The University of Chicago
FOUNDED BY JOHN D. ROCKEFELLER

THE SPERMATOGENESIS OF HYDRA

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL
OF SCIENCE IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

(DEPARTMENT OF BOTANY)

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CHICAGO
1905

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With plate 22–24.

Introduction.

Hydra and the coelenterates have occupied a conspicuous place in the development of biological theory. The first experiments on regeneration were performed on hydra. It and other coelenterates have been subjects of many investigations looking to the settlement of the gastraea theory and the mooted germ layer hypothesis. WEISMANN obtained his evidence for continuity of the germ plasm largely from investigation of this group. While therefore there is much literature on the ovogenesis and spermatogenesis of the coelenterates, it is nearly all for the express purpose of simply determining from which germ layer the sex cells are derived; very little has been done in following the nuclear changes during spermatogenesis and nothing has been published on the reduction phenomena of this or the lower groups of animals. The present study was undertaken to determine if in so simple an animal as *Hydra* there occurs any simplification of the process of spermatogenesis as known in the higher animals.

Historical.

In 1743 BAKER (2) published a drawing of *Hydra* with testicles. This is reproduced in Fig. 1. It is the first mention of these organs

of *Hydra*, though their nature was not then recognized. The projecting tubercles he considered abnormalities. In 1744 TREMBLEY figured the male (79); so RÖSEL (73), SCHRANK and others observed and described the male organs but all considered them pathological structures or parasites. In 1836 EHRENBERG (18) described both the egg and sperm. Still LAURENT (58) writes in 1844 in regard to these pustules that he can not consider them analogous to testes, but with TREMBLEY and RÖSEL he regards these tumors as maladies and thinks the eggs develop without fecundation. (Voyage de la Bonite, Nouvelle Recherches sur l'Hydre, p. 20 and 40.)

In 1872 KLEINENBERG (47) published his volume on *Hydra*. Regarding the spermatogenesis he says that the formation of the spermary begins by the rapid growth of the interstitial cells over a limited circular area. These divide rapidly a number of times, then become amoeboid. Ultimately they pack closely together and elongate. The nucleus then disintegrates while the cell substance becomes granular. There then appear in the cells, one to four strongly refractive oval bodies, whether formed from the disintegrated nucleus or not he does not know. These refractive bodies become the nuclei of smaller cells from which the sperm form by the growth of the tail from one end.

The results of BERGH and KOROTNEFF (52) are very similar except they claim that the sperm are formed directly from the nuclei of the multinucleate sperm mother-cells. In 1885 THALLWITZ (80) published an investigation "Über die Entwicklung der männlichen Keimzellen bei den Hydroiden". He is the first to undertake to trace nuclear and cellular changes among hydroids during spermatogenesis. In the several which he investigated he says the course of spermatogenesis is somewhat as follows: — The primary spermatoblasts are derived from the ectoderm and are characterized by their reaction toward reagents, their sharp nuclear contour and their wealth of protoplasm. They multiply always mitotically, and emigrate to the gonophore, where multiplication continues, until the gonophore is packed full. Then the protoplasm of the germ cells is lost so that the cell is little larger than the nucleus. The nucleus which heretofore has stained with difficulty now takes on a deep stain, while the nucleolus heretofore distinct disappears. Division follows and from the small cells formed the sperm are evolved, the nucleus becoming the head of the sperm. His points of advance are, then, the recognition of a series of complex nuclear changes

preceding actual division; appreciation of the purpose of multiplication of the interstitial or germ cells, namely to increase the number of sperm; and that the head of the sperm is the cell nucleus. Such was the condition of our knowledge of spermatogenesis among the coelenterates when the present investigation was undertaken.

Species of *Hydra*.

