, appear even before the sexual bud, and their presence seems to determine the formation of the latter.

Kleinenberg (1881) was perhaps the first to suggest a migration of egg cells. In a certain period of development eggs can be found regularly in ectoderm as well as in endoderm of Eudendrium. Since no intermediate stages are found between endoderm cell and egg, he was inclined to think that the eggs are transformed ectoderm cells which, in all or in large part, would migrate into the endoderm through the mesolamella.

De Varenne (1882) studied the origin of egg and sperm in Campanularia flexuosa, Gonothyraea loveni and Podocoryne carnea, and of the egg alone of Plumularia echinulata, Sertularia pumila and Obelia geniculata. They were chosen to represent three types of sexual generation: sporosac, gonophore, and medusa. He concluded that male as well as female germ cells take origin in the endoderm of the coenosarc. The transformation of differentiated endoderm cells into germ cells is especially clear in the female. It was incidental that the species he examined were all coenogonic.

Hamann (1882) described the formation of genital products of Tubularia coronata. He found them to be derived from the inner layer of the

entocodon, which in turn is formed from a proliferation of ectoderm cells. He recognized Gonothyraea loveni and Plumularia fragilis as coenogonic, while Hydractinia echinata and the species of Tubularia (indivisa, coronata, mesembryanthemum, larynx),as blastogonic.

Jickeli, (1883) found certain cells in the ectoderm of Tubularia mesembryanthemum similar to what others described indiscriminately as interstitial cells. He was inclined to consider them as prospective egg cells and would later on move into the entocodon.

Weismann (1883) conducted a detailed investigation on the germ cell formation in Hydrozoa belonging to 14 different families. Among the 33 species he studied, 29 are true hydroids and 4 are siphonophores. His data is condensed as follows: 12 species have their sex cells, both male and female, originated from young ectodermal cells: Hydra (sp. not given), Dendroclava dohrnii, Cordylophora lacustris, Syncoryne sarsii, Cladocoryne floccosa, Heterocordyle conybeari, Bougainvillia ramosa, Perigonimus cidaritis, Cladonema radiatum, Pennaria cavolini, Corymorpha sp. and Tubularia mesembryanthemum.

13 species have their sex cells, both male and female, derived from either young endoderm cells or from those ectodermal cells which have migrated into the endoderm: Corydendrium parasiticum, Coryne pusilla, Hydractinia echinata, Pachycordyle conybeari, Gonothyraea loveni, Opercularella lacerata, Halecium tenellum, Halecium halecinum, Sertularella polyzonias, Sertularia pumila, Plumularia echinulata, Plumularia halecioides and Antennularia antennina.

4 species have an endodermal origin; they are all siphonophores: Hippopodius neapolitanus, Galeolaria aurantiaca, Forskalia contorta and Agalma rubrum,

3 species have their male germ cells formed from young ectoderm cells while their female germ cells from either young endoderm cells or those ectoderm cells which have migrated into the endoderm: Clava squamata, Podocoryne carnea and Campanularia flexuosa.

Lastly, 1 odd species, Eudendrium racemosum, has just the reverse arrangement: i.e., the eggs come from young ectoderm cells whereas the spermatozoa arise either from young endoderm cells or from migrated ectoderm cells. It is to be noted that when Weismann states "either from young endoderm cells or from those ectoderm cells which migrated into the endoderm", he means that they arise from young endoderm cells in direct observation, but that they should in theory be considered as immigrated ectoderm cells. This investigation has usually been cited as supplying evidence to his germ-plasm theory, and will be subject to analysis elsewhere in this thesis.

Thallwitz (1885) studied the development of male germ cells of several hydroids. An ectodermal origin was reported for Tubularia mesembryanthemum, Podocoryne carnea and Cladoryne floccosa, an endodermal origin for Sertularella polyzonias, Plumularia echinulata, Gonothyraea loveni and Hydractinia echinata. In the last-named species the male germ cells, as well as the eggs, arise from the endoderm of the blastostyle, migrate subsequently to the sex bud and then to the entocodon. In Gonothyrea loveni, the male germ cells differentiate in the endoderm of the stem and later find their way into the entocodon, which is itself of ectodermal origin.

Tichomiroff (1887) was of the opinion that the entocodon is of

ectodermal nature, but that the genital cells are formed by the endoderm. He observed that the tip of the endoderm of the medusa bud at the earliest stage divides into an upper half, formed by cubical cells, and a lower half, formed by cylindrical cells. The upper part is the germinal zone, thickens at the base of the entocodon and, in doing so, projects into the gastric cavity. The germ cells, however , gradually migrate into the entocodon.

Ishikawa (1887) attempted to prove Weismann's hypothesis by showing that in male Eudendrium racemosum, the young germ cells which can be seen in the endoderm of very early gonophores arise actually from the ectoderm. Their migration through the mesolamella, however, could not be observed. In a later study (1888) he obtains same results in female Podocoryne carnea. In his drawings for both species, the primordial germ cell cannot be distinguished from the nematocytes.

Korotneff (1888), hitherto an advocate of an endodermal origin, reported that in Myriothela the starting point of a male as well as a female is a primordial germ cell of ectodermal origin. The germinal mass is later enclosed by an endodermal sheath.

Brauer (1891) studied the development of Tubularia mesembryanthemum, and concluded that the sexual products arise from the interstitial cells in the ectoderm of the gonophore peduncle. The germ cells then pass close to the base of the peduncle, across the mesolamella into the endoderm, then finally into the entocodon.

Hardy (1891), in Myriothela phrygia, observed free rounded cells in the deeper part of the ectoderm, usually occurring in small groups. They frequently show sign of active proliferation, and are regarded as

primordial germ cells. He also believed that they later travel into the gonophores.

Bunting (1894) concluded that in Hydractinia the ova are first seen in the endoderm of the blastostyle and therefore apparently endodermal in origin; the spermatozoa arise from the inner layer of the entocodon, hence are ectodermal in origin. In Podocoryne, ova first make their appearance in the endoderm of the manubrium, and reach maturity in ectoderm of the same. Spermatozoa, however, arise in the ectoderm of the manubrium and mature in the same place.

Allen (1900) traced the germ cell origin of Parypha crocea, both male and female, to the entocodon, which she regarded as ectodermal.

Wulfert ('02) claimed that the primordial germ cells can be observed at a very early stage of development in Gonothyreae loveni. They originate from the interstitial cells of the ectoderm, soon invade the endoderm of the young stem and later migrate into the ectodermal entocodon.

Schneider ('02) confirmed the ectodermal origin of the entocodon in Tubularia mesembryanthemum, but asserted that the entocodon does not give rise to the germ cells. According to his observation, the germ cells arise from the ectoderm of the gonophoral peduncle, later migrate by different routes into the interior of the gonophore.

C. W. Hargitt ('04 b) observed eggs of undisputable nature in the endoderm of the stem in a new species, Pachycordyle weismanni. He was ready to accept the migration of the eggs into the gonophore as suggested by Weismann, but denied their origin from the ectoderm as

Weismann proposed on a purely theoretical ground. For Tubularia mesembryanthemum, he reconciled the discrepancy between Brauer on the one hand and Ciamician and Weismann on the other hand by regarding both cases as valid, because germ cells have been found in the ectoderm of both the gonophoral peduncle and the spadix.

Downing ('05) worked on the spermatogenesis of four species of Hydra, and came to the conclusion that the spermatozoa are derived from the interstitial cells. He regarded the interstitial layer as representing a mesoderm in nascendi. Moreover, he believed the sex cells to be a distinct group of cells and that the germ track is therefore continuous in Hydra.

Trinci ('05) disclosed the transformation of ordinary cells into ovocytes in Phialium variabile and Tiarella parthenopoea. It appeared to him that the germ cells do not have a definite, exclusive origin in any one or the other layer. The transformation of somatic cells into germ cells at the beginning of germ cell formation may take place in different positions according to species, or even in the same species, sometimes in ectoderm, sometimes in endoderm.

Goette ('07) made a detailed study on the origin of germ cells in a large number of hydroids (21 species of Leptomedusae and 14 species of Anthomedusae), of which 16 species are the same as have been examined by Weismann (1883). His observation and especially his interpretation turned out to be very different from Weismann's. His conclusion was that the origin of germ cells can be variable---from one germ layer to another, from one region to another, from species to species as well as from sex to sex of the same species--- and, above all, germ cells can actually be formed

from histologically differentiated cells, that Weismann's hypothesis of germ-site shift is unnecessary and bound to be fruitless.

Goette's work was so extensive and his evidence so strong that it virtually put an end to any further attempt of seeking a general law governing the topographical origin of the germ cells. The problem as such therefore ceased to receive attention unless it was undertaken with the view of corroborating or else refuting Weismann's germ-plasm theory. G. T. Hargitt's work ('13, '16, '19) has been of such nature, and his conclusion is in full agreement with that of Goette. In associating the germ cell problem with the theory of germinal continuity, zoologists naturally shift their interest from the place of origin to the time of origin.

2. ORIGIN IN TIME.

In the case of hydroids giving off free medusa, the germ cells are usually formed after its liberation (Weismann, 1883). In a few genera, e.g., Dendroclava, Perigonimus, germ cells may differentiate before the medusa is detached, but they do so only by the end of the medusa-bud development and almost invariably are functionally limited to the sexual generation itself.[+] A demonstratable continuity of germ plasm is obviously out of ~~the~~ question. The situation is, however, complicated in those

---

[+] Millepora marrayi, as reported by Hardy (1891), might be an exception. The spermatozoa are said to originate in the ectoderm of the coenosarc, migrate into the ectoderm of the zooids where they aggregate into spermarium.

species whose sexual generation is structurally reduced to the state of gonophore, sporosac, or altogether wanting. Many of them show a tendency toward a precocious development of germ cells in various parts of the polypoid structure, starting even before the rudiment of the sexual bud makes its appearance. However, it must be emphasized that even in these species germ cells are not recognizable until approaching maturity. Therefore, despite their precocious appearance, they are nevertheless far too late in their differentiation to establish a visible germ track as has been described in other group of animals. Weismann himself admitted: "The germ cells of hydroids do not come into being during embryonic development; they build up only during later life." (1883).

Yet there are a few over-enthusiastic followers of Weismann who tried to identify primordial germ cells in early developmental periods of the polyp and have claimed their success; among them Wulfert, Harm, Stschelkanowjew and Downing are representative.

Wulfert ('02) was able to trace the sex cells back to the primordial germ cells in Gonothyraea loveni. These supposedly primordial germ cells, wandering in nature, were observed as early as the stage when the planula attached itself to the substratum. They were admitted as originating from the interstitial cells, and all gradations from interstitial cells to germ cells were found. When Wulfert's drawing is examined, it will be seen that the ganglion cells, interstitial cells and germ cells look so alike that distinction was highly subjective. Interstitial cells are produced in the ectoderm and the endoderm while the planula is still within the gonophore, and these were followed through their differentiation into

species whose sexual generation is structurally reduced to the state of gonophore, sporosac, or altogether wanting. Many of them show a tendency toward a precocious development of germ cells in various parts of the polypoid structure, starting even before the rudiment of the sexual bud makes its appearance. However, it must be emphasized that even in these species germ cells are not recognizable until approaching maturity. Therefore, despite their precocious appearance, they are nevertheless far too late in their differentiation to establish a visible germ track as has been described in other group of animals. Weismann himself admitted: "The germ cells of hydroids do not come into being during embryonic development; they build up only during later life." (1883).

Yet there are a few over-enthusiastic followers of Weismann who tried to identify primordial germ cells in early developmental periods of the polyp and have claimed their success; among them Wulfert, Harm, Stschelkanowjew and Downing are representative.

Wulfert ('02) was able to trace the sex cells back to the primordial germ cells in Gonothyraea loveni. These supposedly primordial germ cells, wandering in nature, were observed as early as the stage when the planula attached itself to the substratum. They were admitted as originating from the interstitial cells, and all gradations from interstitial cells to germ cells were found. When Wulfert's drawing is examined, it will be seen that the ganglion cells, interstitial cells and germ cells look so alike that distinction was highly subjective. Interstitial cells are produced in the ectoderm and the endoderm while the planula is still within the gonophore, and these were followed through their differentiation into

nematocysts and ganglion cells. Hargitt's special study on the same species ('19) reveals that by the time the planula has been perfected there are no cells in the ectoderm or endoderm which have even the remotest resemblance to germ cell.

Harm ('02) studied the young polyp of Clava squamata just developing from planula. Under the interstitial cells of the subtentacular region, and closely applied to the mesolamella are some cells distinguishable by their large nucleus and rich cytoplasm. These cells later on give off long processes when the embryo grows in size. It is these cells which Harm referred to as primordial germ cells. He did not follow up their further development but believed they were the same type of cells which he saw at a later stage passing from ectoderm to endoderm through the mesoglea to become the real oocytes. While the primordial germ cells in his figures give an impression of ganglion cells, his contention has been borne out in the main by a recent paper (Brien, '42). However, as Brien clearly indicated, these cells together with some other kinds of cells are derived from the interstitial cells.

Stschelkanowjew ('06) described the presence of germ cell in late cleavage stages of Cunina proboscidea. At that stage the embryo is in the form of a solid mass of cells arranged into two layers of one-cell thickness. Between the ectoderm and the endoderm layers, he finds one or two cells which he believes to be germ cells. No characteristics have been given to these cells. Except for the position, these cells, as judged from his figure, seem to be the same as the ectoderm cells in size, form, color reaction and the size of the nucleus. G. T. Hargitt ('19),

observing similar situation in the morula formation of Tubularia crocea, indicates that these cells are very likely none other than the interstitial cells.

Downing ('05) having studied the spermatogenesis of Hydra, upheld the view that "the sex cells are a distinct group of cells and that the germ plasm is then continuous in Hydra." He considered the spermatogonia to be derived from the interstitial cells, but maintained that the interstitial cells giving rise to sex cells could be distinguished from those giving rise to nematocysts and ganglion cells by their "very large nuclei, extremely granular, and often by the presence of a 'Nebenkern'". From this he came to the conclusion that "at some stage of the embryonic development certain cells are stamped with these characters and that they and their progeny form the sex cells distinct throughout the life of the individual." It is surprising to note that Downing reached this conclusion even without actually examining the embryonic stages. Moreover, the criterion which he used to differentiate sex cells from interstitials has been found to be applicable to most interstitial cells, and his conclusion has been refuted by other investigators working on the same material (Wagner, '09; Tanreuther, '09).

Recently, Brien has reinvestigated the germ cell differentiation of Clava squamata. About the time of attachment of the oozoids, when the latter acquire their first tentacles, the epithelial cells of the ectoderm begin to lose their flagella and become covered with a thin layer of perisarc. At their base, especially in the subtentacular region, interstitial cells become very abundant, forming a layer of uneven thickness. Some of

these interstitial cells dissociate from this layer to transform into epithelial cells; others disperse into small groups intercalating between the epithelial cells to constitute the future cnidoblasts, and still others finally form amoeboid basophilic cells with large nucleus and prominent nucleolus, which he asserts to be the primordial germ cells. His evidence lies in the fact that some of the amoeboid cells are seen in the midst of traversing the mesolamella toward the endoderm, and that in the ectoderm these cells are readily traceable to the germ cells of later stages. Brien, though supporting an early differentiation of sex cells in this species, denies the presence of a continuous germ line basically different from that of the soma. Thus he says: "Il ne peut être question chez les Clava (ni chez des Hydroïdes en général) d'une lignée sexuelle continue et distincte fondamentaement du soma. Leur differention se fait à la même source que toutes les autres lignées somatiques."

From this review it is obvious that even in those coenogonic species claimed to have extremely precocious formation of germ cells the so-called primordial germ cells are either interstitial cells themselves or their derivatives. Since interstitial cells in these species also give rise to cnidoblasts, ganglion cells, glandular cells, and sometimes epithelial cells ~~as~~ before, during, as well as after the formation of the sex cells, their identification as primordial germ cells is unjustified, and a distinct and exclusive germ line is manifestly absent.

It should be mentioned here that not all coenogonic species show such early differentiation of sex cells as that Harm and Brien have claimed for Clava squamata. Hargitt ('19), in order to test the question of the

presence of germ cells in the embryo, has made a careful and extensive study of the cleavage stages and the planulae of such coenogonic species like Campanularia flexuosa and Gonothyraea loveni. In neither does a differentiation of germ cells occur in these stages.

3. THE INTERSTITIAL CELL.

From the foregoing it can be seen that the interstitial cell has often been involved in the controversy of germ cell or germ plasm problems. It seems advisable therefore to give an account of this type of cell, which is one that has been heavily relied upon for a solution to the complexities of morphogenesis like regeneration and budding.

The interstitial cell, or I-cell for short as the German workers prefer to call it, was first described by Kleinenberg (1872) in Hydra as a kind of small cell in the ectoderm, between the epithelio-muscular cells and not quite reaching the mesolamella, with a relatively large nucleus and very sparse, but deeply staining cytoplasm. Kleinenberg observed the participation of I-cells in the formation of the gonad, and accordingly I-cells have at times been designated also as primordial germ cells. Nussbaum (1887) considered them as totipotent reserve cells and ascribed the extraordinary regenerative capacity of Hydra to the totipotency of these cells, but it was left for Weismann (1892) to postulate that the totipotency was due to the germ plasm which these cells were supposed to contain. Two years later, Lang (1894) investigated the budding of hydroids and reported the buds were built of I-cells, thus verifying Weismann's hypothesis that budding again is due to the presence of germ plasm, which found its carrier in these cells. I-cells were supposed, therefore, to

be totipotent, responsible for germ cell formation as well as for formation of other cell types, for regeneration and for budding in Hydroids, all due to their contained germ plasm.

The significance of I-cells in regeneration was first questioned by Rowley ('02). Rowley found that the regeneration of the tentacle is chiefly through the appropriation of the neighboring differentiated cells and their proliferation. Thus she stated: "The new cells which appear during the regeneration of Hydra are formed by division of the old cells throughout the entire piece as in the normal growing animal, and the tentacles are formed from old cells that have arisen by division of the already differentiated cells of the old part." I-cells therefore play no significant role in this case. Later investigators, however, rather favoured the importance or even indispensibility of these cells in regeneration. Thus Hadzi ('10), who had attributed extraordinary significance to I-cells in bud formation, thought the same situation might hold true in regeneration. He believed that I-cells migrate into the endoderm and thereby transform into endoderm cells. His conclusion reads: "It is certain that I-cells are chiefly if not exclusively concerned." Schulze ('18) was in complete agreement with Hadzi, and found proof in the inability of regeneration from severed tentacle, which does not contain I-cells. Mattes ('25) stated: "Through the proliferation and growth of particularly the interstitial cells the normal proportions are restored, and then chiefly through the remodeling at the concerned ~~psi~~ position are the missing parts replaced." Goetsch ('29), based on his own research, was of the opinion that in all regeneration processes,

I-cells play the chief role. According to his conception, the regenerated part was built up of the interstitial cells that migrate to that spot of regeneration. The situation would seem to be settled in view of the experiment of Zawasin and Strelin ('29). By subjecting Hydra to a determined dose of X-ray, the interstitial cells were the first to succumb; such individuals thus deprived of I-cells were found to have lost their regenerative capacity. But it must be stressed that in this particular experiment, the possible effect of irradiation upon the surviving tissue cells should not be lost sight of in evaluating the result.

Despite the strong tendency toward favoring the importance of I-cells in regeneration, the result of Kanajew's experiment ('30) seems sufficient to outweigh it. By a combination of vital staining and transplantation technique, Kanajew, working on the same material (Hydra), came to the inevitable conclusion that it is the already differentiated cells of both layers that serve as the principal material for regeneration. I-cells are used chiefly as the material for nematocysts. His result is in line with that of Gelei ('25), who reported briefly that the regeneration of the pedal disc is accomplished without the help of the interstitial cell.

The importance of I-cell in bud formation was first contested by Braem (1894), who asserted that the migration into the endoderm and their subsequent formation of both ectodermal and endodermal elements as claimed by Lang (1894) was non-existent, and that the bud was built up at the expense of both layers of the parent. Lang's contention, however, was upheld by the renowned work of Hadzi's ('10). According to Hadzi, bud formation begins with the active division and aggregation of the interstitial

cells in the ectoderm followed by the migration of a part of them into the endoderm to build up the endodermal cells, while those remaining in the ectoderm transform into ectodermal cells. He concluded that in the formation of the bud, body layers need not take part, I-cell alone being sufficient. The migration of I-cell was again attacked by Braem ('10), and Hadzi ('11) retorted with the evidence supplied by Boulenger ('10), who, inspired by Hadzi's paper, found similar migration in Merisia. Tannreuther ('09), while denying the migration of I-cells, nevertheless considered them important in budding. However, Kanajew ('30) has been able to show with unequivocal evidence that the bud formation in Hydra is principally a process of morphallaxis. The bud is made of parental body layers without appreciable extent of mitosis which, paradoxically, is abundant in the interstitial cells. Migration of I-cells across the mesolamella does occur, but by far too rare to assume any important function in bud formation. Kanajew thus concluded that the role of I-cell in budding as well as regeneration is entirely subordinate.

The budding of another hydroid, Cladonema, recently studied by Brien ('42) yields similar results. This hydroid has its epidermis composed of only one single layer of cells, and I-cells are rare and dispersed. At the blastogenetic point, the epidermal cells as well as gastrodermal cells undergo morphological de-differentiation and constitute respectively the ectoblast and endoblast of the bud. The endoblast retains its original property, forming the gastrodermis of the new polyp, while the ectoblast becomes polystratified, the peripheral layer reconstituting the epidermis, the deeper layers, consisting of indifferent cells, segregating

into a variety of cells: epidermal, glandular, cnidoblasts and germ cells. The bud is therefore formed through the de-differentiation of the somatic cells, and not through the activity of the pre-existing interstitial cells.

In the previous section (p. 15) we have already seen that the identification of I-cells as primordial germ cells is misleading and incorrect in the first place. Now, even if we accept, for the sake of argument, that I-cells do carry germ plasm as Weismann thought, the fact that regeneration and budding can take place in defiance of I-cell would serve to destroy the corresponding evidence which Weismann has been giving in support of his germ-plasm theory.

B. AN ANALYSIS OF WEISMANN'S INVESTIGATION ON THE SEX CELLS OF HYDROIDS.

It has been generally held that Weismann's investigation on the sex cells of the hydroids, embodied in his monograph "Die Entstehung der Sexualzellen bei den Hydromedusen" (1883) gives substantial support to his theory of continuity of germ plasm (Romanes, 1899; Richards, '31; Everett, '42). Actually, in his classical work --- The Germ-Plasm (1892) --- the case of Hydroids is cited as evidence of the theory of the composition of germ plasm. In the meantime, other workers (Goette, '07; Hargitt, '19) on the same group of animals, have found much to contradict his main contention that there is fundamental difference between the germ plasm and the soma. It is therefore desirable to re-examine Weismann's paper to see in which way and to what extent that piece of work actually substantiates his famed theory. That paper, unfortunately, has not been available in English, and its contents are rather inadequately known. In order to understand his reasoning intelligibly as a basis for criticism, I deem it appropriate to give in the following sections the salient features of his original work.