The spermatogenesis of three species of *Hydra* has been studied. In undertaking to discover which species these were, a tangle of terminology has been encountered. NUSSBAUM, 1887 (70) after carefully reviewing the literature on the various species of *Hydra* concludes that all the forms described can be included under four species: *viridis*, *grisea*, *fusca*, and *attenuata*. He says however that he cannot vouch for the latter species as he has not seen it. It is one described by RÖSEL (73) and PALLAS (71). BRAUER, 1891 (5) also recognizes four species, but not the same ones as NUSSBAUM gives, for he drops *attenuata* and points out that under *H. fusca* have been included two species, *fusca* proper, and a species which he indicates as "*spec*?" He demonstrates its right to a spearate specific name, for it alone is unisexual and moreover is characterized by differences of structure, gross anatomy and peculiarities of the egg. HARGITT, 1901 (30) says in the synopsis of the hydromedusae that "of the genus *Hydra* there are two well distinguished species, *H. fusca* and *H. viridis*".

We have found four species and have studied the spermatogenesis of all but *grisea*. Combining the specific characters established by JICKELI, NUSSBAUM, BRAUER and others, these species would be as follows:

I. *Hydra viridis* L. Synonomy: Polype verd, TREMBLEY: grüner Polyp, RÖSEL; *Hydra viridissima*, PALLAS. Body green. Tentacles 5—12, seldom as high as 18; shorter than the body. When body is stretched it is 1—1,5 cm long. Nettle cells small. Hermaphrodite; sexually mature April—Oct. Eggs fall off from parent. Egg spherical and its sheath almost smooth.

A smaller variety, *H. viridis var. bakeri* is found in salt marshes.

II. *H. grisea* L. Synonomy: Polype de la seconde espèce, TREMBLEY; oraniengelber Polyp mit langen, hörnerförmigen Armen, RÖSEL; *H. vulgaris*, *H. aurantica*, *H. grisea*, LEYDIG and KLEINENBERG; *H. trembleyi*, HAACKE. Gray, pale yellow or reddish. (The color, except in *H. viridis*, is so dependent on food that no reliance

can be placed upon it as a specific character.) 5—18 tentacles. Tentacles scarcely as long as the body. The latter cylindrical, 2 cm or slightly more in length when expanded. Foot usually enlarged. Nettle cells larger than *viridis* or *fusca*. Hermaphrodite. Sexually mature April—August and occasionally as late as December. Eggs fall off after forming the membrane. Membrane covered with large projections which are mostly divided at the tip.

III. *H. fusca* L. Syonomy: Polype de la troisième espèce, TREMBLEY, though probably most of those described by TREMBLEY belong to the next species; brauner Polyp mit langen hörnerförmigen Armen, RÖSEL; *H. oligactes* PALLAS; *H. roeselii* HAACKE; *H. vulgaris* JICKELI. Color brown, sometimes almost white. Tentacles 10—6 or possibly fewer, longer than the body when expanded. Nettle cells larger than in *viridis*. Body about 2 cm long. Hermaphroditic. Sexually mature Sept.—Oct. Eggs remain attached to parent until both germ layers are formed. Then the mother contracts bringing egg into contact with supporting object. To this the egg is attached by an ectodermal secretion of the mother. When attached, egg is flat below, convex above, membrane beset on upper side of egg with short points.

IV. *H. dioecia*, nomen novum. Synonomy: *H. spec?* BRAUER, *H. monoecia*, DOWNING [1]), BROWN. Tentacles 5—8, and capable of much expansion, as much as ten times length of body. Body 2.5 cm long when expanded. Sexually mature Oct.—Dec. Sexes distinct. Eggs remain attached to parent until germ layers are formed. Mother then contracts, remaining until the eggs are well developed. Eggs are glued to the surface on which the mother rests. Egg spherical; membrane set with small knob.