The first important observation which Weismann made after examining some 35 species of Hydroids is that there is a definite relation between the distribution of the germ-site (Keimstätte), i.e., the place where the germ cell differentiates, and the status of the sexual generation. He classified the sex generation into 6 stages with increasing order of morphological retrogression:

Stage I. Free living medusa.

II. Medusoid with radial canals but

no marginal tentacles, mostly also without velum and sense organs; manubrium without mouth opening; liberating mostly when mature.

Stage III. Sessile medusoid,,radial canal mostly absent or incomplete; subumbrella cavity present.

Stage IV. Sessile gonophore, wall still with endoderm lamella and two ectodermal layers but no canals and mouth opening; manubrium directly enclosed.

Stage V. Sessile gonophore whose wall consists of incomplete layers.

Stage VI. Sporosac, without any trace of medusoid structures.

Likewise, the distribution of the germ site was arranged into 6 stages with increasing order of centripetal shift, i.e., shift toward the proximal end of the colony.

Stage I. Germ site in the ectoderm of the manubrium.

Stage II. In entocodon.

Stage III. In endoderm of gonophore bud.

Stage IV. In endoderm of blastostyle.

Stage V. In coenosarc of lateral hydranth.

Stage VI. In coenosarc of main hydranth.

It must be agreed that while an exact stage-to-stage correspondence does not necessarily hold, the parallelism between the degree of germ-site shift and that of morphological regression on the part of the sexual generation is quite obvious. The cause of this shift in germ site is interpreted by Weismann as an acceleration of sexual maturity.

As the transformation of the free living medusa to the sessile

sporosac is a matter of phylogenetic retrogression, Weismann immediately attaches phylogenetic significance to the shift of the germ-site. The state of germ-site in those species producing free medusa is taken as the starting point, because in them the medusae have undergone little regression and their germ-site should represent most the primitive pattern. In nearly all tubularids with free medusa, Weismann finds the germ-site to be the ectoderm of the manubrium (Stage I). From there on a tendency of precocious differentiation of germ cells prevails. Instead of differentiating after the manubrium is well established (in most cases even after the medusa is liberated from the polyp), the germ cells differentiate in the entocodon at a time when the medusoid is yet only a rudiment. This shift (Stage II) is essentially one of time; the topography is not altered to any appreciable extent because the inner layer of the entocodon, where the germ cells differentiate, will soon develop into the ectoderm of the manubrium. Further shift of germ site is thought to take place along one of two alternative lines: 1) germ site still confined to the ectoderm but retreats into the wall of the gonophore bud, or 2) germ site diverted from ectoderm to endoderm of the gonophore bud. Both procedures have been adopted by tubularids as well as by campanularids. Following up the second alternative, one gets Stage III: the germ site now lies in the endoderm of the gonophore bud. Further intensification results in Stage IV, here the germ site pushes back to the endoderm of the blastostyle. Stage V, with the germ site situated further back in the coenosarc of the lateral hydranth, is a heterogeneous group with reference to germ layers, because germ cells may differentiate in either ectoderm or endoderm. Weismann does

sporosac is a matter of phylogenetic retrogression, Weismann immediately attaches phylogenetic significance to the shift of the germ-site. The state of germ-site in those species producing free medusa is taken as the starting point, because in them the medusae have undergone little regression and their germ-site should represent most the primitive pattern. In nearly all tubularids with free medusa, Weismann finds the germ-site to be the ectoderm of the manubrium (Stage I). From there on a tendency of precocious differentiation of germ cells prevails. Instead of differentiating after the manubrium is well established (in most cases even after the medusa is liberated from the polyp), the germ cells differentiate in the entocodon at a time when the medusoid is yet only a rudiment. This shift (Stage II) is essentially one of time; the topography is not altered to any appreciable extent because the inner layer of the entocodon, where the germ cells differentiate, will soon develop into the ectoderm of the manubrium. Further shift of germ site is thought to take place along one of two alternative lines: 1) germ site still confined to the ectoderm but retreats into the wall of the gonophore bud, or 2) germ site diverted from ectoderm to endoderm of the gonophore bud. Both procedures have been adopted by tubularids as well as by campanularids. Following up the second alternative, one gets Stage III: the germ site now lies in the endoderm of the gonophore bud. Further intensification results in Stage IV, here the germ site pushes back to the endoderm of the blastostyle. Stage V, with the germ site situated further back in the coenosarc of the lateral hydranth, is a heterogeneous group with reference to germ layers, because germ cells may differentiate in either ectoderm or endoderm. Weismann does

not specify whether this stage should have descended from Stage II or not, in case the germ cells should differentiate in the ectoderm instead of in the endoderm. Finally, the culmination of shift is reach in Stage VI, where the germ site lies in the coenosarc of the main hydranth. Of all the species Weismann examined, only the female of Eudendrium racemosum attains this stage; since in this case the germ cell differentiates in the ectoderm, Stage VI is presumably descended from Stage II through adopting the first alternative and its subsequent intensification.

It should be commented here that while a precocious development of germ cells and a tendency of centripetal displacement of the germ site is an undeniable phenomenon, the over-emphasis of phylogeny alone incurs unnecessary predicaments in explaining many facts which Weismann confronted, among which the wide difference in germ site frequently occurring between the two sexes of the same species is but one. He interprets this on the ground of utility purpose on the part of the animal.

The significance of the germ-site shift as seen by Weismann, however, does not end here. It remains for two more important observations to complete its implications. The first observation consists of two factors:

1. The germ site is stably fixed at a certain place in a given species (or, in some cases, a given sex of that species). It never varies from one region to another, or one germ layer to another.
2. Histologically differentiated cells never transform into sex cells; only cells of embryonic character can give rise to germ cells.

Putting aside the question whether these two facts are correct or not (which Goette and Hargitt show not), it is surprising to see Weismann plunge into the immediate deduction that "not any cells can become germ cell under certain circumstances, but only those cells that are determined to do so in previous cell generations undergo this transformation" (W* p.226). For this deduction in itself is the keynote of the theory of germinal continuity which he later formulated in 1885. In view of the induction phenomenon which plays so important a role in histogenesis and morphogenesis as disclosed by Spemann ('38), it is easy to see that the two premises he presented, even if they were correct, could hardly justify this weighty conclusion.

The other observation which he made is that, in sharp contrast to the phylogenetic shift of the germ site, the place where the germ cells mature, or, the maturation site (Reifungstätte) is remarkably constant and coincides with the phylogenetically oldest germ site (Germ-site Stage I), i.e., the ectoderm of the manubrium. Even those species with most extensive shift of germ-site nowadays still retain their maturation site in the ectoderm of the manubrium.

With (1) the deduction which he obtained from the first observation that only predetermined cells can form germ cells, (2) the fact that germ site varies with species and on the whole exhibits a tendency of centrepetal shift, and (3) that the maturation site is nevertheless fixed at the ectoderm of the manubrium, Weismann visualized a migration of sex cells (including primordial germ cell and germ cell) which is the most important theme of his monograph.

---

*Translation beginning with W. refers to Weismann's monograph, 1892

The method which Weismann used in constructing his theme is none other than to project phylogeny into ontogeny. In other words, he conceived the ontogenetic development of the sex cell to be a recapitulation of the evolutional history. Since in those species with free medusa the ectoderm of manubrium is at once the germ site and maturation site, he reasoned that the germ cell must have been originated there (as primordial germ cell), differentiated there (as germ cell) and matured there. Despite that nowadays the germ site may be shifted to various extents, the primordial germ cell, in view of its predetermined nature, must still arise from the archaic position --- the ectoderm of manubrium, or the rudiment which is going to develop into the ectoderm of manubrium, and it is on this ground that he maintains the germ cells of hydroids should be always ectodermal in origin, no matter whether they differentiate in the ectoderm or in the endoderm.

Since the primordial germ cells have arisen from the ectoderm of manubrium, they must have undergone a migration so that they could reach the new germ site where they are to differentiate into germ cells. This constitutes one phase of the migration. The other phase is the migration of the germ cells from the germ site to the maturation site; it seems that a migration of this kind is inevitable if the germ cell which has been differentiating at some other place is eventually to lie in the ectoderm of manubrium as already mentioned. The two phases of migration could be considered therefore as the efferent and afferent paths of the sex cells with reference to the definite maturation site. Accordingly, Weismann states there is no migration in species whose germ

site is also the maturation site, as is the case with those giving free medusa. Nor is a migration to be expected in forms which have germ site in the entocodon, such as in Tubularia (W. p. 219), because the germ cell is driven to the final position simply by the formative force of the gonophore bud (W. p. 270). Once the shift of germ site carries beyond Stage II (Stages III, IV and V), an active migration of the primordial germ cells becomes necessary, and in doing so they work their way through the mesolamella to reach their germ site, only to penetrate the mesolamella once again later on, this time as germ cells, in order to return to the age-old site of maturation. Weismann has some evidence to show that the mesolamella does not constitute a mechanical barrier for such migration, in or out. Moreover, even in the case where germ site shows extensive shift yet is still confined within the ectoderm (Stage VI), the germ cells on their way back nevertheless break through the mesolamella twice, first entering and then quitting the endoderm, to reach the homologous layer of the ectoderm of manubrium.

Such is the essence of his hypothesis of germ-site shift, obviously with the migration of primordial germ cell and of germ cell as its nucleus. Therefore before proceeding to his conception of descent of sex cells in Hydroids, it is advisable to determine what actual observations Weismann has had at his disposal to warrant this hypothesis.

For the identity of the primordial germ cells, Weismann indicates that they are a kind of embryonic cells which give rise to germ cells. He admits, however, there is no morphological distinction

between the primordial germ cells on the one hand and other embryonic cells on the other. The primordial germ cells therefore have no morphological characteristics of their own. What makes them primordial germ cells is that they, and only they, can give rise to germ cells. This criterion, however, is taken for granted by Weismann as a deduction of his two observations (see p. 22). It has been shown that this deduction is logically unsound even if the premises be correct. The investigations of Goette ('07) and Hargitt ('19), furthermore, revealed facts which are incompatible with these premises. The presence in the hydroids of a kind of primordial germ cells in the sense of Weismann is therefore purely imaginary and not supported by evidence of any kind in his original paper.

As a proof of the migration of the supposed primordial germ cells Weismann presents the evidence he found in Podocoryne, Hydractinia, and Pachycordyle. In Podocoryne, in the young gonophore not yet containing any egg or having only very few small eggs, separate cells may be seen in the ectoderm, larger than the rest, with a somewhat large, light nucleus and a deeply-stained nucleolus. Sometimes these cells are seen to apply closely to the mesolamella. Similar cells, separate or in groups, may be found on the other side of the mesolamella, in the endoderm, thus indicating they have migrated from the ectoderm. These cells later develop into eggs.

In Hydractinia as well as in Podocoryne, where Weismann made particularly detailed observations, neither in female nor in male does the germ cell originate through transformation of the differentiated endoderm cells. Since the endoderm could not form germ cells, and yet germ cells are formed in the endoderm of these species (female at least), they must

have migrated from the ectoderm.

In Pachycordyle, where the spermarium (only the male is known) is found to mature in the spadix (the maturation site is accordingly in endoderm and hence an exception to the general rule), cells similar to those constituting the spermarium are found in the ectoderm, Weismann considers this to be a strong morphological proof of a migration from the ectoderm.

Of these evidences, the first(from Podocoryne) and the third (from Pachycordyle) are along the same line of argument, namely, the presence in the ectoderm of cells similar in appearance to the developing germ cells in the endoderm. When his original drawing of Podocoryne is consulted, it will be found that the ectoderm cell which he labels (ekt') and states in the legend to be similar to the developing eggs in the endoderm bears little resemblance to the latter (W. Pl. 19, fig. 1B), and a linking up between them is rather far-fetched. The said ectoderm cell could well have been an interstitial cell. His drawing for Pachycordyle (W. Pl. 6, fig. 6) shows the primordial germ cell (ukz) similar to the developing male germ cell (kz) of the endoderm, but here the male germ cell shows resemblance to the ordinary ectoderm cell (ekt) just as well. It is obvious from his drawing that he was dealing with an interstitial cell again. Since interstitial cells have been reported to be present in both ectoderm and endoderm (Hargitt, '19), the assumption of a migration becomes completely unnecessary.

The second evidence has been shown to rest on a false premise. Goette ('07) and Hargitt ('13, '16, '19) found numerous cases where

division of an endoderm cell results in the formation of two cells, one of which becomes a germ cell while the other persists as an epithelial cell, thus supplying the facts demanded by Weismann himself[+] to prove his contention incorrect.

It should be mentioned that Weismann never succeeded in finding the supposed primordial germ cells passing through the mesolamella, but he found that ectoderm cell generally are capable of pressing into the latter (W. p. 237). In view of the actual findings of Goette and Hargitt, there is obviously no need of assuming, in the absence of direct evidence, a migration of "primordial germ cell" into the endoderm, in order to give rise to the germ cells therein.

Emphasis should be made here that the existence and the migration of the primordial germ cells are of utmost importance to Weismann's hypothesis of germ-site shift, but a review of his evidence reveals that the identity of the primordial germ cell is speculative in the first place, and the migration of the so-called primordial germ cells is likewise not accompanied by direct proof, which he himself admitted (W. p. 254). We might then well ask on what ground did Weismann defend his idea in the absence of direct evidence? In order to understand his reasoning, it is necessary to give his arguments in his own words: (W. p. 288).

[+] "The egg in no case arises from accomplished endoderm cell; indeed, the cells from which the eggs differentiate lie long before at the depth of the endoderm which is otherwise single-layered. If the eggs were of endodermal origin, so they would have to arise from division of ordinary endoderm cells; and it would follow that, turning toward the gastrocoele, the distal half remains epithelial cell, the basal half becomes germ cell. Nothing has been proved of such division ..... " (W. p. 237).

"Such being the case, no explanation for the displacement of germ site from the ectoderm to the endoderm other than the one assumed before could exist, namely, a migration of the primordial germ cells from the ectoderm into the endoderm. In Podocoryne the male germ site today lies in the ectoderm of the manubrium, the female, however, in the endoderm of the gonophore bud. When once it is established that the latter position is derived from the former, how otherwise could one explain that suddenly cells of endoderm took over the function which previously were possessed by those ectoderm cells? It would be a different matter if in some species it occurred that germ cells differentiate at indiscriminate place in the stalk, now in ectoderm, now in endoderm. But this never occurs; of all the data communicated above it is evident that the germ site of present-day species is rigidly localized, and what else can this mean other than that certain cell generations alone possess the ability to produce sex cells, that a strict law of heredity governs here and nothing is arbitrary and accidental? How, then, under such circumstances could the endoderm cells of a gonophore bud take over the inherited properties of the ectoderm cells of the same bud? A long series of cell generations separates two cells, one of which originated from ectoderm cells, the other from endoderm cells lying on the other side of the mesolamella; they connected only at the root of the whole polyp stalk; in other words, in the cleavage process of the egg, from which the first hydranth and colony originate. How and whereby could it become possible that suddenly the endoderm cell should differentiate into sex cells as the ectoderm cell has hitherto done? It is no exageration to regard this as impossible. When certain cells of the endoderm of the gonophore bud show the ability to differentiate into germ cell, the conclusion is undeniable that they must have migrated from the ectoderm, whether this be confirmed by observation or not."

In the above passage we can see that the footing for such an idea of migration is the supposed ectodermal origin of the primordial germ cells which in turn is based on the doctrine of recapitulation. But does recapitulation necessarily occur for every character of phyletic significance? To-day we know very well that organism does not have to look back to its remote history for its ontogeny, and the biogenetic law is inaccurate and misleading (Shumway, '32; de Beer, '28). But it was primarily on the basis of recapitulation that Weismann propounded the migration of primordial germ cells to which he so stubbornly adhered that he seemed to have defended it to the extent of disregarding truth. His interpretation of the germ cell origin of Coryne (W. p. 238) serves to

illustrate how far imagination can be pushed to suit a preconceived idea. In this hydroid the germ cells arise from the entocodon, which he observed. In contrast to the entocodon of most hydroids, that of this genus is formed of endoderm instead of ectoderm, which he also admitted. However, he contends that the endodermal entocodon of Coryne must have descended from the phyletically old ectodermal entocodon, because Syncoryne is the nearest relative of Coryne, and it has the usual ectodermal entocodon. His interpretation of the formation of this endodermal entocodon is that, in this case, not only the primordial germ cells but all cells which would normally constitute the entocodon have detached from the ectoderm together and have invaded the endoderm, only to rebuild there an entocodon just like the true ectodermal one (W. p. 238). Such assumption of an unrecognizable, wholesale migration involving primordial germ cells as well as tissue cells and their subsequent reassembling seems of course fantastic in view of the actual formative process of the endodermal entocodon in Tubularia crocea, as will be presented later (p. 51).

Proof for a migration of the germ cells to the maturation site seems ample; any hydroid whose germ site differs from its maturation site could be taken as a circumstantial evidence of such migration. A most extensive migration of this kind is found in female Eudendrium racemosum, in which Weismann shows that the germ cells differentiate in the ectoderm of the main hydranth, therefrom migrate in the same germ layer to the lateral hydranth, where they invade the endoderm, proceed to the endoderm of the blastostyle and sporosac, and within the latter re-enter the ectoderm to mature there. However, the situation is by no means as striking and

as unequivocal as it appears. In those forms having their germ site in the endoderm within the gonophore bud the germ cells merely step over the mesolamella and lodge themselves in a position characteristic of a large number of hydroids, which actually lies inbetween the endoderm and ectoderm, but is cleverly homologized to the ectoderm of manubrium by him to convey the idea of "historical reminiscense" (W. p. 290). Migration of this category is, at best, very limited. In those forms having germ site centripetal to the gonophore and yet nearby it, the evagination process of the body wall during the formation of the gonophore could easily carry the germ cells into the latter. An active migration so much emphasized by Weismann is therefore open to serious doubt. Then, in the case of Hydractinia, where the male germ cells are shown on the way of migration in his drawing (W. Pl. 23, fig. 6), it is questionable whether the cells which he regards as germ cells are really germ cells. It will be seen later (p.66) that unjustified interpretation of certain cells as germ cell is a common source of error in papers purporting to demonstrate germ cell migration. Finally, in those species with an extensive shift of germ site, it remains to be shown that the definitive germ cells are not developed in situ; for it is not inconceivable that the germ site might not be so strictly limited to the coenosarc, and that only those carried to the gonophore bud would reach maturity while the remainder may be resorbed or otherwise disintegrated. However, before any of these possibilities are established, the fact that germ cells differentiate in the coenosarc at a distance and eventually make their appearance in the medusa equivalents (gonophore or sporosac) tends to favor a migration of

some sort.

But Weismann's conception about migration of germ cells involves much more than that. From the observation that none of the germ cells of female Podocoryne are left behind in the endoderm (W. 270), that in general the germ cells of campanularids and plumularids arrange themselves in rather definite order from the advanced stages below to the young stages up the germinal zone (W. 272), and that in species like Eudendrium the germ cells quit and enter one body layer after another at definite location during the course of their migration (W. 275), Weismann went so far in his deduction as to think that the route of procession for each individual germ cell is predetermined in a rather specific way, not impossibly that "a given germ cell of the coenosarc migrates only to a determined gonophore." Each individual germ cell is viewed by Weismann to act as an independent being which "strives for a definite aim."

Summing up the evidence at Weismann's disposal, it can be briefly stated that there is fair basis (though no entrenched) for a migration of germ cell less the teleological part, little basis for the alleged migration of the would-be "primordial germ cell", and none at all for the identity of primordial germ cell in Weismann's sense. It is important to note that a migration of germ cells to the maturation site, even if we regard it as definitely established, does not lend support to the main theme, because this migration in itself can by no means prove the occurrence of the other phase of migration --- the migration of the primordial germ cell --- which, however, is the crux of the whole problem and of which evidence is sadly meagre.

After all these observations and speculations Weismann brought

forth his conception about the descent of germ cell in hydroids. Paradoxically as it may seem, he made it very clear that the germ cells of the hydroids arise late in their life cycle as descendants of ordinary young tissue cells, and in no case are special cells set apart in early embryonic stages for that purpose (W. p. 279). He himself also refuted the idea of Nussbaum ('80), who maintained that germ cells are separated from the remaining cells in a very early stage before any histological differentiation takes place, which Weismann's over-enthusiastic followers have nevertheless tried to defend in vain.

However, Weismann's negation of Nussbaum's idea only made the issue more subtle. For basically, he and Nussbaum believed the same principle, that there is a fundamental difference between the "sex molecule" on the one hand and the "somatic molecule" on the other hand. What discrepancy they had is only a matter of time for the expression of the "sex molecule". While Nussbaum contended the early separation and consequently an absolute independence of sex cells of the germ layer, Weismann contested that the sex molecule may mix with soma for a long time before it splits off the germ layer. Since the sex molecule could occur in diffuse state and intermingle with the soma, the same principle is rendered much less vulnerable to attack in Weismann's version than in Nussbaum's.

Such a delay in split of germ from soma is nothing out of the way, according to Weismann. Reviewing the then known cases of early segregation he pointed out that Diptera has its primordial germ cell segregated in the first cleavage; Moina (Daphnidae), in the fifth cleavage;

and Sagitta, during the invagination of the archenteron. A delay is apparent even in this series; in the case of Sagitta, the sex molecules must have been mixed first with the collective somatic molecules, then with the endoderm molecules, only later become separate as such (W. p.280). It is not surprising therefore the segregation should possibly undergo further delay. Weismann also emphasized that soma containing sex molecule is not the same as soma becoming sex cell. A direct transformation of histologically differentiated cells into sex cells is considered in this paper+ as theoretically impossible, whereas the bringing forth of a brood of cells which differentiate partly into sex cells is entirely justified.

As to why the sex molecule should lie diffuse in the somatic cells for a considerable number of cell generations, Weismann suggests that a general advantage of this kind is to enhance propagative capacity of the individual which arises from the fertilized egg. In animals with alternation of generation this advantage is especially apparent, inasmuch as numerous individuals can be brought forward from a single egg.

Such is a broad outline of Weismann's investigation on the sex cells of the hydroids. An analysis reveals that he had a too strong leaning upon the theory of recapitulation, and too little facts to warrant his conclusions. It is supposition upon supposition that makes up the hypothesis of germ-site shift, which was then taken as evidence for his theme in his subsequent work (Weismann, '92, p. 189). As far as the

---

+ In his later work, this conception is changed (Weismann, '92, p.197).

factual part goes, his investigation on hydroids can in no way lend any support to his theory of germ-plasm. In the meantime, he does hear out one fundamental fact, to wit, the germ cells of this group arise very late in their life cycle, and there is therefore no recognizable continuity of germ track. Accordingly he asserts that the sex molecule, or germ plasm, remains in this case mixed with the soma over a long period of time. However, it is obvious that if his distinction between germ plasm and soma is to have any meaning at all in this group of animals, there must exist a time, sooner or later, when we should expect the segregation to occur. Does that ever occur in reality? The situation will be taken up under "Discussion."