BRAUER distinguishes this species as '*H. spec?*' presumably because it is exceedingly difficult to determine what name should be applied to it. He says it has been both described and figured. Certainly some if not all of the figures which TREMBLEY gives of his third species and characterizes as the 'Polypes à long bras' are this species. But TREMBLEY of course does not apply a specific name. This was first done by LINNAEUS, and in his description '*H. spec?*' was confused with *H. fusca*. This confusion has continued

1) I suggested the name *H. monoecia* in my preliminary announcement (in: Science, V. 12, p. 228). This would be a use of the term monoecious directly contrary to its customary use in botany. I therefore use the specific name given above in place of that which I first proposed.

until BRAUER clearly recognized the difference. If any of the numerous attempts to establish new species of *Hydra* have been based on observations of this species the descriptions are not sufficiently accurate to permit of its recognition.

It seems almost invidious to introduce another name into the already confused mass of specific names heretofore applied to *Hydra*, and yet it seems impossible after going over the literature to adopt any of the specific names already used without again confusing this clearly marked species with the others previously recognized, especially *H. fusca*. The most striking character of the species is that the sexes are distinct. BRAUER, who noticed this first, had the animals under observation for many months. It is not uncommon to find individuals of the other species with only testes or ovaries present, i. e. proterogynous, but the majority have both at the same time. In the hundred and more individuals of '*H. spec.*?' observed sexually mature in the present investigation, none have been seen with both ovary and spermary, so that these observations, though not as extensive as BRAUER'S, confirm his in this character. The figures of male and female are given in Fig. 6 and 7. I would prepose for this '*H. spec*?' of BRAUER the specific name *dioecia*.

The three species *grisea*, *fusca* and *dioecia*, according to HARGITT as quoted, would have to be considered the same. But the characters just given are sufficiently exact to warrant terming them distinct species. Possibly they may be found to be the same animal assuming different form, structure, relative size of parts and habits under varying conditions. But it would seem less probable than that *H. viridis* was merely a *H. fusca* containing symbiotic algae. Until they are demonstrated to be varieties, it will be wise to recognize the four species.

Zone of the testes.

The testes develop in the three hermaphrodite species in a zone extending from just below the tentacles to a point perhaps a half, though usually not over a third of the way to the aboral end. Those testes nearest the tentacles are the least mature. The ovaries are produced on that third of the body just below the zone of the testes.

Proterogony.

In these hermaphroditic species, the animals are usually proterogynous, the ovaries maturing before the testes are formed.

Specimens of *H. fusca* were observed from the time the first indications of testes could be detected as minute swellings. In early September a testis is formed, matured, discharges its sperm and disappears in about twenty-four hours. Within a short time after the first appears, others follow, perhaps are synchronous in appearance with the first. The testes usually begin to appear about the time the ovaries are matured. The sequence of events is the same for *H. viridis*, though so far as my observations go it seems less proterogynous, the testes appearing about the time the egg is discharged. This statement contradicts the one made by W. MARSHALL (64) who says in regard to *H. viridis* that by the end of May the testicles discharge their sperm, although the eggs do not show themselves before the end of September. It seems probable that altering conditions change the breeding habits of *Hydra*. At least it is difficult to harmonize the statements made by different observers as to times and conditions of breeding on any other basis. I think this statement of MARSHALL's might readily be accounted for by assuming that he saw the end of one reproduction cycle — the testes being present — and the beginning of the next when the ovaries appeared. These three species produce few ovaries and testes — one or two of the former and four or five of the latter being the rule. If an individual produces an unusually large number of ovaries, testes and ovaries will both be present, and it must be remembered that it is a frequent occurrence to have testes and ovary appear synchronously.

Testes in *H. dioecia.*

In *H. dioecia* the testes may be very numerous and appear on the entire body except the very foot and immediately under the tentacles. Fig. 10 shows such an animal copied from BRAUER. It is more completely covered than any animal observed by the present writer, although twenty-eight testes on a single *Hydra* have been counted. In this species the ovaries may be numerous too, and each may give rise to several eggs (Fig. 6 and 7). We have then in these closely related species conditions varying from synchronous appearance of the testes and ovaries through proterogyny to distinct sexes.

Form of testes.

The testes appear in surface views as minute elevations, conical at first but as growth occurs they become mammiform. Toward maturity the tip is seen to be full of sperm freely and rapidly

swimming in the contained fluid. The activity of the sperm is very great, so that it gives to the fluid in the tip the appearance of boiling. Within the basal part of the spermary lines of yellowish brown granules may be seen extending up into the otherwise colorless testes among the rapidly dividing cells. These are found in sections to be nutritive material, and their fate will be discussed later.

Rupture of the spermary.

When the spermary is well distended with active sperm, the tip ruptures and the sperm are set free. After the rupture of the spermary and the discharge of its sperm there still remain many interstitial cells and some of the spermatocytes and spermatogonia. Some of these continue to divide and form spermatids and so spermatozoa, but there soon ensues among them a degeneration.

Phagacytosis.

Giant multinucleate cells, possibly phagocytes, are formed, apparently by the fusion of adjacent cells. The outer thin lamella-like portions of the ectoderm cells slough off. The giant cells seem to be slowly resolved; at least they disappear by some process; the elongated ectoderm cells resume their normal proportions, and thus the tissue regains its normal appearance.

Adult spermary.

The adult spermary varies greatly in size. Section through a *Hydra* in the plane of an average sized testis is given in Fig. 11. The dimensions are as follows:

Height of spermary	along	the	dotted	line, 122.0 μ
Diam. of base	„	„	„	223.0 „
Thickness of ectoderm	„	„	„	23.5 „
„ „ endoderm	„	„	„	94.0 „
Diam. of body cavity	„	„	„	164.0 „

When the spermary is well matured a section through it gives a figure like Fig. 9. The very elongate ectoderm cells form supporting strands, their outer plate like ends forming the outer wall of the spermary. The basal part of the spermary is occupied by the spermatogonia. The other cell generations are to be noted more peripherally, usually in no well defined order. The peripheral portion is occupied by sperm free swimming in a fluid. This is probably nutritive in nature, for with fixing agents it forms considerable coagulum.

Budding, Regeneration and sexual Reproduction.

During most of the year *Hydra* reproduces by budding. Only for a short time is it reproducing sexually. The processes seem to be antagonistic. Certainly they are seldom contemporaneous in the same animal. Once I observed a hydra budding in which a nearly mature bud had the testes developing. LAURENT records that in one instance vigorous budding followed the formation of the 'tumors' so that he inclined to account for the budding by the irritation produced by these pustules, an explanation which is nullified by the recognition of these pustules as testes. My observations are that sexual reproduction is an exhausting process in *Hydra*. It is followed by vigorous feeding which induces budding. TREMBLEY also observed that the process of regeneration was checked by the appearance of a bud. Regeneration, budding and sexual reproduction seem then to be mutually exclusive — a condition that is likely due to the fact that each of these processes primarily utilizes the same cells, the interstitials. However the relations of these processes have not yet been satisfactorily determined and the subject is one still open for observation and experiment.

Experiments.

Some experiments have been made in the course of these studies with a view to determining under what conditions *Hydra* would reproduce sexually, but thus far only negative results have been obtained.

Changed temperature.

It was noticed that *H. fusca* and *H. dioecia* begin their sexual reproduction in the fall and the decreased temperature was thought of as the possible occasion. Fifty experiments were conducted by placing a few *Hydra* in a dish with water and some plant life, imitating natural conditions as closely as possible, and subjecting them to decreased temperature, for varying times from a few hours to a week, and under varying conditions of light and darkness. Alternations of heat and cold were also tried. In only one case out of the fifty experiments did the sexual organs develop. This was on one animal which had been kept in a refrigerator in darkness at about 12 degrees C. for twelve hours. As there appeared at the same time sexual organs on several control *Hydra* in the laboratory which were kept in the light at the temperature of the room, it can only be concluded that light and temperature are not

determining factors in the appearance of the testes. When the *Hydra* were placed in the cold water they contracted at first but remained so only a short time, when they expanded as usual and resumed their normal life. They reduced rapidly in size, becoming emaciated more quickly than the animals in control jars at the room temperature.