C. A SUMMARY OF LITERATURE ON GONOPHORE AND GERM CELL OF TUBULARIA

1. GONOPHORE FORMATION AND GERM CELL ORIGIN

I. The Gonophore Peduncle

The nature of the gonophore peduncle, or gonophore bearer, of Tubularia has been disputed by Bonnevie (1898) and Kuhn ('10). Bonnevie tried to establish the homology between the peduncle and the blastostyle. Consequently she suggested that the peduncle can be compared to a reduced polyp which is borne on the gastrozooid and which has lost its polypoid structures. This homology has been considered as unjustified by Kuhn. Kuhn contested that the typical blastostyle always arises from a position where a normal polyp could also have occurred, and has more structures to it to be comparable to a polyp. Both requirements are not met with in the peduncle concerned, because in no case has Tubularia been reported to bear a normal polyp at a position in between the two circlets of tentacles, and there is no part of the peduncle that recalls the anatomy of a polyp. Kuhn rather thought the peduncle is an organ of the polyp, which carries it in adaptation to the production of gonophores. To him, therefore, the polyp itself is the blastostyle. There is apparent advantage in growing the gonophores on the peduncle rather than directly on the polyp wall, in view of the considerably more space acquired and the possibility of successive generations of gonophore production. Besides, the tendency as found in Clava where the polyp wall lifts up somewhat at the base of the gonophore cluster seems to favor Kuhn's suggestion. The peduncle of Tubularia is therefore not to be called as "blastostyle".

II. The Entocodon and Germ Cell Origin

For the formation of the entocodon of Tubularia, authors have been almost unanimous that it is formed by proliferation and later isolation of the ectodermal cells. All of them saw the close relation between the entocodon and the germ cells, and in fact, the earliest worker mistook the entire entocodon as germinal mass (Agassiz, 1860). For the origin of the germ cells, most authors agreed that the germ cells, male and female alike, are formed in the inner layer of the entocodon. This group comprises of Ciamician (1878), Weismann (1880, 1883), Hamann (1882), C.W.Hargitt ('04), Goette ('07), on Tubularia mesembryanthemum, Allen ('00) and G.T.Hargitt ('09) on T. crocea, and Perez ('13) on T. indivisa. Others believed that germ cells arise in the ectoderm of the peduncle, and later migrate up into the entocodon. This group includes Jickeli (1883), Brauer (1891), Schneider ('02), C.W.Hargitt ('04) on T. memembryanthemum, and Lowe ('26) on T. larynx. Lastly, Koch (1876) was the only one who, working on T. larynx, supported van Beneden's assumption, i.e., male cell from ectoderm and female from endoderm.

In contrast to all others Benoit ('25), while agreeing that the germ cells are formed in the inner layer of the entocodon in T. mesembryanthemum, challenged the statement that the entocodon is endodermal instead of ectodermal in origin, and hence also the germ cells. He traced the entire entocodon to a single interstitial cell lying among the endoderm cells.

2. FORMATION OF MALE GERM CELL

Owing to the small dimension of the male elements and the lack

in the variety of form and size of the chromosomes, Tubularia, like many hydroids, makes very unfavorable material for the study of spermatogenesis. The only two papers that made such an attempt were written by Thallwitz (1885) and Benoit ('25), both on T. mesembryanthemum. Thallwitz followed up the differentiation of entocodon into epithelial cells and germ cells. The germ cells multiply intensely without much diminishment in size to form the spermatogonia, which are characterized by very chromatic linin in the nucleus. Thallwitz also noticed that development of the spermatozoa takes place from the periphery of the gonophore toward the interior. Benoit reported that the last generation of spermatogonia shows synaptic stages characteristic of primary spermatocytes. Reduction division progresses from the base of the gonophore at the periphery to the apical and interior part. The secondary spermatocytes resulted in having a nucleus of 2-3μ in diameter. The nucleus of spermatids measures about 1μ. The spermatids start to transform into spermatozoa from the periphery, progressing gradually toward the center. The spermatozoa have a very chromatic head of about 1μ in length and a tail about 25μ. They are grouped in bundles lying in various directions.

3. FORMATION OF FEMALE GERM CELL

The nature of the female germ cells of Tubularia was a puzzle to the early investigators. Agassiz (1860) failed to see the eggs of T. crocea, but observed that embryos develop from a large spherical mass which has been split off from a granular mass of protoplasm which he called germ bases. Allman (1871) likewise found 4 to 8 protoplasmic masses detached from the "generative plasma" and developing into actinulae.

Ciamician (1878) reported that among the numerous germ cells in a gonophore (of T. mesembryanthemum), only 4-8 become real eggs, the surrounding germ cells serving only as nutritive material for their development. Benoit ('25) however, had reason to believe that what Ciamician described as real eggs should actually have been the "plasmodial area" which he later discovered in the same species. Balfour (1883) stated that the egg attains its final size by an active ingestion of the neighboring germ cells like the way an amoeba engulfs the micro-organism for food.

It is now clear that the egg of Tubularia is formed by a process of annexation of numerous primary oocytes and not by active phagocytosis on the part of the privileged oocyte. The egg increases in size mainly through the summation of new protoplasm as a result of the fusion process, and not by simple absorption of water and vacuolization of cytoplasm as Grönberg (1897) suggested. As to which oocyte is to become the privileged one, whose nucleus will be the nucleus of the definitive egg, opinions differ. Hamann (1887), Brauer (1891) and Schneider ('02) asserted that there was very early differentiation of the germ cells into egg cell and "vitelline cells", whereas the majority of investigators agreed that all the oocytes in a gonophore are potentially the same, and the differentiation of a certain oocyte into the egg cell is because that oocyte is most favored by advantage of position or nutrition, and hence is dominant over all the rest in development (Doflein, 1896; Allen, '00; Perez, '13; Hargitt, '19; Benoit, '25).

The nuclei of the annexed oocytes are assimilated by the developing ovum very rapidly during the early phase of its growth, but in later

stages they survive digestion and appear as "pseudocells" in the cytoplasm of the egg (Hargitt, '09; Benoit, '25). "Pseudocells" are mostly seen lying in vacuoles; many of them may be found in the process of division within the vacuoles, and they may divide again and again before the daughter "pseudocells" completely separate from each other (Allen, '00). Portions of the division products are often in the process of being absorbed into the cytoplasm of the ovum. Doflein thought the "pseudocells" serve to take the place of the yolk granules which are wanting in this genus, and they are broken down to serve as food for the developing egg.

The changes in the egg nucleus incidental to maturation process will be dealt with in the next section, but the relation between the relative size of the nucleus and the mode of nourishment of the egg may be mentioned here. According to Jörgensen ('13), who based this claim on observation of egg-cells in a number of different animals, eggs nourished by nurse cells or follicle cells, or by the absorption of adjoining ova and oocytes, have very small nuclei; eggs without special nourishing apparatus but which absorb their food directly, possess relatively large nuclei. Hargitt ('19) tested the egg of 15 species of coelenterate, for which he calculated the ratio of egg volume to nucleus volume. Tubularia crocea has been one among those tested, and was shown to have a ratio of about 6,000, in contrast to 17 of Hydractinia echinata, whose egg, unlike that of Tubularia secures nourishment from the adjoining enteric cavity. Hargitt's result is therefore consistent with the suggestion of Jörgensen's.

4. NORMAL EGG VS. GIANT EGG; THEIR MATURATION

Benoit ('25) distinguishes in young T. mesembryanthemum two

varieties of female gonophores; small and large. The small variety gives rise to the normal egg, and may contain as many as 4 generations of egg in the same gonophore, whereas the large variety gives rise to the giant egg, and contains at most only 2 generations in the same. There are intermediate gradations between these two types of gonophores; furthermore, the small variety eventually catches up and reaches about the same size as the large one in the long run.

For the formation of the normal egg, there are two ways. One is by a single oocyte, which is usually situated in the apical zone of the gonophore. This oocyte starts to develop, enlarge, and its cytoplasm vacuolizes and fuses progressively with the neighboring oocytes until a large amoeba-like egg is formed surrounding most of the spadix. Unabsorbed oocytes may start eggs for the succeeding generations. The other way is by several oocytes (usually 4 or 5), situated at the side of the spadix. They start to grow, their cytoplasm becomes likewise vacuolized and fuses progressively with the neighboring oocytes, forming "plasmodial areas" (each growing oocyte forms one plasmodial area). Growing, then fusion of these "plasmodial areas" takes place, slowly from center outward, until one single egg is formed surrounding the spadix. In both cases, the absorbed nuclei turn to "pseudocells", hence there is only one real nucleus to the egg in the final stage of fusion.

The process of giant egg formation is the same as that of the normal egg; it may start either from one single oocyte, or from several oocytes which form corresponding number of "plasmodial areas" before their final fusion into one. The distinctive feature of a giant egg is that

transverse incision, usually one and occasionally two, appears in the egg, deepens and eventually divides the giant egg into 2 (or 3 in case of two lines of incision) secondary eggs. Since a second generation of giant egg may occur in the same gonophore, and which in turn segments into secondary eggs, there may be up to 6 secondary eggs in one gonophore. The secondary eggs from the same giant egg are of the same developmental stage. Each secondary egg, which will give rise to one embryo upon fertilization, is said to possess one privileged nucleus along with many nuclei of the newly absorbed oocytes (those previously absorbed have been turned into "pseudocells"). Unfortunately, Benoit gave no indication as to whether the privileged nucleus of each secondary egg is derived from the nucleus of the previous "plasmodial area" or from a division of the single nucleus of the completed giant egg.

The maturation division of the normal egg of T. mesembryanthemum conforms to that of other animals to a large extent. The germinal vesicle, of which the nucleolus disappears very early during the growth of the egg, approaches the periphery, surrounded by a layer of fine, granular cytoplasm, and is very apt to be overlooked. Chromatin hitherto dispersed becomes arranged in moniliform filament to form a spireme. With the breakdown of the nuclear membrane the spireme segments into 16 chromosomes, eventually to 8 tetrads by longitudinal splitting of each chromosome. The spindle now appears perpendicular to the surface of the egg. The first polar body is given off, and the ovum becomes the secondary oocyte. The second polar body is given off immediately after the first. Reduction of chromosomes occurs at the second maturation division.

In the case of the giant egg, each secondary egg performs its

own maturation division in the same way as the normal egg as far as the privileged nucleus (germinal vesicle) is concerned; the first and second polar bodies are given off in the usual manner. The noteworthy part is that the other ten or so nuclei contained in the cytoplasm and not yet transformed into "pseudocells" also enter into maturation division simultaneously with the privileged nucleus, and each gives off its 1st and 2nd polar bodies. Consequently, when the maturation is complete, each secondary egg contains about a dozen female monovalent nuclei (1 normal nucleus of the privileged egg and 10 or so nuclei of the absorbed oocytes). The normal nucleus remains separate as the monovalent pronucleus, while others fuse into 2 or 3 polyvalent pronuclei of various valency. Polyspermy is the rule during fertilization. One spermatozoan fuses with the monovalent pronucleus of the egg, the other spermatozoa, however, vacuolize, approach each other and unite to form one or more male polyvalent pronuclei. Surrounding each of such pronuclei centrosomes appear, in equal number to that of the spermatozoa that went to make up that pronucleus. Male and female polyvalent pronuclei eventually fuse with each other. There are therefore after fertilization in each secondary egg one conjugation nucleus of the normal male and female pronucleus, giving rise to a bipolar spindle and normal mitosis, and one or more conjugation nuclei formed by the copulation of male and female polyvalent pronuclei, giving rise to multipolar spindle and multipolar mitosis. With the advancement of the cleavage process, multipolar mitosis gradually gives way to typical mitosis.

5. HERMAPHRODITISM

Tubularia, like most hydroids, is strictly dioecious, and all the gonophores and sex cells produced by one colony are of the same sex. How-

ever, Kleinenberg (1881) in his paper on Eudendrium, casually mentioned that hermaphroditism is not infrequent in T. mesembryanthemum at times in between its two main breeding seasons of the year. In the one and same gonophore, some germ cells may be seen to develop into eggs, while others at the same time develop into spermatozoa. Sometimes developing embryos, unfertilized eggs, spermatocytes and mature spermatozoa all may coexist in the same gonophore. Perez ('13) reported a case of accidental hermaphroditism in T. bellis. The colony which he chanced to observe was a female one. Nearly all the clusters on the hydranth were female, but among them there was one male cluster bearing two well-developed male gonophores. Beside this male cluster a mixed cluster was found, bearing 3 gonophores. Microscopic examination revealed that one of them was still in a very early stage, the second was a pure male and the third a definite female containing an embryo; all three attached to the same peduncle. This led Perez to the conclusion that in Tubularia at least, the determination of sex is contemporaneous to, and not much earlier than, the morphological emergence of the medusa-bud. Regular hermaphroditism has been claimed to be characteristic of a species which Bonnevie (1898) described as T. asymmetrica. According to this author, the polyp of this species carries side by side gonophores of different sexes; male and female may even develop simultaneously within the same gonophore.

MATERIAL AND METHODS

The material used in this study is the gymnoblastic hydroid Tubularia crocea*. It was obtained from the Marine Biological Laboratory, Woods Hole, where the writer also had the opportunity of working on living material. Both male and female colonies were collected in June and were fixed in Bouin's fluid or other fixatives according to the stains to be used. Hydranths containing gonophores of various developing stages were chosen for microscopical study. Paraffin sections 8 μ in thickness were cut in series, starting from the apical end to the peduncle. Since the gonophores run in all directions from the gonophoral peduncle with reference to the main axis of the hydranth, cross, oblique, as well as longitudinal sections of gonophore could be obtained even though the sections were made only along one dimension of the hydranth. For general purpose, Delafield's haematoxylin and eosin, Heidenhain's iron haematoxylin,

---

*There are three species of Tubularia off Woods Hole, which can be distinguished from one another by the following key:

I. Colony branched; stems extensively annulated.
   T. larynx, on piles and algae. Stem yellow, hydranths and gonophores bright pink; height 1 to 1½ inch.

II. Colony branched sparsely or not at all; stems annulated only at intervals.
   IIA. Colony formed of unbranched clusters of 5 to 10 individuals; 30 to 40 tentacles in proximal whorl.
      T. couthouyi, sandy and stony bottoms; dead in summer in shallow water. Stem and gonophores scarlet; height 5 to 7 inches, spread of tentacles 1 inch.
   IIB. Colony dense, sparsely branched tuft; 20 to 25 tentacles in proximal whorl.
      T. crocea, pilings just below low tide, sometimes in brackish water. Stems pale, hydranths and gonophores rose; height 3 to 5 inches.

Chlorazol black (Pantin, '46), and Mallory's triple stain have been used with good results on material fixed in Bouin's fluid. For differentiating chromatin from non-chromatin cellular contents, the latest modification of Feulgen's method (Rafalko, '46) counterstained with fast green has been used with considerable success. Ribonuclease digestion followed by Unna's methyl green-pyronin (Brachet, '42) or toluidin blue (Brachet, personal communication) was also used as a comparison. Since the result of these stains depends to large extent on the way they are handled, it is desirable to state the procedures followed in this study.

Rafalko's modification of Feulgen's method: This method is intended for small and diffuse chromatin elements which are not usually revealed by the conventional Feulgen's method. Instead of the hydrochloric acid and the sulphites ordinarily used, the staining solution and the sulphurous acid bath are prepared by directly charging basic fuchsin and distilled water respectively with sulfurdioxide gas. As for the fixative, the present writer found saturated mercuric chloride with 5% glacial acetic acid works much better than strong Flemming's in Tubularia. A minimum time of fixation and washing in water is considered by Rafalko important in bringing about favorable result, and in the present case 1 hour was alloted for each. Other details were carried out as outlined in his original paper.

Material for methyl green-pyronin or for toluidin blue stain was fixed either in Helly's Zenker formol or in Serra's fluid (Serra, '47) for 5 hours, followed by a washing period of 12 hours for Helly's or 5

hours for Serra's. The slides were introduced into a ribonuclease solution of 10 mgs. per cent (prepared by Dr. M. Kunitz's laboratory) which has been buffered to a pH of 7.7, and were kept at 35° C. for 1 hour. After this treatment they were stained either in Unna's methyl green-pyronin or toluidin blue for 20 minutes, followed by a brief washing in running water. From water the slides were transferred directly to 95% alcohol, 5 minutes for toluidin blue and a few seconds for Unna's stain, thence to absolute alcohol and toluene.

It was found that Unna's stain did not give the desired color reaction in this instance. For the toluidin blue stain, the nuclease treated slides showed a differentiation between chromatin material, which stained blue, and nucleolus, which stained purple, and the result was consistent with that obtained from Feulgen's reaction. In this way chromatin, nucleolus and cytoplasmic inclusions could be readily distinguished from one another, which is particularly important in studying the "pseudocell" formation of the female germ cell.

OBSERVATION

1. THE FORMATION OF GONOPHORAL PEDUNCLE

The gonophoral peduncle when well developed is a profusely branched, slightly contractile stalk attached to the hydranth immediately above the proximal tentacles. There are generally 6-10 of them in either sex, arranged more or less in a circlet (Fig. 1). As the hydranth becomes larger and larger, new peduncles may be given off in between the old ones, but they do not reach the same prominence as their earlier part-

ners. Each well-formed peduncle consists of a fairly stout and long stalk which gives off lateral branches freely, with no definite pattern, and near the top it resolves itself into many branches. A branch may in turn give rise to sub-branches. Each branch or sub-branch may bear one or more gonophores, and there may be therefore up to some twenty maturing gonophores on a single peduncle. When two or more gonophores occur on the same branch or sub-branch, the distal one is always the more advanced in development. This rule, however, does not apply to the gonophores between different branches or sub-branches, because they may originate at various levels down the peduncle and yet are about equal in their stage of development. In life the peduncle appears to have a core of red diffuse pigment which intensifies into salmon color as it continues its way into the spadix. The pigment granules are actually contained in the endoderm cells, but are discharged to the "lumen" of the peduncle which is apparently an extension of the main gastrocoele.

The peduncle begins as a local evagination from the wall of the gastral region close to the inner base of the proximal tentacles, and contains ectoderm and endoderm with mesolamella in between (Fig. 2). While the ectoderm of the gastral region is many layered, that of the peduncle wall starts as a single-layer and merges with that of the tentacle. Nematocytes later occur in great abundance all over the ectoderm, often in nests. The mesolamella of the peduncle is continuous with that of the gastral region on the one hand and with that of the gonophore on the other hand. The endoderm consists of a single layer of columnar, highly vacuolated cells, interspersed with a type of large glandular cell which has dense cytoplasm and large nucleus. The secretory granules are as a rule

accumulated at the end toward the "lumen".

The peduncle begins to form a number of gonophores at its tip by branching almost immediately after its emergence and before it has time to elongate (Fig. 2). Young peduncle therefore carries a cluster of minute gonophores with no noticeable stalk. The stalk appears later, giving off branches in all directions. On the tip of each branch or sub-branch a gonophore is borne.

2. THE FORMATION OF THE GONOPHORE

At the point where gonophore is to form, the endoderm cells of the peduncle cease to be vacuolar and change rather abruptly to a layer of compactly arranged, low columnar cells. Glandular cells are yet absent from this new region. The ectoderm of the peduncle, having already reached appreciable thickness, also thins out at this region to form a single layer, which contains discrete nematocytes instead of in nests. The cells constituting the ectoderm have slightly less dense cytoplasm than those of the endoderm, hence it shows less basophilism than the latter. The nuclei of the ectodermal and endodermal cells are, however, almost identical, both having a size of about 6 μ in diameter and a prominent nucleolus of about 1.6 μ.

The entocodon (or primordium of the subumbrella) in this species is definitely formed from endoderm and not by ectoderm as has been reported in most hydroids, including the same species. The earliest indication of entocodon formation is a proliferation of the single-layered endoderm (Fig. 3 at the apical portion of the gonophoral bud, resulting in a mass of cell about 3 layers in thickness. This proliferation is rather transient in

nature, very soon a delamination occurs, separating the original endodermal layer from the cells it produced (Fig. 4). The delamination is possibly instituted by the mesolamella, which seems to send a new, secondary sheet across that mass. In any event, a secondary mesolamella is now present between the new endoderm layer and the beginning entocodon; the latter, however, is internal to the original mesolamella, and remains in such a position in relation to the endoderm that its derivation from it is self-evident. In the meantime the constituent cells increase rapidly in number by division.

Shortly afterwards the endoderm cells at the junction of the original endoderm and the newly delimited endoderm start to give rise to a layer of cells which works its way in between the ectoderm and the entocodon, toward the apical end of the gonophore (Fig. 5, 6). This is the endoderm lamella, which forms a dome with a central perforation. The endoderm lamella will later give rise by a process of splitting to 4 radial canals, one at each corner of the central perforation (Fig. 11). The latter apparently represents the only remnants of the large space within the rim of umbrella in free-swimming medusa.

As the endoderm lamella is progressing apically, or slightly later, the endoderm beneath the center of the germ mass begins to exert pressure against the latter, forcing that region upward so that the germ mass now assumes kidney-shaped in longitudinal section ~~(Fig. 5B)~~. The intruding endoderm continues to push apically, and the peripheral zone of the rapidly growing germ mass is forced to extend toward the direction of the peduncle, the whole germ mass thus appearing horse-shoe-shaped in

longitudinal section (Fig. 6). That portion of endoderm (with its gastrocoele) partially surrounded by the germ mass constitutes the spadix. In the meantime, a narrow crack occurs in the entocodon in such a way as to separate a thin superior cell-layer, or the "inner ectoderm," from the rest of the entocodon that consists of the "germ mass" (Fig. 7). That crack represents the subumbrellar cavity.

The development of the tentacles of the gonophore is first indicated by a local thickening of the endoderm lamella at four equidistant points of the apical end. These thickenings push the overlying ectoderm and protrude from the surface of the gonophore. The tentacles, four in number, are more prominent in female than in male, from which they may be absent altogether.

A completed gonophore ~~(Fig. 7)~~ therefore consists of, from outside inward, the following layers: a single layer of ectoderm, a mesolamella (original), a layer of endoderm lamella which splits at top to form four radial canals, a layer of inner ectoderm, a narrow space representing the subumbrella cavity, a germ mass, a mesolamella (secondary), and a spadix lined by endoderm cells. The spadix encloses a gastrocoele which is continuous with the main gastrocoele of the hydranth through the channel of the peduncle.

3. THE FORMATION OF MALE GERM CELL

When the gonophore is as small as 64 μ in its major diameter, a subspherical entocodon is already seen embedded in the endoderm lamella. The cells constituting the entocodon are not different from the generalized cells of the ectoderm, of the endoderm lamella or of the endoderm proper,

having a large nucleus of 5-6.4 μ and a nucleolus of 1.6-1.8 μ. It is only later on, when the entocodon becomes kidney-shaped in longitudinal section on account of the pressure asserted by the tip of spadix, that a differentiation into an outer, or superior, layer of "inner ectoderm" and an inner, or inferior group of cells --- the "germ mass" --- takes place, although a space in between these two may not yet be present (Fig. 6). The cells of the inner ectoderm become flattened and their nuclei correspondingly depressed to about 7 x 3.2 μ. The cells constituting the germ mass remain unchanged in shape, and so are their nuclei, which are still indistinguishable from those of the endoderm proper. These cells are designated here as the primordial germ cells. Gonophores up to 135 μ in major diameter may have their germ mass still in this stage. From this onward the nuclei of the primordial germ cells begin to take deeper Feulgen stain than those of the endoderm cells because of the condensation of chromatin material upon the nuclear reticulum. The primordial germ cells then pass gradually to the spermatogonia stage. The spermatogonia are distinctly smaller than their predecessor, having a nucleus of about 3.2-3.6 μ and a nucleolus of 1.2 μ. Gonophores up to 260 μ may still contain nothing more advanced than spermatogonia in their germ mass.