H. viridis and *H. grisea* have been found to be sexually active in spring and summer, while *H. fusca* and *H. dioecia* are sexually active in the fall. It would seem likely that the cause which initiates sexual reproduction, whatever it may be, is the same in all four species, and it was thought possible that spring and fall rains might act as the immediate cause.

Distilled water.

Experiments were tried by placing animals in distilled water for varying times and then removing to ordinary pond water. And it was observed that animals kept for from six to twenty four hours in distilled water and then placed in pond water do develop speedily elevations along the sides of the body similar in external appearance to young testes. But they disappeared in the course of twelve hours. with no consequent developments. The elevation would be readily explained by osmosis. The reverse process is also effective in producing these external protrusions, *Hydra* developing them frequently when placed in distilled water. The swellings are not ectodermal thickenings, but bulgings of both walls. It is interesting to note that they always appear symmetrically on opposite sides of the animals as buds tend to appear.

Change in ion concentration.

To see if changes in ion concentration would be effective in starting the development of sexual organs, a number of solutions in varying concentrations were used. But no results have as yet been obtained, aside from demonstrating the extreme sensitiveness of *Hydra* to the action of solutions, it being necessary to work with $\frac{N}{100}$ solution or still more dilute to prevent immediate death. Thus a $\frac{N}{100}$ NH_4Cl kills *H. dioecia* instantly or nearly so. But *H. viridis* will endure more than twice as strong a solution for several hours. A $\frac{3}{1000}$ N KCl is fatal usually. But individual *Hydra* differ remarkably, some ani-

mals surviving in a $\frac{N}{100}$ KCl longer than others will in a $\frac{3}{1000}$ N. The varying effects of different solutions are interesting, but have no bearing on spermatogenesis; the results also are yet very incomplete.

It has been noticed by many observers that budding occurs when *Hydra* is well fed. Thus Laurent says „La production des bourgeons d'après les observations de Trembley et de tous ses successeurs, est fréquente pendant la belle saison chez les individues très bien nourris et nulle ou rare pendant l'arriéresaison surtout sur les *Hydres* qui se reproduisent par oeufs."

Food.

I have found also that during the spermatogenesis animals are nearly devoid of surplus food material. Figures will make apparent this difference. Fig. 14 is taken from a budding animal. It will be noted that both endoderm and ectoderm cells are filled with granules, and distended with protoplasm. A layer of droplets staining intensely black with osmic acid occupies the peripheral margin of the ectoderm cells. Among the endoderm cells, too, appear certain granular masses, staining intensely with gentian violet especially, which mark the gland cells. These are absent except during digestive processes. The ectoderm and endoderm cells are so full of granules, that it is extremely difficult to follow cell walls. The cells too are often multinucleate. Fig. 3 is taken from a *Hydra* on which testes were developed. The cells are not granular now, but the protoplasm even is so decreased that most cells are very vacuolate. Having observed this starved condition repeatedly in sections of animals bearing reproductive organs it seemed possible that the absence of appropriate food might be the cause of the sexual reproduction. So animals were kept without food for varying lengths of time up to three months, were fed sparingly on various crustacea and oligochaeta, were alternately fed and starved, but all to no effect as far as the development of sexual organs was concerned. It was concluded therefore that the starved condition was an effect rather than a cause, and was due to the rapid use of nutritive material during development of ovaries and spermaries. These grow so rapidly that digestive processes can not keep pace. Food is seldom ingested during the sexual reproduction.

It will be necessary to continue the experiments to determine

what are those factors in the animal's life history which cause the sexual organs to appear. It may be that one of the causes suggested above is the true cause in spite of the apparent negative results, and that it has not been allowed to operate under appropriate conditions. Until experiments shall be found for *Hydra* that lead to the production of sexual reproduction at the will of the experimenter, it would be unwise to consider the experiments here recorded as sufficient to exclude from further consideration temperature changes, changes in ion concentration and osmotic pressure or varying conditions of food supply. Sufficient to say that thus far the results have been negative.