From spermatogonia the developing germ cells pass onto the meiocyte stage. This lasts a relatively long period in the process of spermatogenesis, and the cells are characterized by their hexagonal shape, the relative refractoriness of their nuclei to iron-haematoxylin, and the absence of a nucleolus. The nucleus of the meiocyte is about 3.2 μ. Gonophores up to 300 μ in major diameter may have their germ mass still in this stage. The

meiocytes are seen to be grouped into compartments (Fig.8,9) which are separated by extremely fine septa. The origin of the septum will be described shortly.

Owing to the minuteness of the chromosomes the synaptic process could not be made out in detail. The secondary spermatocytes, with a nucleus measuring 1.8 μ and still refractory to iron-haematoxylin, can be seen transforming into spermatids, which show strong affinity for this stain, and measure 1.4 μ. This transformation may occur in gonophores of 240 μ or above, often seen to progress from the interior to the periphery. From spermatids the spermatozoa are formed, their head measuring 2.5 μ in length. Developing spermatozoa are arranged in a feather-like pattern. Mature spermatozoa are liberated from the "cyst" or compartment containing them and become aggregated into a dense and smooth layer at the periphery of the gonophore (Figs. 11 & 12). In life the mature male gonophore is milky in color owing to the sperm inside.

Thus far the development of the first generation of germ cell is outlined. But beside this first generation, a second crop of germ cell is invariably present in gonophores above 250 μ in major diameter, which has escaped the notice of earlier workers. There are two centers of formation (Fig.11,13), to be merged later into one. The first center is the lower rim of the germ mass; the second is at the center of the same, next to the tip of spadix. They usually start to develop when the first generation reaches the meiocyte stage, and the gonophore about 250-285 μ in major diameter, the first center being slightly the earlier to appear. The new germ cells produced at these centers have a large nucleus and a prominent nucleolus of

the same magnitude as those of the primordial germ cell, to which they can easily be referred.

The primordial germ cells produced at the first (basal) center have only a slight tendency to advance apically, whereas those produced by the second (apical) center can definitely spread against the margin of spadix toward the basal portion of the gonophore. In the process of spreading, the primordial germ cells gradually change into spermatogonia; thus within the "cap" of the second generation formed at the apical center, the top consists of large-nucleated primordial germ cells while the lower portion is already transforming into small-nucleated spermatogonia (Figs. 12 & 13). The spreading, however, is not of uniform rate around the spadix, and frequently much more advanced on one side than on the other, hence a crescent shape is often the picture for the second generation in cross sections. Eventually the germ cells formed by the apical center extends far down to unite with those from the basal center; in this way the spadix becomes completely surrounded by the second generation (Fig. 14). The latter gradually increases in bulk through multiplication and pushes against the first generation at the periphery, from which it is, however, clearly distinguishable (Figs. 10, 12, 15). Judged from the normalcy of the second generation there is little doubt that it can reach maturity just as the first generation does. Also there is no reason why a tertiary generation should not occur, comparing the relatively short cycle of spermatogenesis and the long breeding season. While it is a fact that a gonophore does not contain more than two generations of germ cell at one time, one cannot be certain whether, in the case of full-sized gonophore, the more advanced generation actually

represents the original first generation or is already the second generation in itself.

The origin of the second generation is of particular interest. The fact that the spadix tip may actually break through the gonophore wall and cells are seen to protrude out of the gonophore suggested that the second generation might be formed by new endoderm cells brought to the germ mass from the spadix through eruption at the tip of spadix. This eruption would not appear altogether impossible when one sees the vigorous contraction of the gonophore wall, which can be easily demonstrated by exposing the gonophore to a beam of strong light. But a closer examination showed that the frequency of the eruption phenomenon is much too low to account for the presence of the second generation in nearly every gonophore of respectable size. On the other hand, in sections stained with modified Feulgen's method where nuclear details were more favorably shown, cells which are evidently referable to the primordial germ cells can always be found. For instance, in a young gonophore with a major diameter of 135 μ, practically all the cells in the germ mass were found in the meiocyte stage, with a nucleus of 3.2 μ, the chromatin material showing condensation and much more Feulgen positive than the nucleus of the endoderm cell in the spadix. There are, however, a small number of cells in the germ mass which have a nucleus of 4.8 μ and a prominent nucleolus (which appeared as a pale vesicle because of Feulgen negative). The chromatin material in their nucleus was just as diffused as in the ordinary endoderm cell. These cells are undoubtedly the primordial germ cells in their resting stage (Fig. 7). They are distributed in the germ

mass mostly on the top of spadix and in the region of the rim, thus corresponding to the position where the second generation is to occur. The second generation therefore is formed from primordial germ cells which hitherto remain dormant in the germ mass.

The grouping of germ cells into radiating cysts (Figs. 8, 9) from the meiocyte stage onward (meiocytes, spermatocytes, spermatids and newly metamorphosed spermatozoa) is due to the presence of thin septa between the cysts. The septum itself was found to be the cytoplasmic process of a cell which may be designated as the septum cell (Fig. 10). Septum cells are mostly located along the outer margin of the germ mass in between two neighboring cysts. Their nuclei tend to orientate in a radial direction, measuring about 6.4 x 4.2 μ and provided with a distinct nucleolus. Their cell body is triangular shaped in section, its base resting on the outer margin of the germ mass and its apex prolonged into a thin process running in between the cords toward the spadix. The process may reach a length of 75 μ or more, stops short at the margin of the second generation.

As to the origin of these septum cells, it was revealed that they are already present, though inconspicuously, in the spermatogonia stage, because they can be distinguished from the spermatogonia by their clearer karyoplasm and larger nucleolus (1.6 μ in contrast to 1.2 μ of spermatogonia). Their distribution along the periphery of the germ mass is also indicative of their nature. These cells sometimes can be distinguished even in the primordial germ cell stage. For instance, in a gonophore of 135 μ, where the germ cell was still of the primordial type, these cells could already be marked out by their clearer karyoplasm and peripheral distribution. No such dis-

tinction can be made in the solid entocodon stage. The septum cells therefore are differentiated from the primordial germ cells of the later phase.

After the mature spermatozoa are liberated from their cysts, the second generation is seen to be surrounded by a thick layer of relatively homogeneous matrix, in which the developing germ cells are advancing toward the periphery. There are already some scattered cells close to the margin of this matrix; these cells are considered to be presumptive septum cells. In gonophores where all the sperm has been discharged and no new generation is forming, as is the case when the breeding season is nearly over, the spadix becomes surrounded by a thin layer of matrix in which only a small number of primordial germ cells are left.

The male germ cell, particularly in the meiocyte stage, is susceptible to a parasitic ciliate belonging to the genus Anophrys, hitherto reported only in sea urchins (Kudo, '46). This ciliate ranging from 30-60 μ in length, penetrates the germ mass, feeds voraciously on the meiocytes, and multiplies by binary fission to such an extent that the whole gonophore can be filled to capacity with this parasite (Fig. 16). The spadix is completely destroyed in heavily infected gonophores. Meiocytes after being ingested retain their individuality for a short time, and the picture of the meiocytes aggregating to form a giant cell with the acquisition of a new common nucleus was very puzzling. It was not until an examination on living gonophoresthat this parasitism was revealed.

Occasionally the spadix of the male gonophore was found to

contain mature spermatozoa in its "lumen" (Fig. 9). These spermatozoa might have gained access to the gastrocoele through the mouth opening of the hydranth, and from the gastrocoele they could reach the spadix through the channel of the peduncle.

4. THE FORMATION OF FEMALE GERM CELL

The primordial germ cells of the female gonophore are formed in the same way as that of the male, namely, they arise from the endoderm cells at the tip of the gonophoral bud and constitute the germ mass after the originally solid entocodon has acquired the subumbrellar cavity. No difference can be made out between male and female, as far as the germ mass is concerned, until the gonophore reaches about 105 μ in major diameter, when a slight asymmetrical development in the germ mass begins to be noticeable in the female. The primordial germ cells at one side of the spadix become less compactly arranged than the other, although the cells themselves do not show increase in size yet. This marks the side where the definitive egg will start its development. In contrast to the male where there is a reduction in size when the primordial germ cell is transforming into the spermatogonium, no corresponding reduction is observed in the female and the developing germ cells pass into oogonia and then meiocytes without sharp morphological distinction.

The meiocytes are all of the same size when they start their growth. Soon, however, those on the side where they are fewer in number gain in their rate of development and outgrow their partners on the other side of the spadix. They frequently reach the size of 27 x 19 μ or more, and may have a nucleus of 13 μ before any fusion takes place. The space

for their increase in size must be gained at the expense of the small meiocytes, which, having a nucleus of only 4.8 μ or less, are seen to be pushed closer and closer to one another. There is a rather clear line of junction between these two classes of meiocytes, large and small (Fig. 17). The former are eventually to be annexed to form the definitive egg (Fig. 20), the latter are under-privileged in this generation and will be left behind on the spadix, waiting for their chance in the second generation. It might be mentioned in advance that primordial germ cells are also present at the tip of spadix as in the male, but unlike the male, they are not directly responsible for the second generation of egg. Cells corresponding to the septum cells of the male are also seen scattered along the peripheral margin of the germ mass ~~(Fig. 7)~~.

A fusion process subsequently takes place among the large meiocytes. There are many centers of fusion, each forming a so-called "plasmodial area." Within each such area, cell membrane of neighboring meiocytes breaks down and their cytoplasm merges together (Fig. 18). A plasmodial area is therefore a syncitium, containing many nuclei. It is temporarily separated from its neighbor by a septum, which is apparently the cytoplasmic process of the septum cell. Vacuolization of the cytoplasm now occurs, starting from near the spadix toward the periphery. A number of the nuclei show sign of degeneration after this cytoplasmic fusion. This degeneration is first seen in a change of the nucleolus which, instead of being round, becomes elongated, drop-shaped, or even branched, and often acquires a vacuole in its center. These deformed nucleoli are frequently seen shift to the periphery of the nucleus, then pass out of the latter and disintegrate (Fig. 19). When the

section is stained with Mallory's triple stain, the nucleolus stains orange against the purple of the nucleus, and after its disintegration fine orange granules may still be seen for a short time in the vicinity of the nucleus from which it was expelled. Enucleolated nuclei likewise disappear very soon. That they actually disappear is proved by the presence among the syncitium of many blurred shadows the size, shape, and staining reaction of a nucleus but lacking a nucleolus. At first they were thought to be a part of a normal nucleus through whose periphery the section had been passing, but a careful mapping of the blurred nuclei on the sections immediately preceding and following revealed that they were actually in the process of disintegration.

In the final stage of meiocyte development the hitherto separate plasmodial areas begin to fuse with one another and eventually unite into one large, amoeboid-shaped oocyte which is seen partially wrapping the spadix. In this final phase of fusion all the septa between the plasmodial areas are broken down. Owing to the extensive vacuolization the entire oocyte appears highly alveolar. Embedded in it are numerous "pseudocells" the formation of which will presently be described. The oocyte up to this moment is still connected with the underdeveloped meiocytes, but soon it severs itself from the spadix to form the definitive egg. The undersized meiocytes remain on the spadix and start to grow after the first egg is detached.

In the majority of cases there is only one detached egg in each mature gonophore. Not rarely, however, the definitive egg is seen in the process of segmentation, either longitudinal or horizontal, or both, which

is very different from the pattern of ordinary cleavage. Sections of these segmenting eggs indicated that they were still unfertilized. Through this segmentation process 2 to 4 secondary eggs are resulted from the original definitive egg. Fig. 21A shows 3 secondary eggs are being formed of the original egg. In another live gonophore, 4 actinulae and 1 egg have been recovered (Fig. 21B), the former apparently derived from the 4 secondary eggs of the first generation while the latter being the egg of the second generation.

As mentioned above, a number of the nuclei disintegrated following the expulsion of their nucleoli, but those which retain the nucleoli are gradually converted into the so-called pseudocells. The process has been followed up with the aid of Feulgen stain as well as toluidin blue (following ribonuclease digestion as described under "Material and Methods"). The nucleus concerned enlarges somewhat first; its karyoplasm changes from apparent homogeneity to coarse granular. Further condensation results in a number of distinct granules which are decidedly chromatin in nature. These granules in turn coalesce into one or two large globules, the size of which depends on the number present in the nucleus. They are much larger (about 4.5 μ) than the nucleolus (about 3.2 μ) when there is only a single globule, but are of about the same size as the nucleolus when two are existing in the same nucleus. As a result of this condensation of chromatin material, the nucleus becomes more refractory in appearance and is now transformed into a typical pseudocell. The chromatin globules of the pseudocell may again break up into numerous small rings or shapeless fragments dispersing in the "cell". The nucleolus, however, survives all these changes.

Prepared section often shows pictures which are suggestive of a division of the pseudocells. An examination on live material proved that this is not an artefact, and both chromatin globule and nucleolus are involved in the division. Daughter pseudocells are formed endogenously, acquiring their own membrane while still within the parent "cell" membrane; they were also seen to be in the process of separation from their parent. Unequal crescentic splitting of the pseudocells frequently occurs, giving off a small, lens-shaped portion which lacks either nucleolus or chromatin globule or both.

5. ACCIDENTAL HERMAPHRODITISM

Tubularia crocea was known to be strictly dioecious and hermaphroditism has not been reported, so far as the writer is aware, for this species. No case of hermaphroditism has been met with among thousands of gonophores which had been cut into serial sections. However, in a regeneration experiment to be described under the next heading, one of the 22 regenerated hydranths distinguished itself by bearing a milky white gonophore which is characteristic of male sex. That hydranth was then fixed and cut into section. It was found to bear 6 maturing gonophores the contents of which are listed below:

1 Pure female, with large oocyte;

1 Pure female, with germ cell in meiocyte stage;

1 Hermaphrodite, containing 1 actinula; second generation consisted of male germ cell already in meiocyte stage;

1 Potential hermaphrodite, containing 1 actinula; second generation showed breakdown of female meiocytes, but no male element yet formed;

1 Pure male, with well developed spermatozoa;

1 Gonophore contents distorted, sex could not be ascertained. The present case is therefore not only a hermaphroditism with reference to the colony at large, but also with reference to the gonophore itself.

EXPERIMENT ON THE REGENERATION OF GERM CELL

It is well known that Tubularia can regenerate its hydranth after the latter is cut off from the stem. Yet it seems that no definite information is available as to the regeneration of the germ cells, particularly whether the regenerated gonophore contains germ cells at all, or, if it does, whether the regenerated germ cells are functional or not. This issue is important, obviously, in view of Weismann's hypothesis of germ-site shift (see Discussion). In order to answer this question, a very simple experiment was made in the following way. A portion of a mature female colony of T. crocea was isolated; the few branches were all removed at their axils, after which the portion consisted of 22 stems each with a hydranth at top and attached at the base to the entangled stolon. All of the 22 hydranths were removed, together with about 1 cm. of stem beneath each hydranth, which was about one-fourth the total length of the stem of that colony. By the time the hydranths were severed, each of them contained maturing gonophores through whose wall actinulae and eggs were clearly visible. The scheme for the male sex was slightly modified. A single stem of a male colony, severed at about 1 cm. below the hydranth and also at a point immediately above the stolon, was used for this experiment. Both the decapitated female colony and the single piece of male stem were put

into running sea-water on June 14, 1947. Emergence of hydranth occurred in both cases by the end of 48 hours (water temperature 18-20°C.), at the end of every stem of the female colony, and at both ends of the male stem. On July 25 the gonophores on both hydranths of the male turned to milky white. Microscopic examination revealed they both contained actively swimming spermatozoa. The male stem was then transferred to the jar containing the female colony. On July 3 the latter was found to have contained actinulae and eggs in most of their hydranths. This result proves beyond any doubt that germ cells can be regenerated from both male and female Tubularia, and the regenerated germ cells are actually functional.

DISCUSSION

From the foregoing description it is clear that the germ cells, male or female, are formed in the inferior layer of the entocodon which in turn is formed by a proliferation of the endoderm cells at the tip of the gonophoral bud. There is no primordial germ cell at all in the ectoderm of peduncle, as has been reported by some authors, and by comparing the ectoderm layer of the present material with what has been shown in Lowe's drawing ('26) it seems likely that those authors who purported an origin in the ectoderm of peduncle and a subsequent migration of germ cells up the peduncle were actually dealing with the nematocytes instead of with the primordial germ cells (Jickeli, 1883; Brauer, 1891; Schneider, '02; Hargitt, '04; Lowe, '26). With regard to entocodon formation, there is no doubt that in this species the ectoderm of the gonophore bud plays no part. No sign whatever can be seen suggestive of its proliferation,

invagination, or isolation. This result stands therefore in sharp contradiction to most authors working on the same genus (Ciamician, 1878; Weismann, 1880, 1883; Hamann, 1882; Allen, '00; C. H. Hargitt, '04; Goette, '07; G. P. Hargitt, '09; Perez, '13; Dupont, '42). It is all the more surprising in view of the result of Allen ('00) who has been working on exactly the same species. The failure to detect the real origin of the entocodon is probably attributable to the possible absence of the earliest stages of gonophore development in her material.

The only paper maintaining an endodermal origin of the entocodon is that of Benoit ('25). While agreeing that the entocodon is formed by the endoderm, no evidence has been found to support his statement that the entocodon is formed from the division of a single interstitial cell which came to lie among the endoderm cells. Observations go to show that no interstitial cell is needed for entocodon formation, and the proliferation is carried out by the ordinary endoderm cells at the apical region of the gonophoral bud. Since primordial germ cells are derived from the entocodon, their origin should undoubtedly be endodermal.

That the germ cells of Tubularia crocea are formed independently of the interstitial cells is of particular advantage in elucidating the origin and nature of the primordial germ cells. In many instances (Wulfert, '02; Harm, '02; Downing, '05; Stschelkanowjew, '06; Brien, '42) the origin of the germ cell of hydrozoa has been traced back to the interstitial cell. But once the primordial germ cell merges itself into the so-called I-cell, any argument about a germ track or about the nature

of the primordial germ cell becomes futile. As the I-cell can give rise to many different types of cell beside the germ cell (p. 15), one can never be sure, during embryonic stage in particular, that the cell one is dealing with is a presumptive germ cell, or something else. The identification of certain interstitial cell as primordial germ cell has already been found to be subjective and unwarranted (Wager, '09; Tanreuther, '09). Yet on the other hand, one also has no way of disproving an early segregation of germ-plasm in this group of animals, because interstitial cells, from which the primordial germ cell is morphologically indistinguishable at least, do arise early in embryonic development. The problem would remain deadlocked so long as the primordial germ cell is tied up with the I-cell.

With a dissociation of these two types of cells, Tubularia crocea enables us to state definitely that its germ line is discontinuous, because they arise only after the gonophoral bud is established. No indication of the slightest degree can be found of the precocious development or migration of the primordial germ cells. This seems necessarily to cast doubt on Brien's recent generalization on the differentiation of sexual cells in the animal series ('42), which reads:

I. Chez les Hydroïdes donnant des méduses, elle est tardive, discontinue et disperse (méduses);

II. Chez les Hydroïdes porteurs de gonophores, elle est précoce, continue et les cellules sexuées se localisent tardivement dans le gonophore = gonade;

III. Chez les Coelomates et selon des degrés variables, elle est très précoce, limitée aux stades embryonnaires, les cellules sexuelles étant immédiatement localisées en gonade.

For Tubularia is a gonophore-bearing hydroid and should come under II in which it, however, does not fit.

The present study also sheds light on the nature of the primordial germ cells of this form. The primordial germ cells, to begin with, are nothing but a part of the endoderm layer of the gonophoral bud. This layer is a direct continuation of the endoderm layer of the peduncle, which in turn is an extension of the endoderm of the hydranth. There can be no doubt about the fact that the primordial germ cell in this case is derived from dedifferentiated somatic cells. The following fact serves to illustrate further the closeness or even inseparableness between the soma and the germ-plasm. The entocodon at first is made up of a solid mass of cells which are derived from the endoderm. The cells constituting the mass are all alike at that time. Only when a subumbrellar cavity develops in the mass and cells of the topmost layer begin to flatten to form the inner ectoderm, does the germ mass begin to take shape, but even then a transitional state can be found between germ mass, which is germinal, and the inner ectoderm, which is somatic. Moreover, the germ mass is supposed to consist of primordial germ cells only. Yet the present investigation reveals that the primordial germ cells do not all differentiate into germ cells; a small number of them transform into septum cells which are found scattered along the peripheral region of the germ mass. These septum cells can not, of course, be germinal. We thus have a clear example of the germ-plasm taking its origin from somatic cells and differentiating into somatic cells side by side with the germ cells. All these seem to point to one interpretation: that there is no innate fundamental difference between germ-plasm and soma

as Weismann postulated, that there are no fatalistic or predetermined germ cells. The cause of differentiation, whether into various somatic cells or into germ cell, is to be sought extrinsically rather than in the "determinants" within each cell, although the intrinsic factor is indispensible as a substratum for the extrinsic factors to work upon.

It has been indicated elsewhere (p. 26) that Weismann's hypothesis of germ-site shift was constructed largely on the doctrine of recapitulation, and there was little evidence to support a migration of the primordial germ cells which, however, had been used to impart the idea that only certain predetermined cells are able to differentiate into germ cells (W.* p. 226). As a test of the validity of his theme, the result obtained from the regeneration of germ cells in the experiment reported above should be conclusive. For according to the hypothesis of germ-site shift, Tubularia, having its primordial germ cells originating in the ectoderm and carried over to the entocodon simply by the formative force of the gonophoral bud, does not have a migration of the primordial germ cells, which Weismann himself mentioned as an example of his Stage II (W. p. 286). Accordingly, if the hydranth is removed from a stem, the latter should never form germ cells, since all the primordial germ cells should have been eliminated. Even when about one-fourth the length of the stem together with the hydranth is cut off, in order to provide an extra safety margin for the exclusion of any primordial germ cell from the stem, the result showed that functional germ cells were actually

---

*For citations beginning with W. refer to Weismann's monograph, 1883.

regenerated. It is especially convincing in the case of the male stem (p. 65) because spermatozoa were formed even in the hydranth low down the stem at the level of the stolon. This simple experiment is therefore sufficient to disprove the essence of the germ-plasm theory. It might be argued, as Weismann did, that the "sex molecule" might remain mixed with the soma over a long period of time. But if they were to separate at all, the germ-plasm ought to be segregated, at the latest, by the time of sexual maturity. Since the material used in this regeneration experiment was all mature, as can be evidenced by the presence of actinulae or spermatozoa in the previous hydranths, there is certainly no reason to defend the concept that the germ-plasm had yet not separated from the soma. The only alternative interpretation is that in this animal at least, the germ-plasm is forever diffuse with and never separate from the soma, or, more correctly, there is no such distinction into germ-plasm and soma until the morphological differentiation of germ cell is under way.

The presence and formation of the second generation of male germ cell is here reported for the first time. Previous workers noticed that the germ cells in the male gonophores were younger in the region near the spadix (Thallwitz, 1885; Benoit, '25), and this fact led them to think that spermatogenesis progressed from periphery inward. The present investigation reveals that the male germ cells around the spadix belong to another generation, which has a dual center of formation. The two generations of germ cell, though coexisting in the same gonophore, are rather distinct from each other in their stage of development. Within each generation, however, the developmental stages are relatively uniform and advance from

inside out (Fig.10) instead of in the reverse order as reported by earlier workers. The origin of the second generation has been traced to some primordial germ cells lying dormant in the germ mass. This does supply some evidence for the conception that germ cells may not all follow the same tempo of development, that the early stages of germ cell retain their property of self-propagation for a certain length of time, which fact has often been used to defend Weismann's theory of germinal continuity (Everett, '45). It must be emphasized, however, that the phenomen of segregation is by no means unique for germ-plasm. Any somatic tissue, after proceeding to a certain degree of differentiation, will "breed true" to their own character, as is well known in tissue culture study, yet this does not imply that these tissues are inherently different from each other before their differentiation. Levander's experiment ('45) is sufficient to show how widely different tissues can be formed from a common source-material, the mesenchyme. For the present case, it suffices to say that segregation of this sort is altogether meaningless as an evidence of the Weismannian principle, because the primordial germ cells themselves are formed from somatic tissue in the first place.

Accidental hermaphroditism has not been hitherto reported in T. crocea, although it is known to occur in some congeneric species (Kleinenberg, 1881; Bonnevie, 1898; Perez, '13). The present case is of interest because of that it occurred after the regeneration of the hydranth, and it seems possible that certain degree of correlation might exist between these two incidents. As Kleinenberg (1881) communicated

long ago, T. mesembryanthemm is normally dioecious but hermaphroditism is not infrequent at the interval between the two main breeding seasons of the year. In both instances, one can suspect that the environment under which the germ cells were being formed was not as favorable as it should normally have been, and it is conceivable that this unfavorable environmental condition might in turn effect the mechanism of sex determination of some hydranth. Of particular interest is the fact that the present case shows a gonophore in which the first generation of germ cell was female whereas the second generation was definitely male. In view of the way the second generation is formed, there can be only one conclusion; namely, the primordial germ cells, even after long been segregated, are still capable of changing their sexuality. In other words, they are not sexually determined even after they have been in the germ mass for a certain length of time. This conclusion would appear, however, to stand in contradiction to the results obtained by Foyn ('27). This author mixed the material expressed from the male and female stems of Clava squamata in equal proportion, and got hermaphroditism in the colony developed from the reunition mass. His result is also in line with the well-known fact that hydrozoa impart their sexuality to the buds, just as dioecious plant does in the course of vegetative multiplication. To reconcile the apparently contradictory results it may be necessary to accept the potential bisexuality of the individual, male or female, as proposed by Witschi ('39), and to assume that the vegetative part of the female is producing a kind of chemical messenger, under whose influence the primordial germ cells would differentiate along female direction, whereas in its absence or

deficit, as presumably in the male individual, they would differentiate into male. It has also to be taken for granted that the primordial germ cells are refractory to the chemical factor so long as they remain dormant. The present case then can be interpreted on the ground that the chemical messenger produced by the vegetative part of that female polyp was sufficient enough at the beginning, so that the first generation of germ cells differentiated into oogonia and eventually to mature egg, yet the same individual failed to keep on producing this chemical in sufficient quantity, presumably owing to unfavorable environment, and under such condition the primordial germ cells of the second generation, hitherto lain dormant and thus refractory to the previous chemical mediator, now differentiated into spermatogonia because of the deficit of the same. At any rate, sex determination in Tubularia, or hydroids in general, is by no means an once-for-all process. It has to be operated locally on each individual gonophore, and on successive generations of the primordial germ cells contained therein.

In conclusion, reference may again be made to Weismannism. It is commonly known that Weismann based his theory of germinal continuity upon his work on the Hydromedusa (Downing, '05; Everett, '45). Paradoxically as it may seem, the Hydromedusa (Hydrozoa) turns out to be the group that furnishes the best evidence to disprove Weismannism, in view of the results of previous workers (Goette, '07; G. T. Hargitt, '19) and the present study. The direct transformation from tissue cell to germ cell as reported by earlier investigators, and the permanent mixing of germ and soma as reported in this paper, are fatally incompatible to his

theory of germ-plasm. Weismann, however, already noticed the phenomena of budding, fission, gemmation and regeneration among animals and plants, and defended with the argument that "a sharp distinction first arose ~~between~~ between the somatic cells and the germ-cells, and the idioplasm of the somatic cells was only subsequently provided with germ-plasm in a latent condition in those cases in which this arrangement was a useful one" (Weismann, 1898, p. 212). However, the existence of this "sharp distinction" was entirely imaginary, and was based on the "differentiation of the idioplasm" which in turn was a corollary of the germ-plasm theory. He was therefore defending a theory with an argument based on the very theory he was attempting to defend. Obviously there is no such distinction as soma and germ-plasm in these animals in the first place, and his theories are roundabout and superfluous. Yet Weismannism is far from dying away. Everett, in his recent review on the germ cell problem in vertebrates ('45), tended to uphold Weismann's principle of germinal continuity on the ground of a probable early segregation of germ cell. It should be emphasized that the claim of an early segregation in vertebrates is to date still much disputed and by no means established, which Everett himself acknowledged. On the other hand, in forms such as Nereis (Wilson, 1892), Crepidula (Conklin, 1897; Costello, '45), Styela (Conklin, '05), etc. where cell lineage has been worked out in considerable detail, it is established beyond any doubt that an early segregation of germ-plasm is non-existent, while somatic cells may actually segregate much earlier than germ cell. Thus in Neries limbata, the germ-plasm is merged with endoderm components in the form of mesentoblasts and still far of sight when the embryo is at 42-cell stage, whereas the

trophoblasts are definitely segregated early at 16-cell stage, the intermediate stages being 23-, 29-, 32-, and 36-cell. It is obvious that early segregation is not characteristic of germ-plasm, and at the same time is not limited to germ-plasm. The point of issue is therefore whether there is a sharp distinction between germ and soma and whether there is a predetermination of germ cell as Weismann postulated, and not merely a matter of the time of segregation. As Weismann considered all tissues capable of producing or regeneration as due to the presence of germ-plasm, the phenomena in the slime mold Dictyostelium discoideum as reported by Raper ('40) and Bonner ('44) should be relevent in elucidating this point. The separate amoebae of that mold have no relation at all at the beginning; a predestination is therefore out of question. These amoebae, when aggregated at random, organize themselves into a multicellular organism, show histological differentiation and turn a part of themselves into spore cells, which ought to be the germ-plasm according to Weismann's sense. Moreover, cells which have a somatic prospective significance (e.g., the stalk cells) are able to form spore cells when isolated from the colony (Raper, '40). The formation of "germ-plasm", therefore, like that of the varicus somatic cells, is a problem of differentiation and not one of predetermination. There is no fundamental difference between germ and soma, and from the regeneration of germ cell in Tubularia, we see clearly that germ-plasm is forever contained in all of the general somatic cells. The problem then boils down to the question of how differentiation could take place among cells which are potentially all alike. Levander ('42) already showed that the prospective significance of the mesenchyme cells can be shifted by exposing to certain

cell-free extract, and it is to be anticipated that work along this line will pave the way for a better understanding of the mechanism of differentiation, germinal as well as somatic.

SUMMARY AND CONCLUSION

1. In contradiction to the results of earlier workers, the germ cell of both sex is found to be of endodermal origin in Tubularia crocea.

2. No interstitial cells are involved in germ cell formation. Germ cells begin and complete their development in the entocodon, and there is neither early segregation nor migration.

3. The entocodon, from which the germ cells arise, is in turn formed of somatic cells.

4. The primordial germ cells beside differentiating into germ cells give rise to septum cells, which are somatic instead of germinal.

5. A second generation of germ cells is found to occur in the maturing male gonophore. This generation has a double center of formation and is traced back to those primordial germ cells which previously lay dormant in the germ mass.

6. The definitive egg is formed by a process of annexation of sister cells. There is no morphological evidence of a particular cell that controls this process.

7. Nuclei of fused cells disintegrate during the early phase of annexation. This disintegration is preceded by an expulsion of nucleolus hitherto unreported.

8. Division of the "pseudocell" can be observed in living

material. The chromatin nature of the globules it contains is confirmed by its positive reaction to Feulgen's stain.

9. Evidence is presented showing that the definitive egg may segment into 2-4 secondary eggs before fertilization.

10. Experiments prove that general tissue cells throughout the entire length of the stem have the potentiality to form germ cells. "Germplasm" and soma remain permanently diffuse in the stem tissue.

11. A case of accidental hermaphroditism occurred after regeneration of a female colony. In the same gonophore an actinula of the first generation coexisted with male germ cells of the second. The possible mechanism of sex determination is discussed.

12. A ciliate, Anophrys, is often found present in gonophores of both sexes, but especially in the male. It devours the immature germ cell and may multiply to such an extent that the whole gonophore can be packed with this parasite.

13. An extensive analysis is made of Weismann's original work on the sex cells of Hydromedusa. It shows that his theory was shaped on the doctrine of recapitulation and there is no factual evidence to justify the predestination of germ cell and the fundamental difference between germ and soma.

14. It is suggested, in view of the results of recent experiments, that the cause of differentiation, somatic as well as germinal, is to be sought in the extrinsic factors rather than in the intrinsic "determinants"

LITERATURE CITED

*Original paper not seen

Agassiz, L. 1860 Contributions of the natural history of the Acaleaphoe of North America, III. Boston.

Allen, C. M. 1900 A contribution to the development of Parypha crocea. Biol. Bull., 1:291-315.

Allman, G. J. 1871 A monograph of the gymnoblastic or tubularian hydroids. London.

1875 On the structure and development of Myriothela. Philos. Transact., 165:549-576.

Balfour, F. M. 1880 A treatise on comparative embryology. Vol. 1. Cambridge Univ. Press.

Beer, G. R. de 1928 Vertebrate zoology. London.

Beneden, E. van 1874 De la distinction originelle du testicule et de l'ovaire; caractere sexuel des deux feuillets primordiaux de l'embryon, hermaphroditisme morphologique de toute individualite animale, essai d'une theorie de la fecondation. Bull. acad. roy. Sci. lett. Belgique, 37:530-595.

Bergh, R. S. 1879 Studien die erste Entwicklung des Eies von Gonothyrea loveni. Morph. Jahrb., 5:22-61.

Bonner, J. T. 1944 A descriptive study of the development of the slime mold Dictyostelium discoideum. Amer. Jour. Bot., 31:175-182.

Bonnevie, K. 1898 Zur Systematik der Hydroiden. Zeitsch. f. wiss. Zool., 63: 465-495.

Benoit, P. 1921 Les gonophores femelles de Tubularia mesembryanthemum. C.R.A.S. Paris, 173:171-174.

1925 L'ovogenese et les premiers stades du developpement chex la Myriothele et chez la Tubulaire. Arch. de Zool. exp. et gen., 64:85-326.

Boulenger, C. L. 1910 On the origin and migration of the stinging cells in craspedote medusae. Quart. Jour. Micro. Sci., 55:763-783.

Brachet, J. 1942 La localisation des acides pentosenucleiques dans les tissus d'animaux et les oeufs d'amphibiens en voie de developpement. Arch. de Biol., 53:207-257.

Braem, F. 1894 Über die Knospung bei mehrschichtigen Tieren, insbesondere bei Hydroiden. Biol. Zentbl., 14:140-161.

1910 Die ungeschlechtliche Fortpflanzung als Vorläufer der geschlechtlichen. Biol. Zentbl., 30:367-379.

Brauer, A. 1891 Über die Entstehung der Geschlechtsproducten und die Entwicklung von Tubularia mesembryanthemum. Zeitsch. f. wiss. Zool., 52:551-579.

Brien, P. 1942 Etudes sur deux hydroides gymnoblastiques, Cladonema radiatum (Duj.) et Clava squamata (O.F. Müller). Memoires Acad. Royale de Belgique, 20:1-116.

Bunting, M. 1894 The origin of the sex-cells in Hydractinia and Podocoryne, and the development of Hydractinia. Journ. Morph., 9:203-236.

Ciamician, J. 1878 Zur Frage uber die Entstehung der Geschlechtstoffe bei den Hydroiden. Zeitsch. f. wiss. Zool., 30:501-508.

Conklin, E. G. 1897 The embryology of Crepidula, a contribution to the cell lineage and early development of some marine gastropods. Journ. Morph., 13:1-226.

1905 The organization and cell lineage of the ascidian egg. Journ. Acad. Nat. Sci. Phil., 13:5-119.

Costello, D. P. 1945 Experimental studies of germinal localization in Nereis. I. The development of isolated blastomeres. Journ. Exp. Zool., 100:20-66.

Doflein, F. 1896 Die Eibildung bei Tubularia. Zeitsch. f. wiss. Zool., 62:61-73.

Downing, E. R. 1905 The spermatogenesis of Hydra. Zool. Jahrb. Abt. Anat., 21:379-426.

Dujardin, F. 1845 Memoire sur le developpement des meduses et des polypes hydraires. Annales des Sciences Naturelles (3) 4:257-281.

Dupont, M. 1942 Origine des cellules germinales et formation du gonophore chez les Tubulariaires. Arch. de Zool., 53:493-511.

Everett, N. B. 1945 The present status of the germ-cell problem in vertebrates Biol. Rev., 20:45-55.

Foyn, B. 1927 Studien über Geschlecht und Geschlechtszellen bei Hydroiden II. Auspressungsversuche an Clava squamata (Müller), mit Mischung von Zellen aus Polypen desselben oder verschiedenen Geschlechts. Roux' Arch. f. Entwmech., 110:89-148.

Fraipont, J. 1880 Recherches sur l'organisation histologique et le developpement de Campanularia angulata. Arch. zool. exp. gen., 8:433-466.

Gelei, J.van 1925 Über die Sprossbildung bei Hydra grisea. Roux' Arch., 105.633-654.

Goetsch, W. 1929 Das Regenerationsmaterial und seine experimentelle Beeinflussung. Roux. Arch., 117:211-311.

Goette, A. 1880 Ein neuer Hydroid-Polyp mit einer neuen Art der Fortpflanzung. Zool. Anz., 3:352-358.

1907 Vergleichende Entwicklungsgeschichte der Geschleschts-individuen der Hydropolypen. Zeitsch. wiss. Zool., 87:1-335.

Gosse, P. H. 1853 A naturalist's rambles on the Devonshire coast. London.

Groblen, C. 1875 Über Rodocoryne carnea. Sitzungsberichte der Wiener Academie der Wissenschaftung, 72:455-487.

Gröneberg, G. 1879 Beitrage zur Kenntniss der Gattung Tubularia. Zool. Jahrb. Abth. Anat., 11:61-76.

Hadzi, J. 1910 Die Entstehung der Knospe bei Hydra. Arbeiten zool. Inst. Univ. Wien, 18:61-80.

1911 Bemerkungen uber die Knospenbildung bei Hydra. Biol. Zentbl., 31:108-111.

*Haeckel, E. 1864 Die Familie der Russelquallen (Medusae Geryonidae). Jenaische Zeitsch. f. Naturwiss., 1.

Hamann, O. 1882 Der Organismus der Hydropolypen. Jena. Zeitsch., 15:473-544

1887 Die Urkeimzellen (Ureier im Tierreich und ihre Bedeutung). Jena. Zeitsch., 21:516-538.

Hardy, W. B. 1891 On some points in the histology and development of Myriothela phrygia. Quart. Journ. Micro. Sci., New series 32:505-537.

Harm, K. 1903 Die Entwicklungsgeschichte von Clava squamata. Zeitsch. f. wiss. Zool., 73:115-165.

Hargitt, C. W. 1904 Notes on some hydromedusae from the Bay of Naples. Mitteil. Zool. Stat. Neapel, 16:553-585.

Hargitt, G. T. 1909 Maturation, Fertilization and Segmentation of Pennaria tiarella and of Tubularia crocea. Bull. Mus. Comp. Zool. Harvard, 53:161-212.

1913 Germ cells of coelenterates. I. Campanularia flexuosa. Journ. Morph., 24:383-419.

1916 Germ cells of coelenterates. II. Clava leptostyla. Journ. Morph., 27:85-96.

1919 Germ cells of coelenterates. III. General consideration, discussion, conclusion. Journ. Morph., 33:1-59.

Huxley, T. H. 1858 The oceanic Hydrozoa. Ray Society. London

Hyman, L. H. 1940 The invertebrates: Protozoa through Porifera. New York.

Ishikawa, C. 1887 Über die Abstammung der männlichen Geschlechtszellen bei Eudendrium racemosum Cav. Zeitschr. f. wiss. Zool., 45: 669-671.

1888 Über die Herkunft der weiblichen Geschlechtszellen bei Podocoryne carnea Sars. Zeitschr. f. wiss. Zool., 47: 621-625.

Jickeli, C. F. 1883 Der Bau der Hydroipolypen. I. Morph. Jahrb., 8:580-676.

Jörgensen, M. 1913 Zellenstudien I, II, III. Arch. f. Zellforsch., 10:1-201.

Kanajew, J. 1930 Zur Frage der Bedeutung der insterstitiellen Zellen bei "Hydra". Arch. Entwickl., 122:736-759.

*Keferstein, W. and Ehlers, E.
1861 Zoologische Beiträge gesammelt im Winter in Neapel und Messina. I. Beobachtungen über die Siphonophoren von Messina. Leipzig.

*Kleinenberg, N. 1872 Hydra. Eine anatomichentwicklungsgeschichliche Untersuchung. Leipzig.

1881 Über die Entstehung der Eier bei Eudendrium. Zeitschr. f. wiss. Zool., 35:326-332.

Koch, G.von 1876 Mittheilungen über Coelenteraten. Morph. Jahrb., 2:83-86

Korotneff, A. 1876 Histologie de l'Hydre et de la Lucernaire. Arch. Zool. exp. et gen., ~~(2), 6:21-31.~~ 5: 369-400.

*Korotneff, A. 1880 Versuch einer vergleichenden Theorie der Coelenteraten (Myriothela und Hydra). Moskau

1888 Contribution a l'etude des Hydraires. Arch. Zool. exp. et gen., (2), 6:21-31.

Kudo, R. R. 1946 Protozoology. 3rd Edition. Illinois.

Kühn, A. 1910 Die Entwicklung der Geschlechtsindividuen der Hydromedusen. Studien zur Ontogenese und Phylogenese der Hydroiden, II. Zool. Jahrb., Abth. Anat., 30:43-174.

Lang, A. 1894 Zur Frage der Knospung der Hydroiden. Biol. Zentbl., 14:682-687.

Levander, G. 1945 Tissue Induction. Nature, 155:148-149.

Lowe, E. 1926 Embryology of Tubularia. Quart. Journ. Micro. Sci., 70:599-627.

Mattes, O. 1925 Die histologischen Vorgänge bei der Wundheilung und Regeneration von Hydra. Zool. Ang., 62:307-312.

Merejkowsky, M. C.
1880 Sur l'origine et le developpement de l'oeuf dans la meduse Eucope avant la fecondation. C.R.A.S. Paris. 90:1012-1014.

Nussbaum, M. 1880 Zur Differenzirung des Geschlechts im Tierreich. Arch. mikr. Anat., 18:1-121.

1887 Über die Teilbarkeit der lebenden Materie, II. Arch. mikr. Anat., 29:265-366.

Pantin, C. F. A.
1946 Notes on microscopical technique for zoologists. Cambridge Univ. Press.

Perez, C. 1913 Observations sur l'ovogenese et la segmentation des Tubulaires. Bull. scient. France-Belgique, 46:249-278.

Rafalko, J. S. 1946 A modified Feulgen technique for small and diffuse chromatin elements. Stain Technology, 21:91-93.

Raper, K. B. 1940 Pseudoplasmodium formation and organization in Dictyostelium discoideum. Journ. Elisha Mitchell Sci. Soc., 56:241-282.

Richards, A. 1931 Outline of comparative embryology. New York.

Romanes, G. J. 1899 An examination of Weismannism. 2nd edition. Chicago.

Rowley, H. T. 1902 Histological changes in Hydra viridis during regeneration. Amer. Naturalist, 36:579-583.

*Schneider, K. 1902 Lehrbuch der vergleichen Histologie der Tiere. Jena.

*Schulze, F. E. 1871 Über den Bau und die Entwicklung von Cordylophora lacustris nebst bemerkungen über vorkommen und Lebensweise dieses Thieres. Leipzig.

Schulze, P. 1918 Die Bedeutung der interstitiellen Zellen für Lebenvorgänge bei "Hydra." Sitzungsberichte der Gesellschaft naturforschender Freunde zu Berlin, 1918:252-277.

Serra, J. A. 1947 Histochemical tests for proteins and amino acids; the characterization of basic proteins. Stain Techn., 21:5-18.

Shumway, W. 1932 The recapitulation theory. Quart. Rev. Biol., 7:93-99.

Spemann, H. 1938 Embryonic development and induction. Yale Univ. Press.

Strelin, G. S. 1929 Röntgenologische Untersuchungen an Hydren, II. Roux' Arch., 115, 27-51.

Stschelkanowzew, J. 1906 Die Entwicklung von Cunina proboscidea Metschn. Mittheil. zool. Stat. Neapel, 17:433-486.

Tannreuther, G. W. 1909 Observations on the germ cells of Hydra. Biol. Bull., 16:205-209.

Thallwitz, J. 1885 Über die Entwicklung der männlichen Keimzellen bei den Hydroiden. Jena. Zeitschr. Naturw., 18:385-444.

*Tichomiroff, 1887 Zur Entwicklungsgeschichte der Hydroiden. Nachtr. d. ges. Liebh. naturst. Anthrop. u. Ethnog. Moscou.

Trinci, G. 1905 L'oocite degli Idroidi durante il periodo di crescita. Monitore zoologico Italiano, 16:212-214.

Varenne, A. de 1882 Recherches sur la reproduction des polypes hydraires. Arch. Zool. exp., 10:611-710.

Wager, R. E. 1909 The oogenesis and early development of Hydra. Biol. Bull., 18:1-38.

Weismann, A. 1880a Über den Ursprung der Geschlechtszellen bei den Hydroiden. Zool. Anz., 3:367-370.

1880b Zur Frage nach des Ursprung der Geschlechtszellen bei den Hydroiden. Zool. Anz., 3:226-233.

1883 Die Entstehung der Sexualzellen bei den Hydromedusen. Jena.

1892 The germ-plasm. English translation by W. N. Parker and H. Rönnfeldt. New York.

Wilson, E. B. 1892 The cell lineage of Nereis. Journ. Morph., 6:361-480.

Witschi, E. 1939 Modification of the development of sex in lower vertebrates and in mammals; in Allen's Sex and Internal Secretion. Chapter 4, Second edition. Baltimore.

Wülfert, J. 1902 Die Embryonalentwicklung von Gonothyraea loveni. Zeitschr. wiss. Zool., 71:296-327.

Zawarzin, A. A. 1929 Röntgenologische Untersuchungen an Hydren, I. Roux' Arch., 115:1-26.

EXPLANATION OF FIGURES

Sections were cut parallel to the main axis of the gonophore unless otherwise stated. All photomicrographs are unretouched.

Fig. 1. Drawing of the top view of a female hydranth, with manubrium removed to show the arrangement of the gonophoral peduncles and gonophores. Note young gonophoral peduncles in between the more advanced ones.

Fig. 2. Longitudinal section through the peripheral part of a female hydranth, showing the origin of the peduncle and the gonophoral buds. The peduncle in this case gave rise to many gonophoral buds (5 of which are shown in this section) before it had time to elongate. The epidermis and gastrodermis of the hydranth, with the mesolamella in between, are seen to pass into the peduncle and then into the gonophoral buds. The highly vacuolar endodermal cells of the peduncle and buds are studded with dark-staining glandular cells. Iron haematoxylin.

Fig. 3. Section of an early gonophore (female), showing endoderm in one single layer. Iron haematoxylin.

Fig. 4. Section of an early gonophore (female), showing entocodon in the process of delamination from the endoderm. Iron haematoxylin.

Fig. 5. Section of a gonophore (female), showing entocodon completely separated from the endoderm; endoderm-lamella barely visible to the left of the entocodon. Iron haematoxylin.

Fig. 6. Section of a more advanced gonophore (female), showing the formation of the spadix by the endoderm and the completion of the endoderm-lamella. The entocodon now appears inverted U-shaped. Delafield's haematoxylin and eosin.

Fig. 7. Section of a male gonophore of more advanced stage. With the formation of the subumbrellar space, the entocodon is differentiated into a germ mass (internal to the space) and a thin "inner ectoderm" (external to the space) which is closely applied to the endoderm-lamella. The germ cells of the germ mass are mostly in the meiocyte stage, but a few scattered primordial germ cells are also present which are, however, not distinctly shown in this picture because of the faintness of their nuclei. Feulgen and fast green.

Fig. 8. Section of a male gonophore, showing the meiocytes being grouped into radially arranged compartments. The septum cells which are responsible for this arrangement are not distinct in this preparation (See Fig. 17). Delafield's haematoxylin and eosin.

Fig. 9. Cross section of a male gonophore, showing the radial arrangement of the compartments which contain developing spermatozoa of the first generation. Mature spermatozoa begin to accumulate at the periphery of the gonophore in a very thin layer. Note also spermatozoa in the lumen of the spadix. Iron haematoxylin.

Fig. 10. A portion of a section of male gonophore, highly magnified to show the septum cells. These cells (marked by arrow-heads), belong to the germ mass and have no relation with the cells forming the "inner ectoderm". Iron haematoxylin.

Fig. 11. Section of two maturing gonophores. The first generation of germ cells in both gonophores are in meiocyte stage. Second generation begins to form at rim of the germ mass in the gonophore on the left, and at tip of spadix in addition to the rim of germ mass in the gonophore on the right. Two radial canals are shown in the latter gonophore. Iron haematoxylin.

Fig. 12. Section (cut oblique) of a male gonophore, showing developing spermatozoa. The second generation of germ cells forms a crown at the tip of the spadix. Iron haematoxylin.

Fig. 13. Section of a male gonophore, showing the downward extension of the crown. The lower center of formation (the rim of the germ mass) is also shown at the base of gonophore where the section passed beyond the spadix. Iron-haematoxylin.

Fig. 14. Section of a male gonophore, showing the merging of two centers of formation. Second generation now surrounds the entire spadix. Within the first generation, germ cells are maturing from near the spadix toward the periphery, especially on the right side of the spadix. Iron haematoxylin.

Fig. 15. Cross section of a mature male gonophore. All germ cells of the first generation have developed into spermatozoa, which are seen to aggregate at the periphery of the gonophore. The second generation is in meiocyte stage. Iron haematoxylin.

Fig. 16. Male gonophore infected with a parasitic ciliate, Anophrys. The spadix is completely destroyed. Delafield's haematoxylin and eosin.

Fig. 17. Section of peripheral part of a female gonophore, showing the demarkation between the fused meiocytes (on the left) and the unsuccessful ones (on the right). Delafield's haematoxylin and eosin.

Fig. 18. Cross section of a female gonophore, showing the fusion of the growing meiocytes into "plasmodial areas", and also the undersized meiocytes which retain their individuality. Delafield's haematoxylin and eosin.

Fig. 19. Cross section of a female gonophore, showing elimination of nucleolus from the degenerating nuclei (marked by arrowheads) of fused meiocytes. Mallory's triple stain.

Fig. 20. Cross section of a female gonophore showing "pseudocells" in an egg (right) completely detached from the spadix (left). The globules within the pseudocells are Feulgen positive. Feulgen and fast green.

Fig. 21. A. Drawing made on a living female gonophore, showing three secondary eggs resulted from the segmentation of a single mature ovum.

B. Actinulae and egg from one single gonophore, indicating that four secondary eggs had been formed. The single egg represents the fusion product of the second generation.

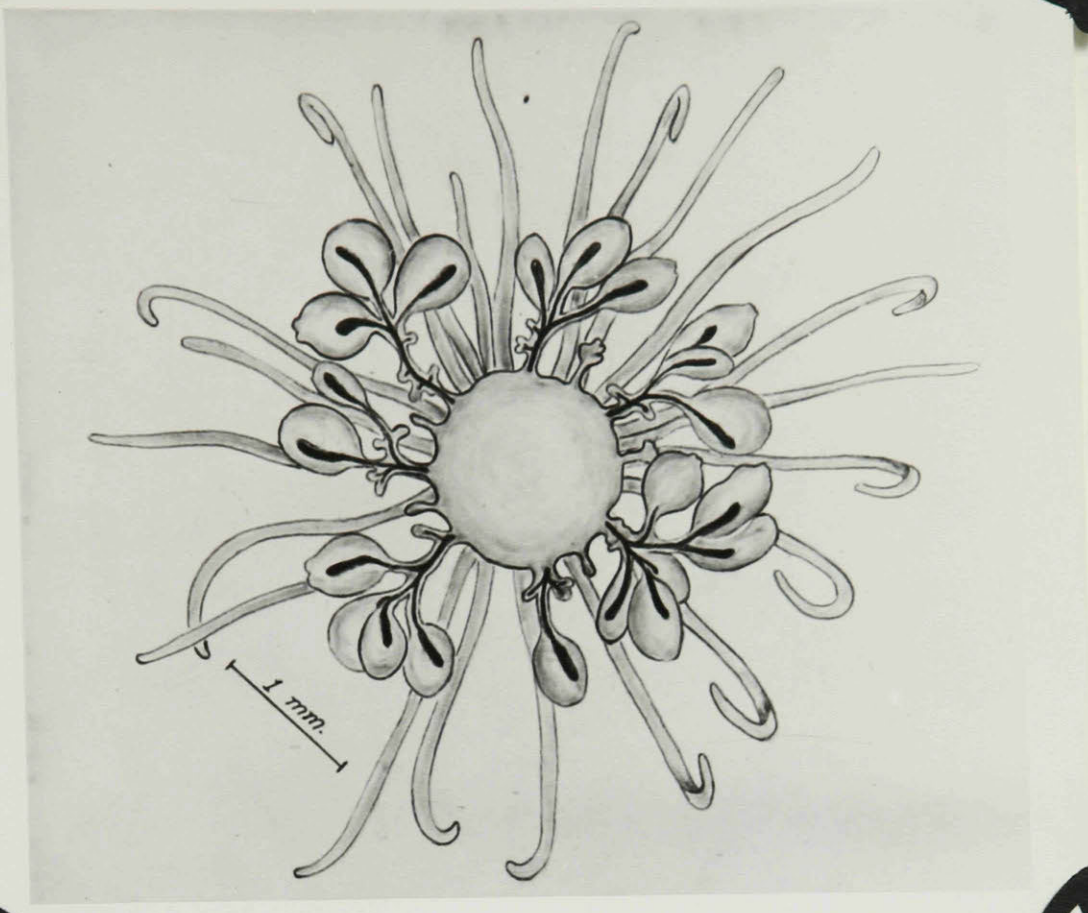

Fig. 1

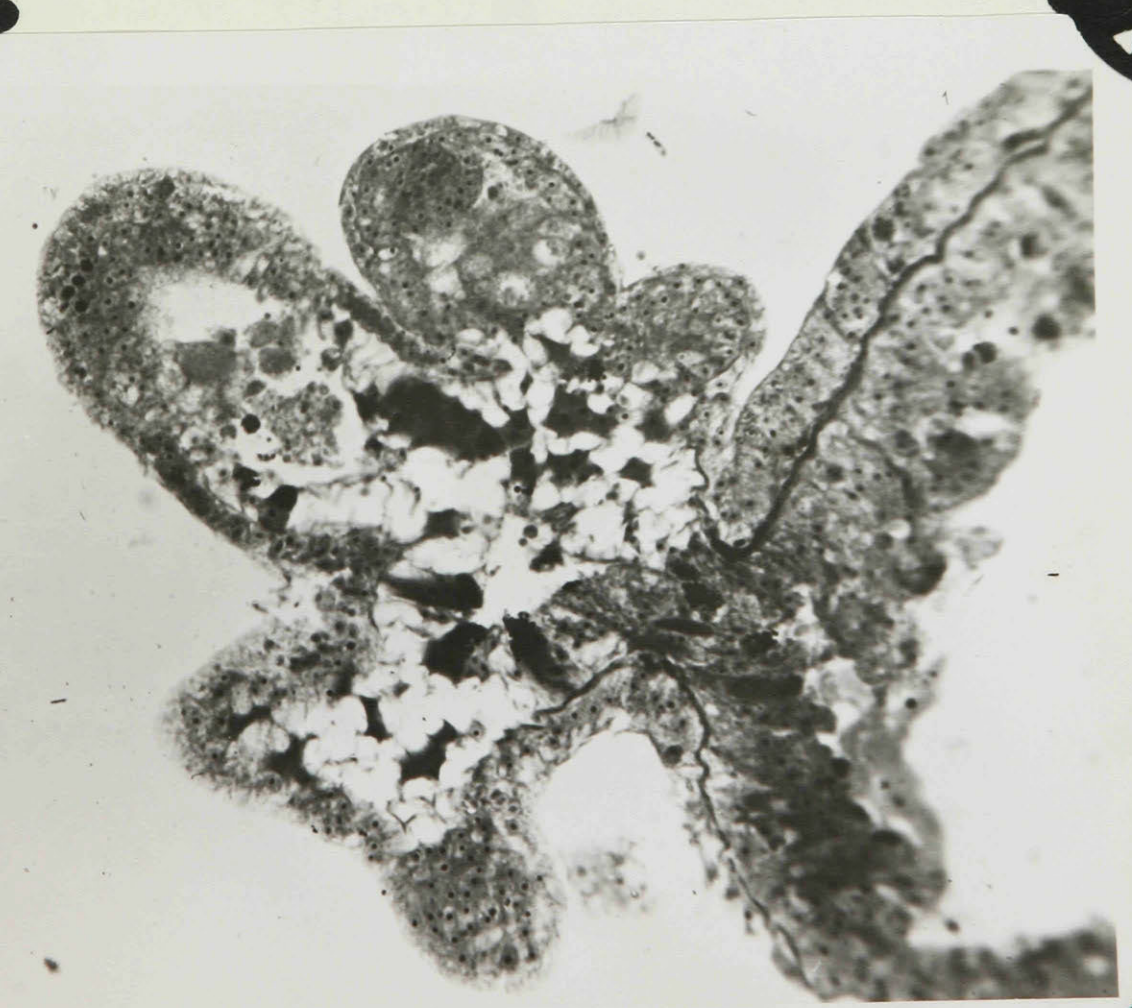

Fig. 2

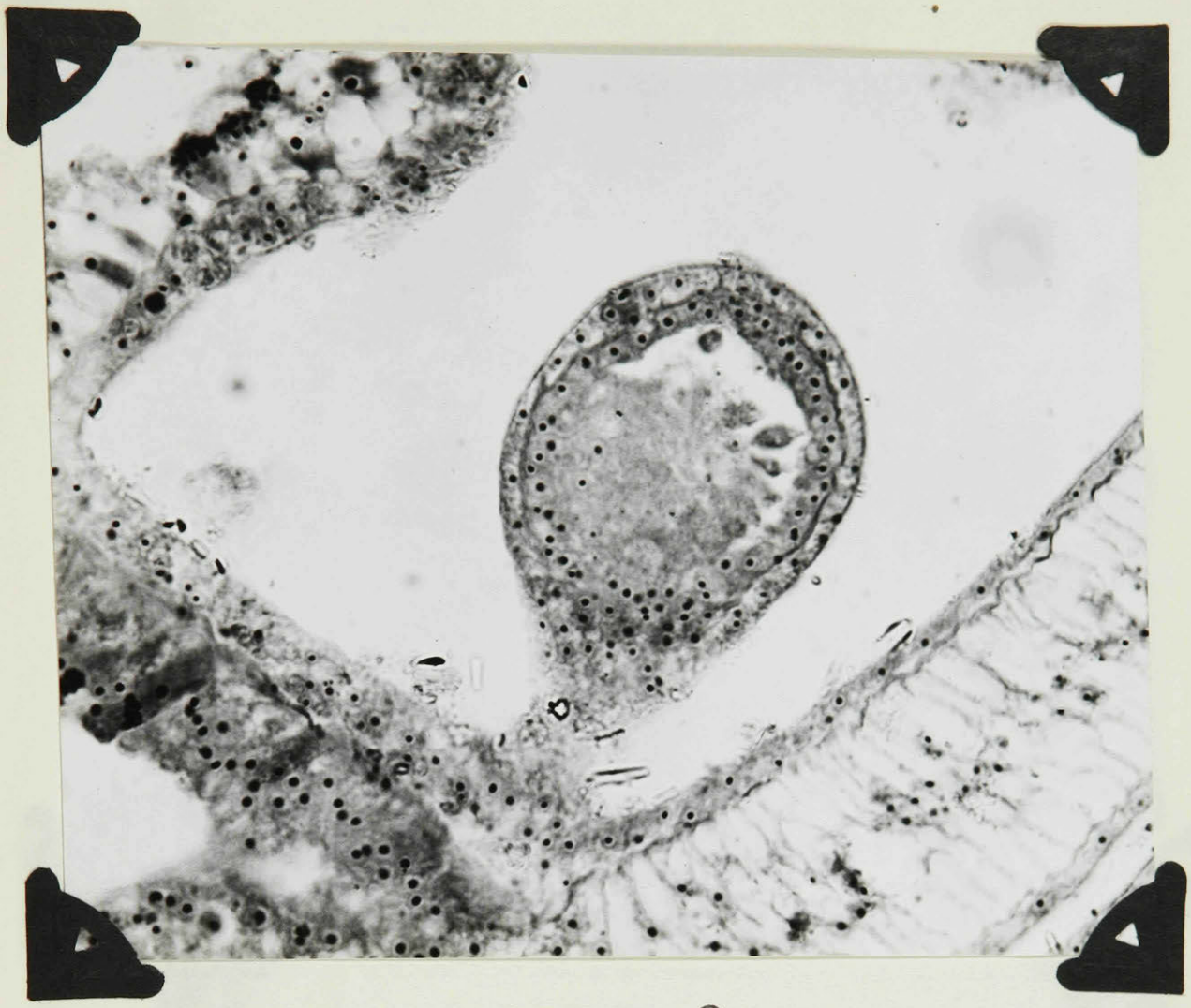

Fig. 3

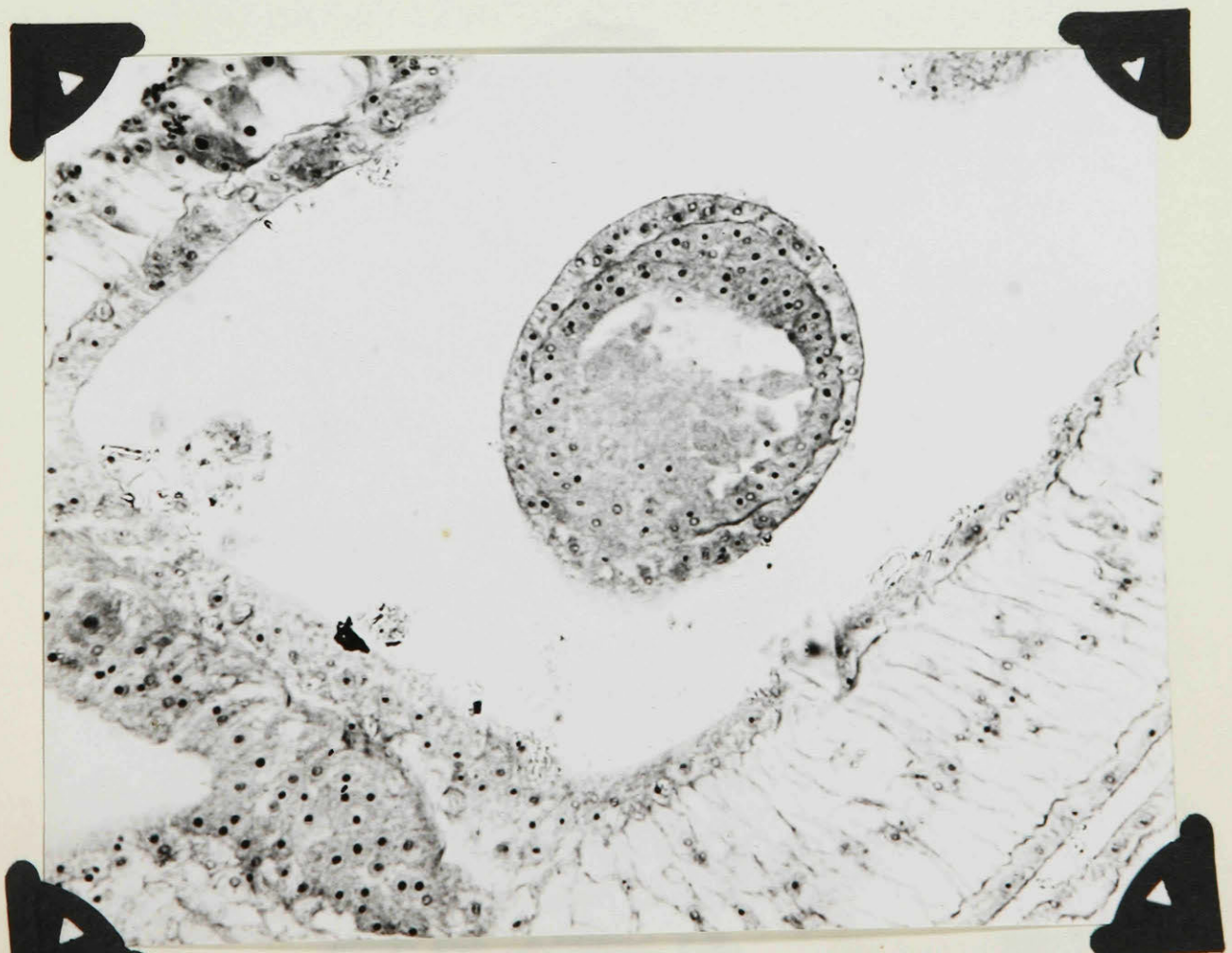

Fig. 4

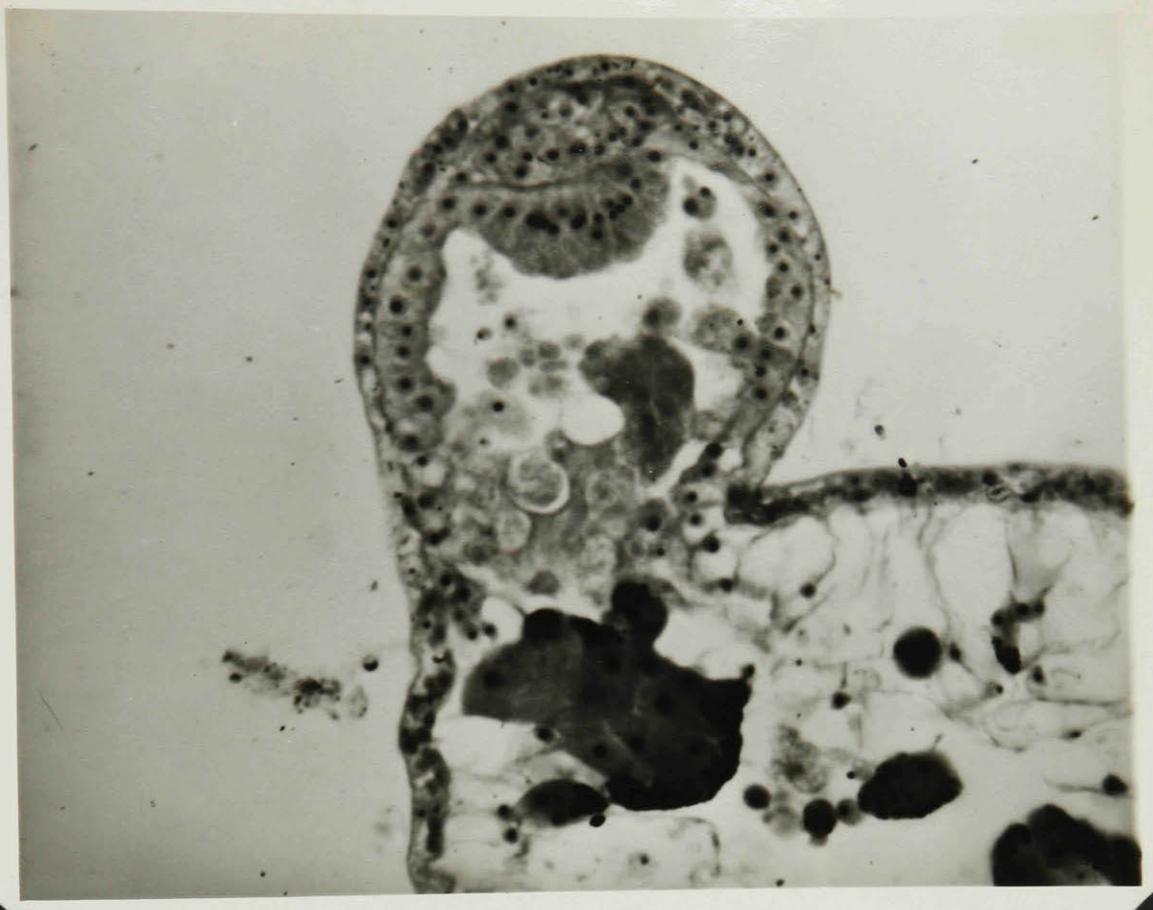

Fig. 5

Fig. 6

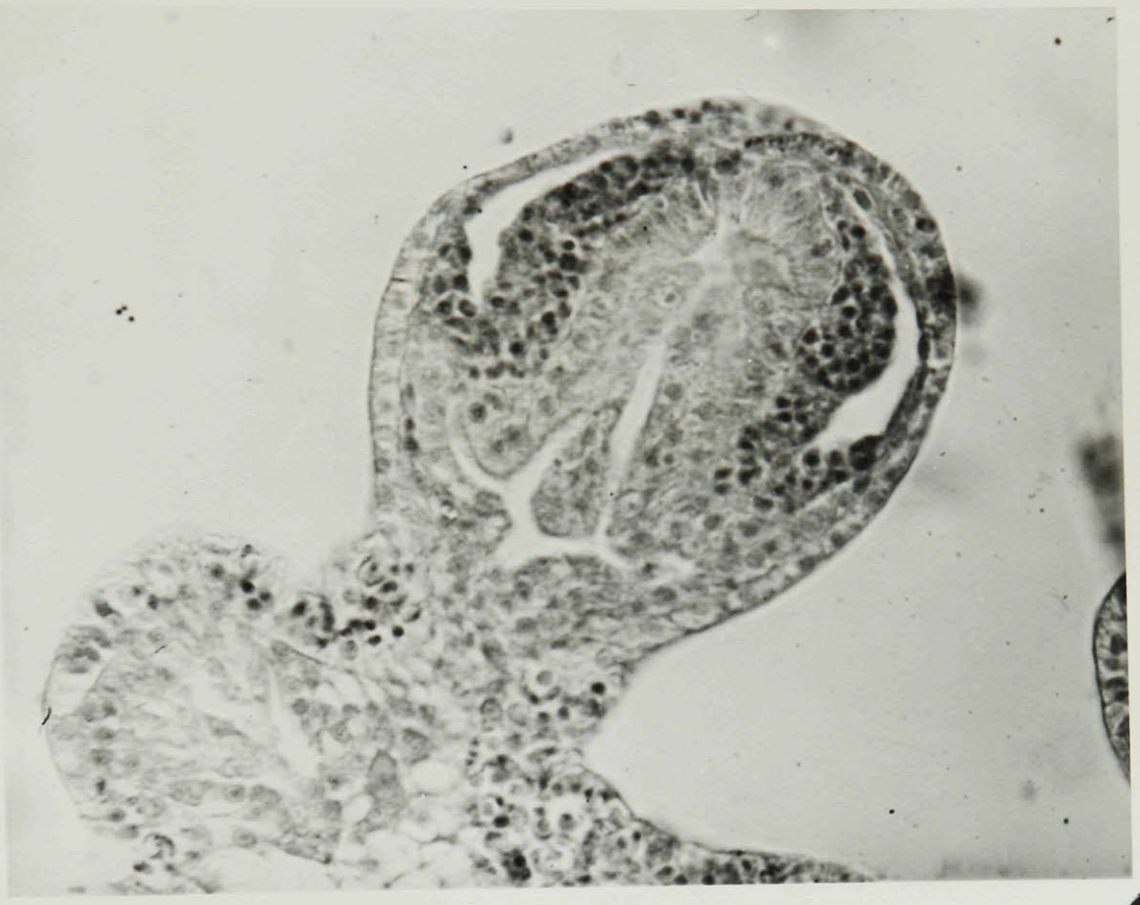

Fig. 7

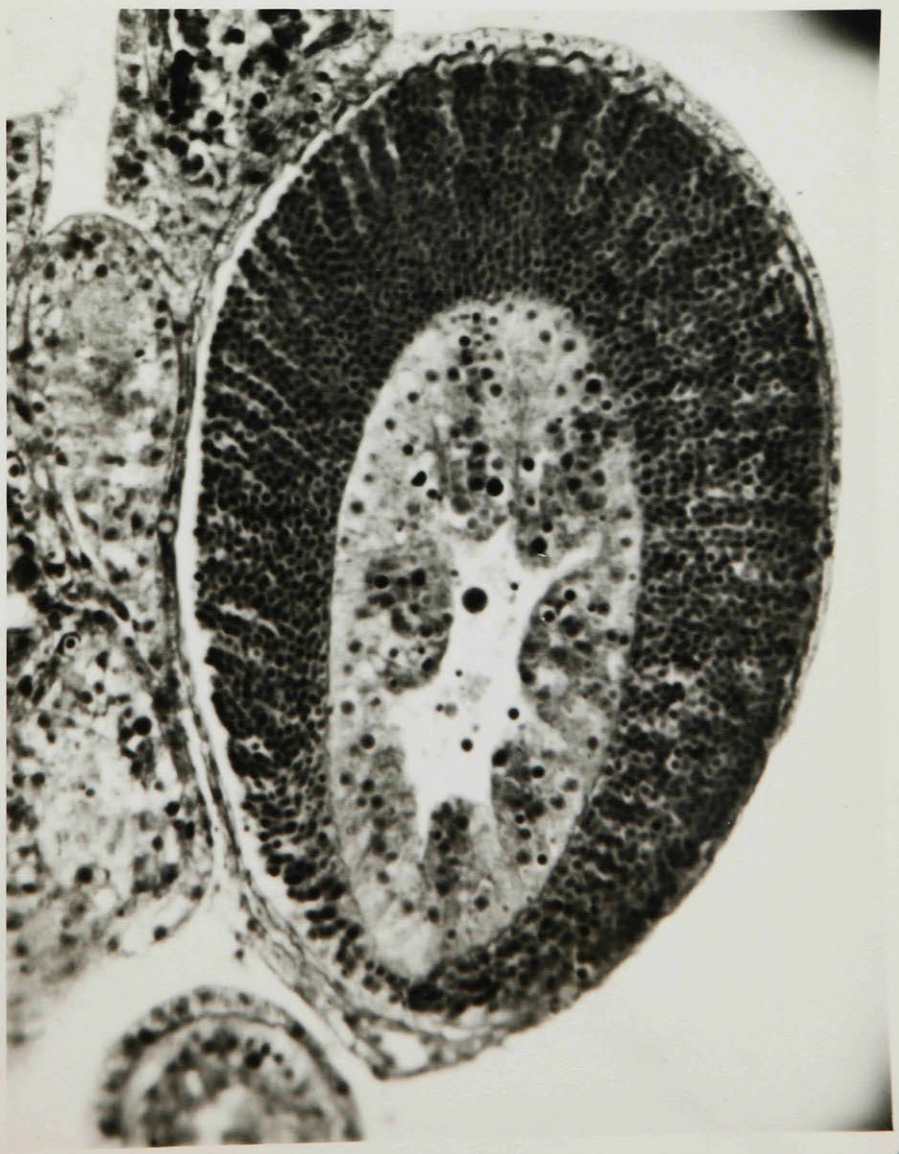

Fig. 8

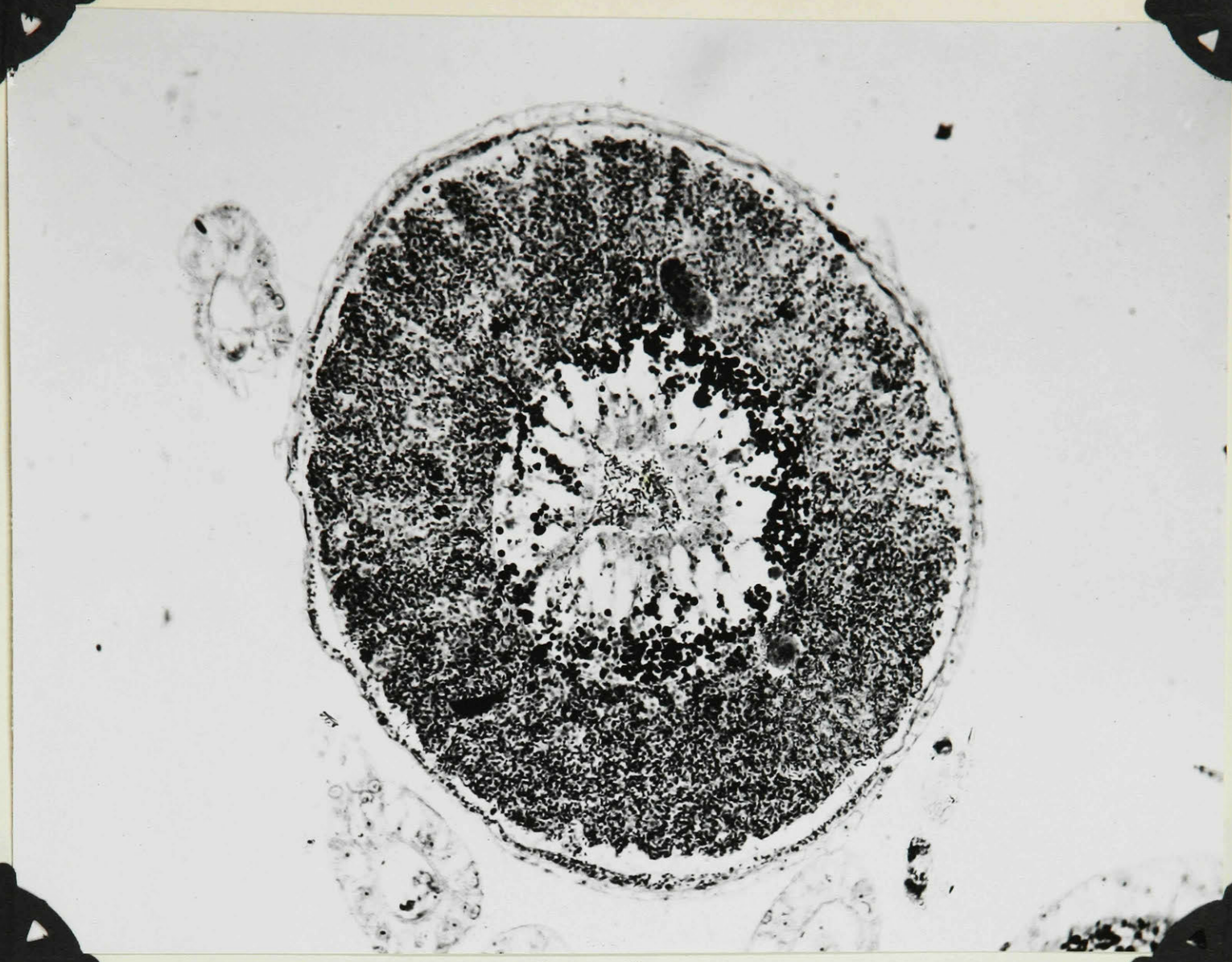

Fig. 9

Fig. 10

Fig. 11

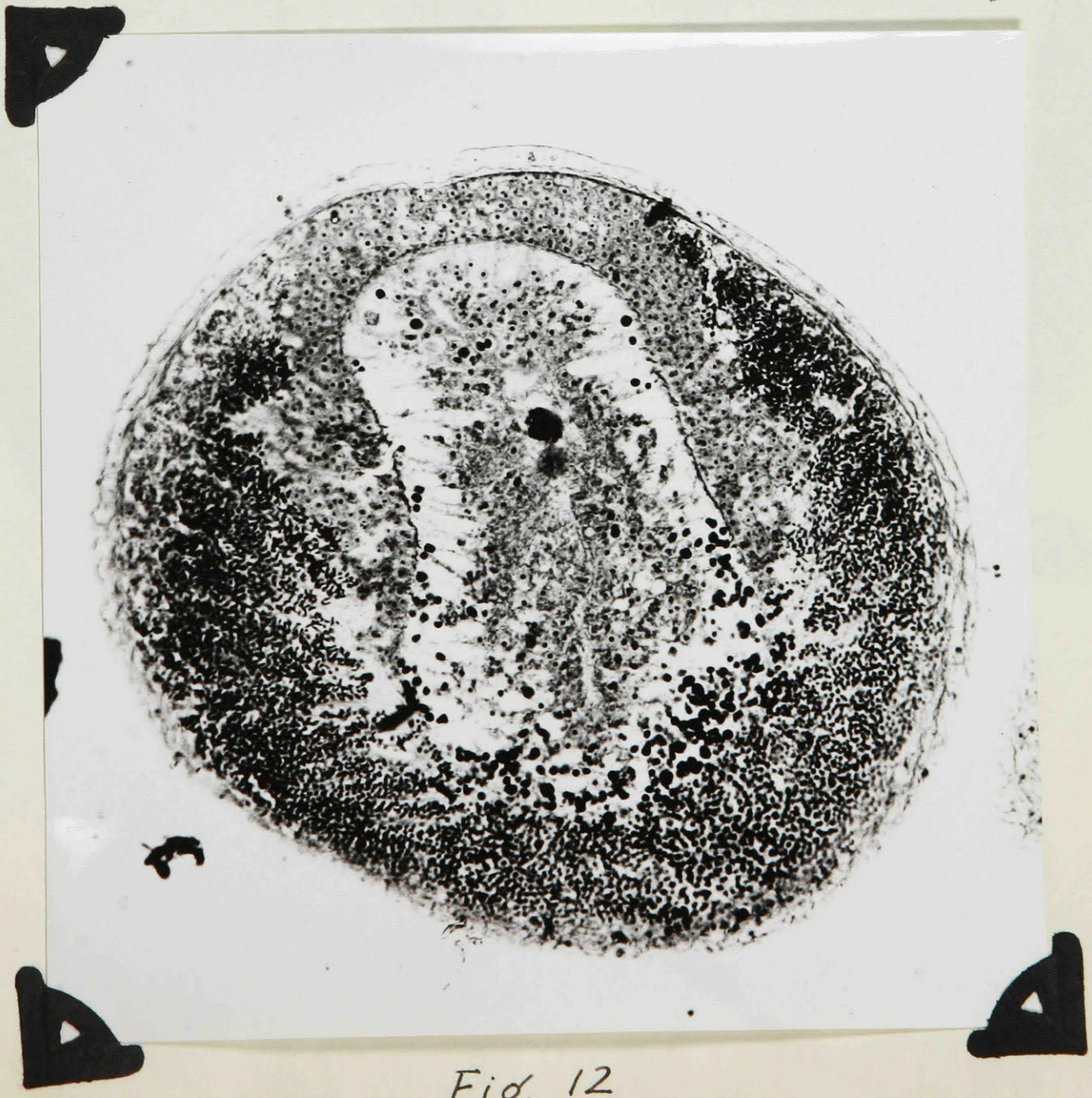

Fig. 12

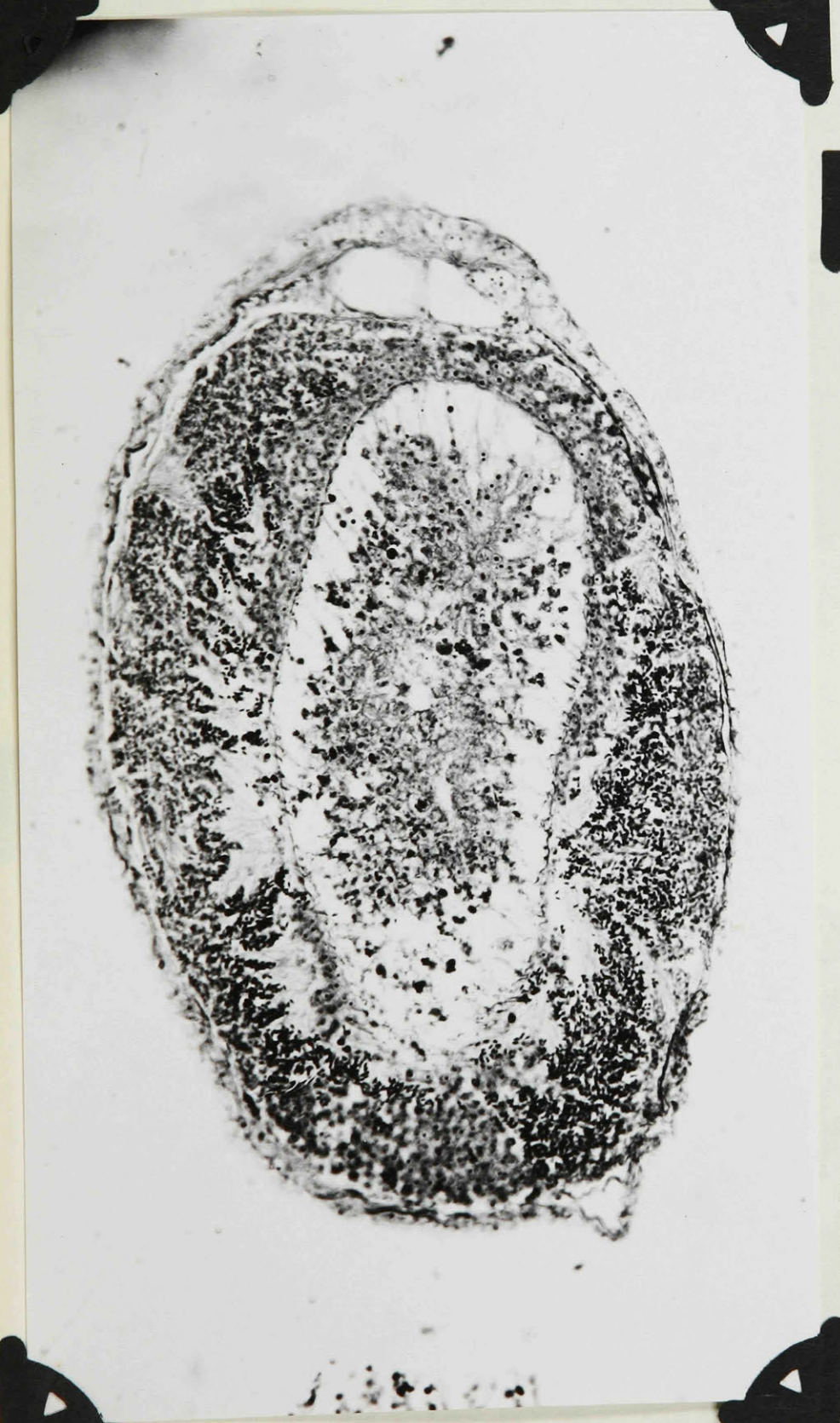

Fig. 13

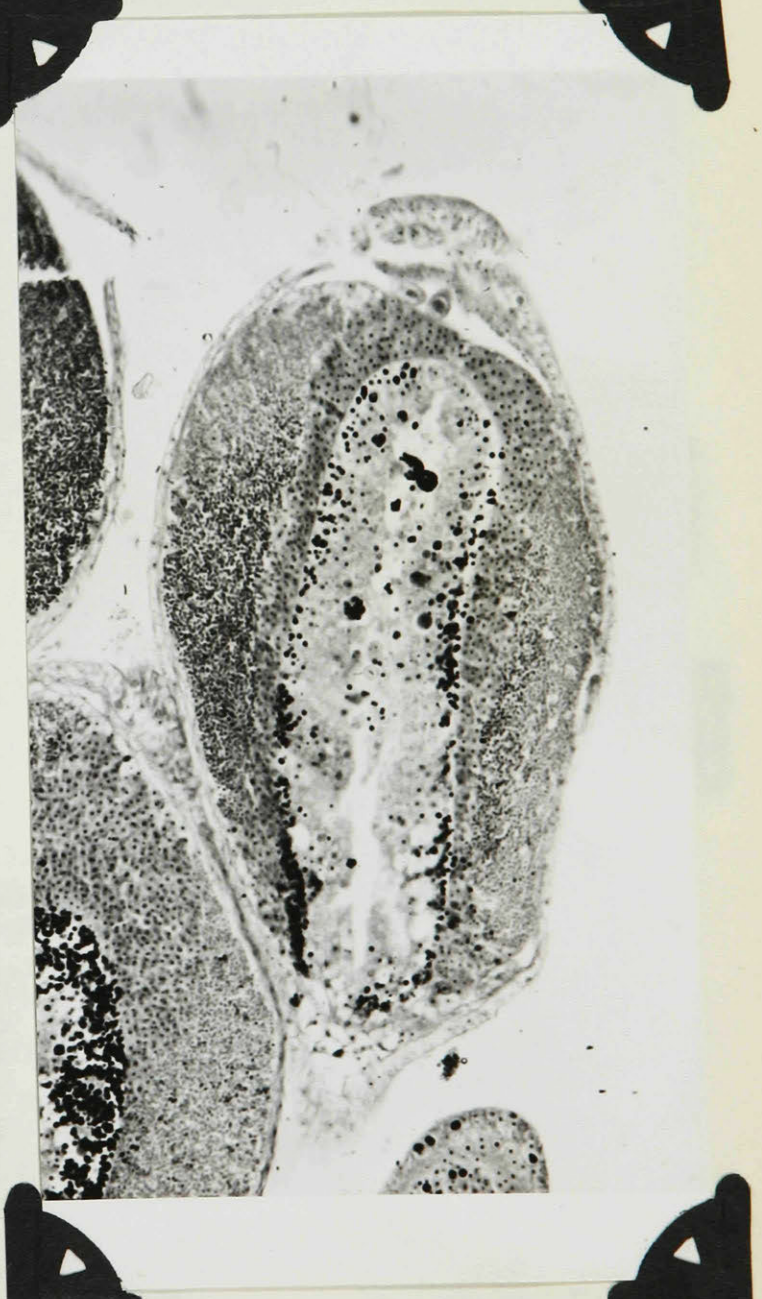

Fig. 14

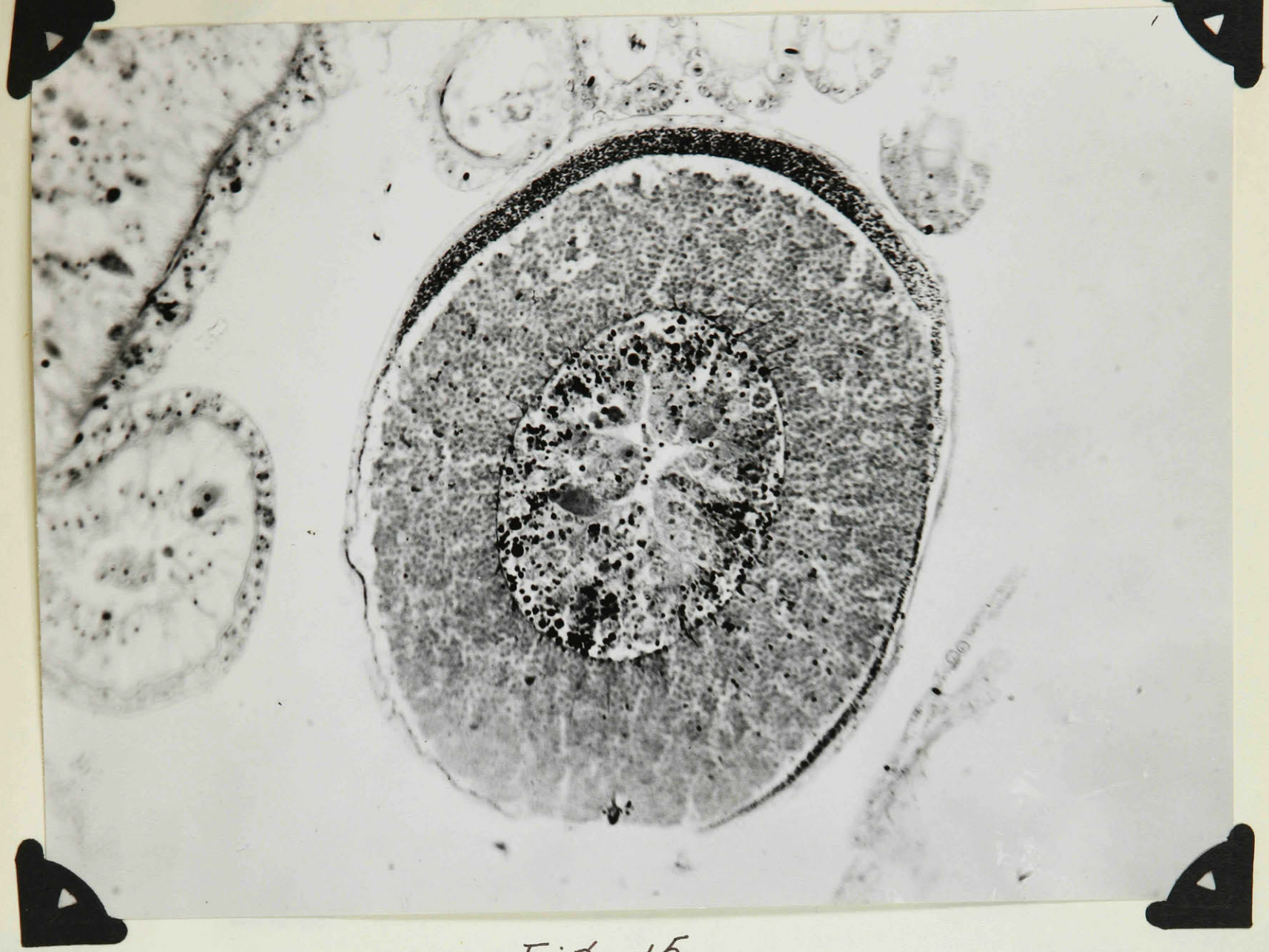

Fig. 15

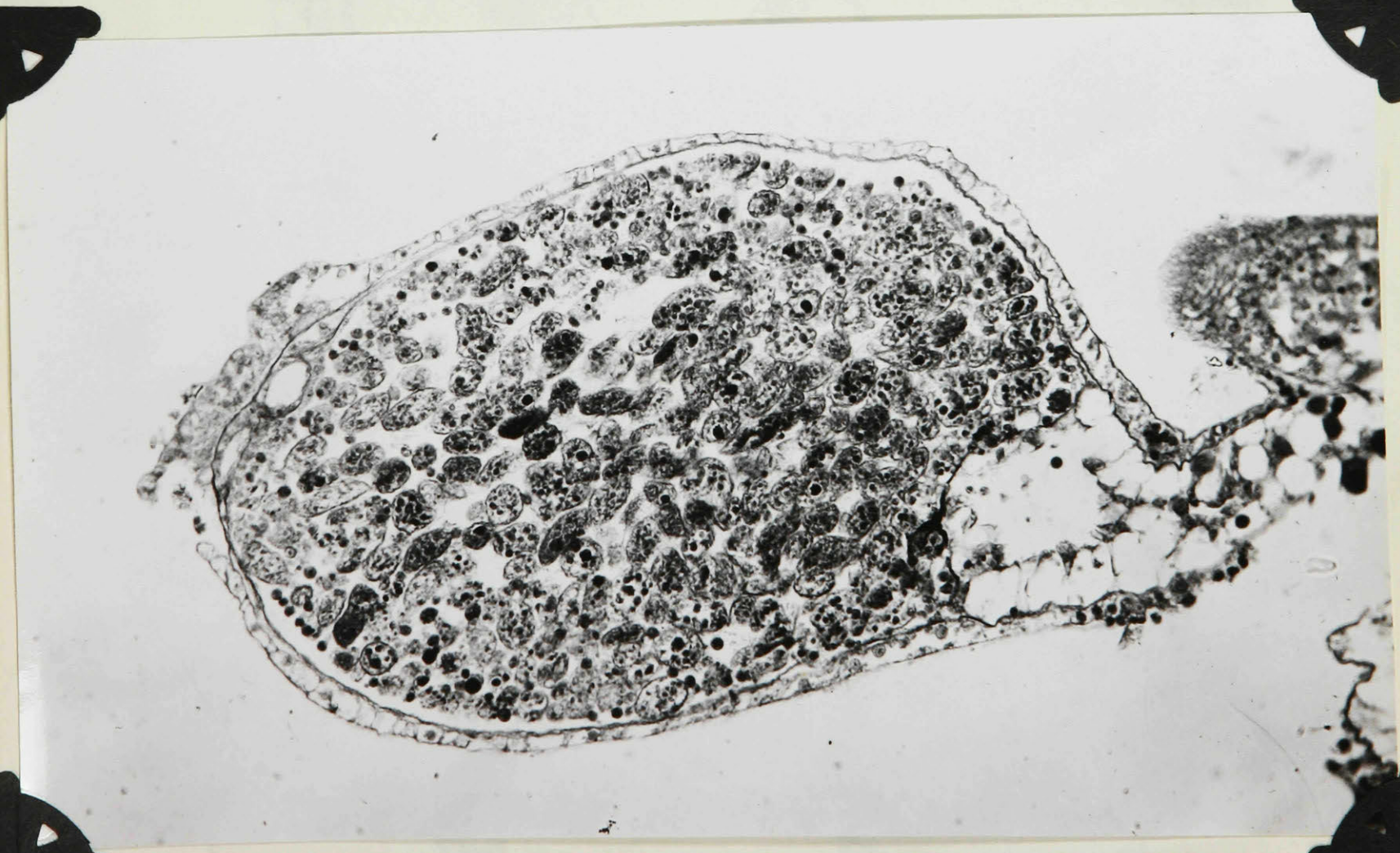

Fig. 16

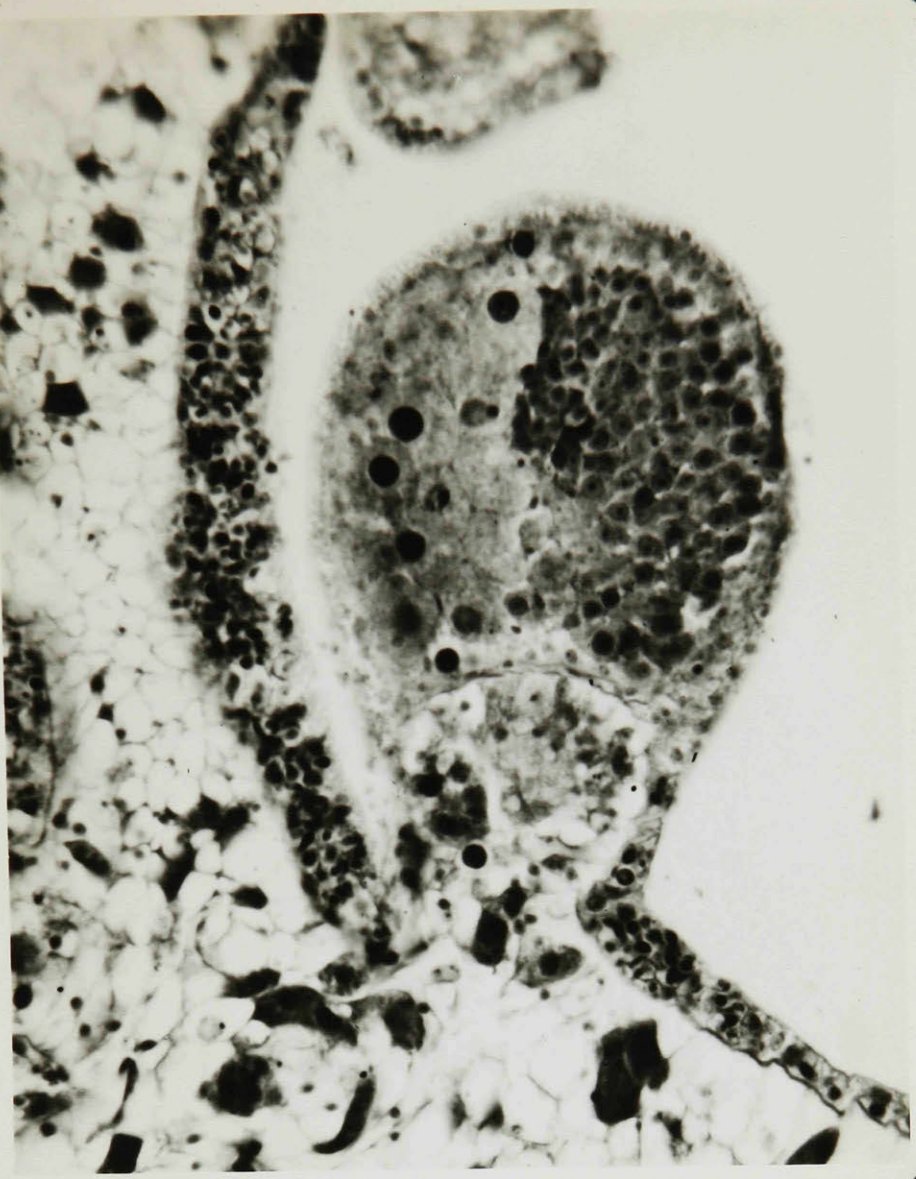

Fig. 17

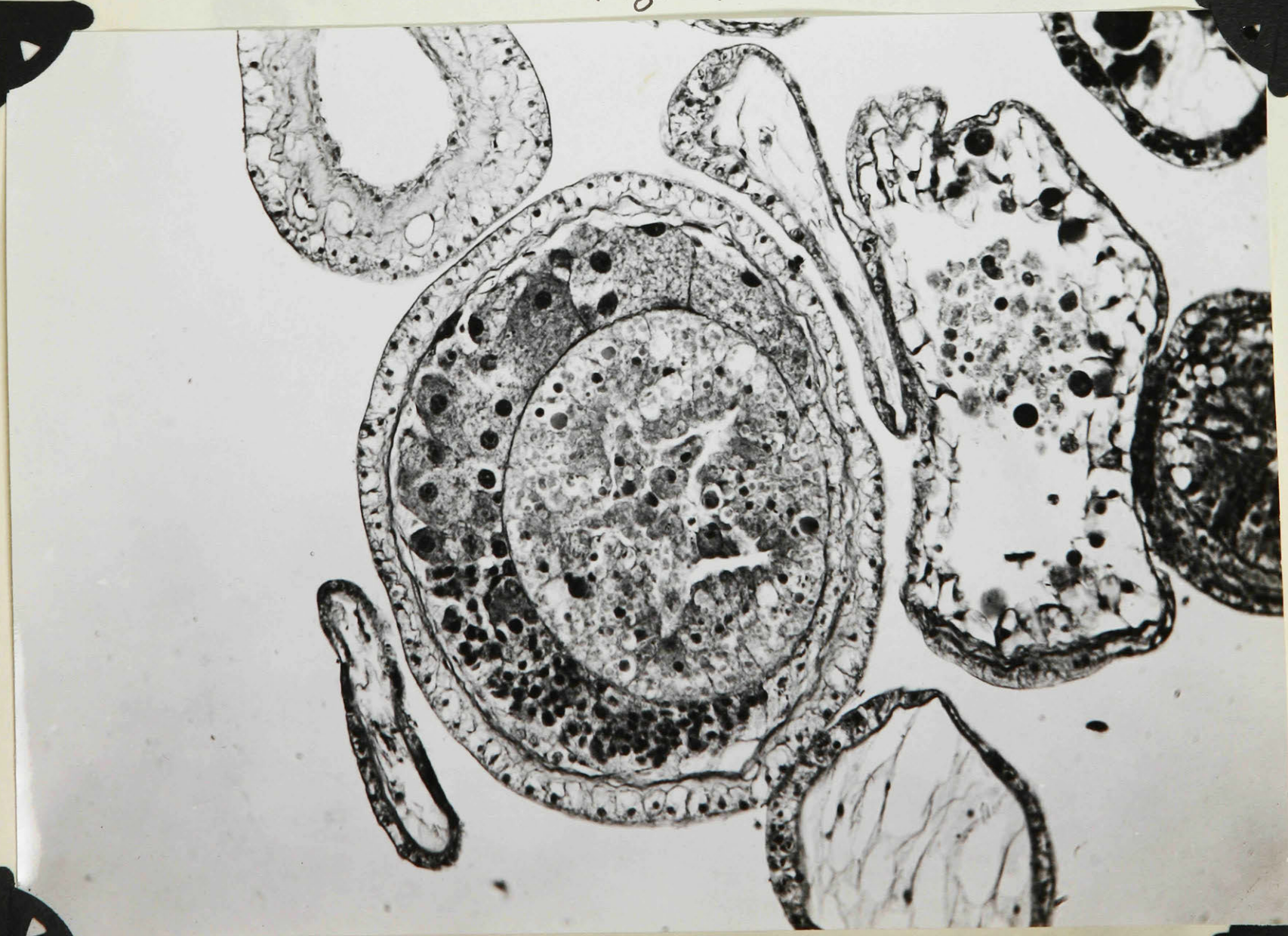

Fig. 18

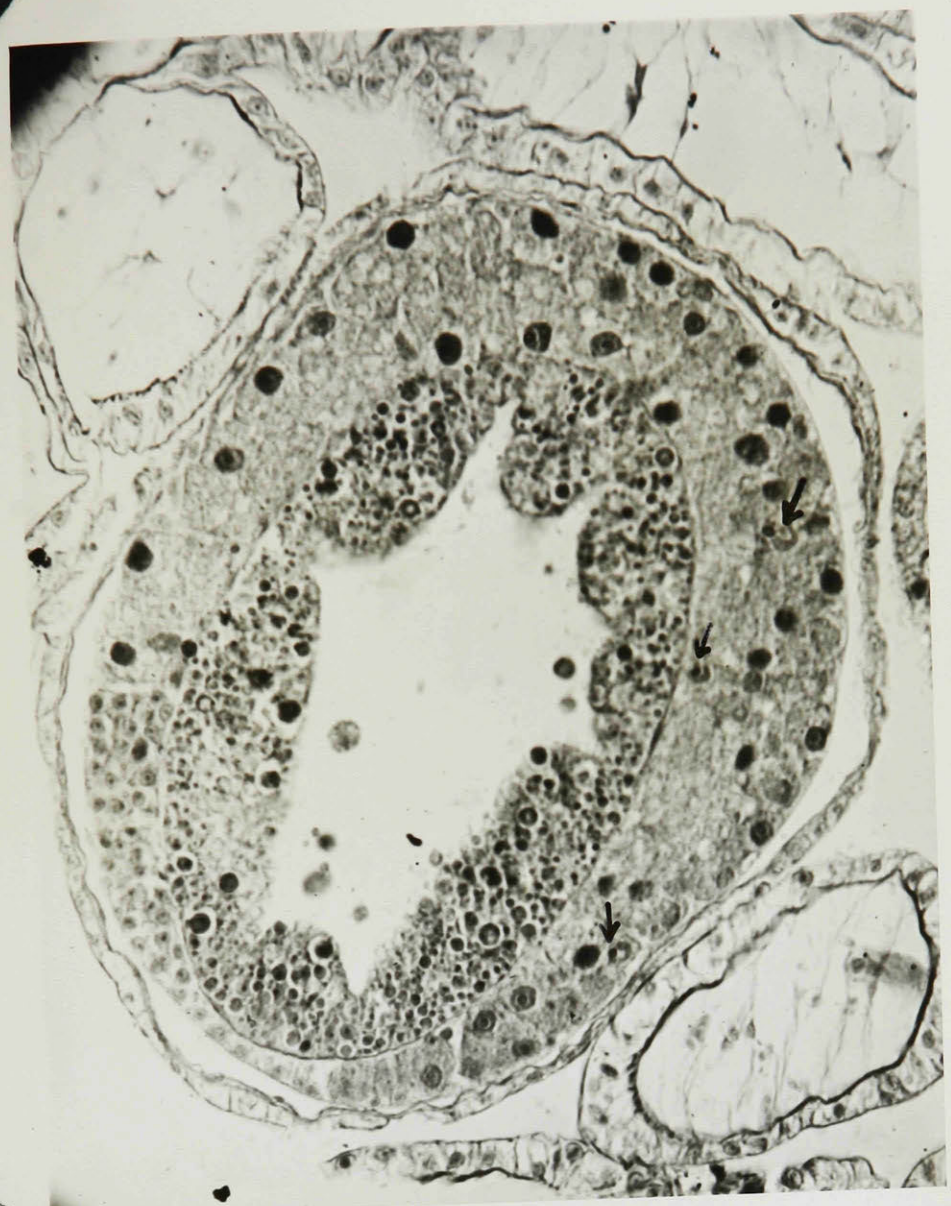

Fig. 19

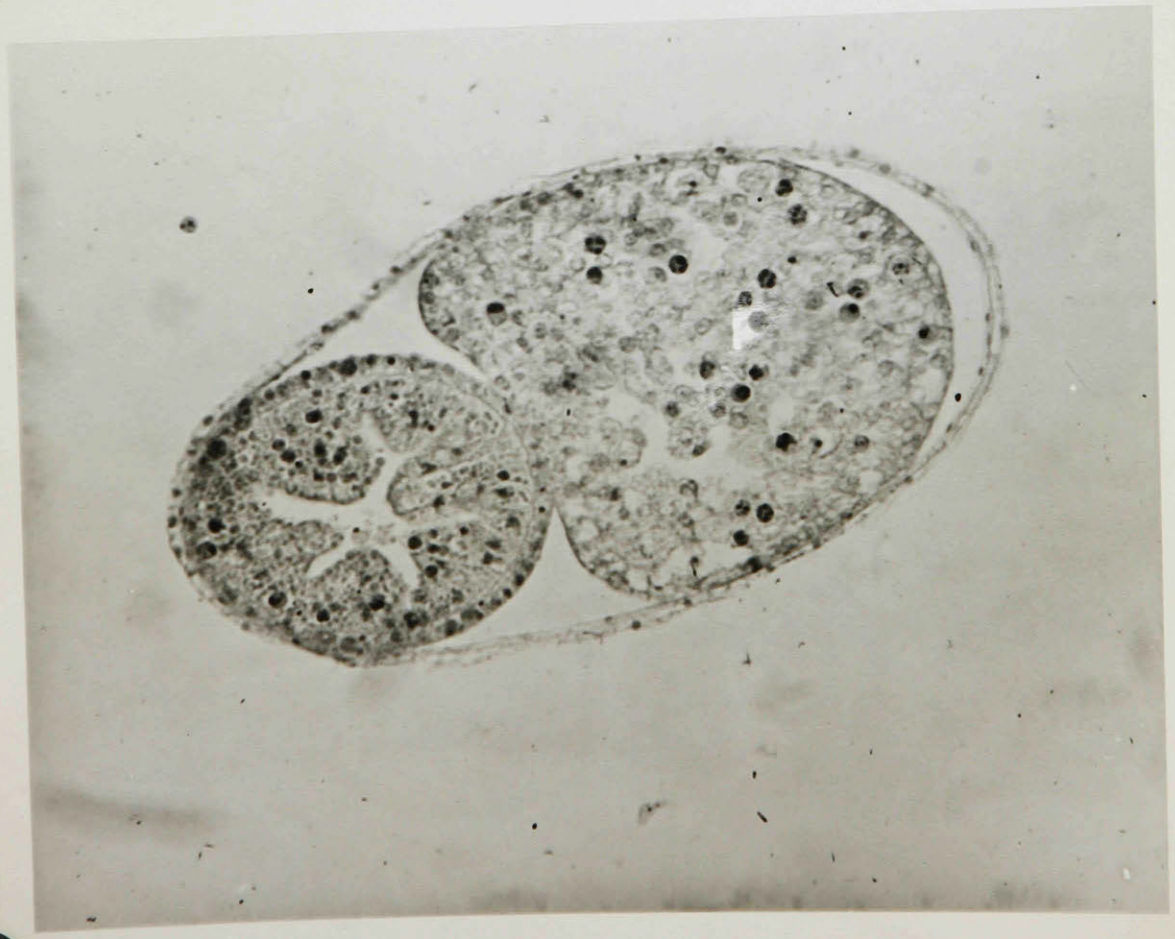

Fig. 20

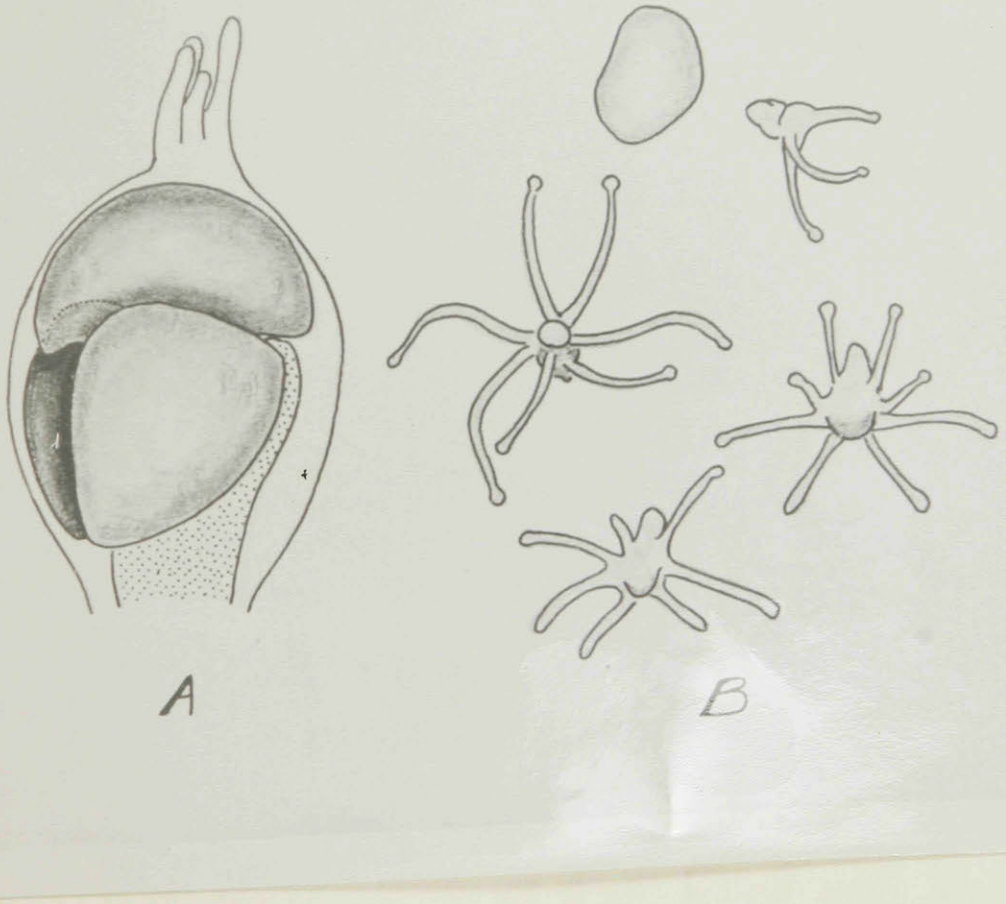

Fig. 21