Material and methods.

The material for the histological study was collected as follows: *H. dioecia*, found sexually mature in park lagoons at Chicago, Oct. and Nov. *H. fusca*, collected sexually mature during Sept. and Oct. at Chicago in park lagoons, also at Beloit, Wis. For some of the material from the latter locality I am indebted to Mr. GRANT SMITH of Beloit. *H. viridis*, collected sexually mature, June and July at Chicago in park lagoons. *H. grisea* found in the park lagoons at Chicago but not in a sexually mature condition.

Fixing agents.

A variety of fixing agents have been used, including Osmic-MERKEL, HERMANN'S, PERENYI'S chromo-nitric, FLEMMING'S, GILSON'S mercuro-nitric, CARNOY'S acetic alcohol, KLEINENBERG'S picro-sulphuric. GRAF'S chrom-oxalic, varying strengths of picro-acetic and hot corrosive. The first three mentioned gave the best fixation, the osmic-MERKEL working especially well. A one-half per cent solution of osmic acid was used to kill the animals. The *Hydra* was placed in a watch crystal in as small a drop of water as possible and still allow the animal to expand well. When expanded about ten ccm of the osmic acid solution was quickly poured over it. In the majority of cases death is so instantaneous as to prevent contraction. The animal is left for a minute in the osmic and then transferred to MERKEL for twenty-four hours. It is afterward dehydrated with alcohol, using fifty per cent. first, cleared in cedar oil and imbedded in paraffin. Sections were cut 7 μ in thickness usually, some 10 μ and some 3. Staining was done on the slide.

Staining.

A variety of stains were used after fixation in each of the above mentioned methods so as to determine what combination would give the best results. Iron haematoxylin, Bordeaux red, orange G, safranin, gentian violent, thionin, Kernschwarz, erythrosin, cyanin, Lyons blue, BIONDI, GRENACHER'S borax carmine, lithium carmine, and paracarmine were among the successful stains tried. The first five gave the best results. The iron haematoxylin was used with the Bordeaux red or the orange G and the safranin with the gentian violet or often alone.

Mounting.

Clearing was done in oil of bergamot or cedar oil and the sections mounted either in thick cedar oil or balsam. The best results were obtained, both regards preservation and stain, from the osmic MERKEL, preferably, or the PERENYI followed by iron haematoxylin and Bordeaux red or for count of chromomeres by safranin. Gentian violet was the best stain to differentiate the gland cells of the endoderm and was used after iron haematoxylin. In using safranin and gentian violet, BIZZOZERO'S adaptation of GRAM'S iodine method was not found to improve the results though tried repeatedly, as were several similar methods.

Ectoderm and endoderm.

The ectoderm and endoderm cells are, roughly speaking, prisms, polygonal in cross sections or as seen from the surface of the animal. These prismatic cells have a greater or less altitude according as the animal is more or less extended. Their free bases are somewhat rounded surfaces. Intercellular spaces are of frequent occurrence, especially in the ectoderm. The protoplasm of the ectoderm cell (of endoderm also) appears to be composed of a mesh work. Indistinct anastomosing strands of a fairly dense material inclose a more fluid substance. Frequently the cell protoplasm is very vacuolate so that there will be a peripheral layer of protoplasm and strands of protoplasm suspending the nucleus, but the bulk of the space between cell wall and nuclear wall is occupied by several large vacuoles. The nucleus also is reticular, is granular and contains one or more, usually one, nucleoli.

Interstitial cells.

Lying between the inner end of the ectoderm cells, though sometimes extending up between them nearly to the surface, are the

interstitial cells. They are much smaller than the ectoderm cells (see Fig. 12 and 15) and are characterized by a relatively large nucleus, which has a nucleolus. Furthermore this nucleus stains deeply and usually differently from the ectoderm nucleus, probably due to the fact that it is so active, dividing often, especially during spermatogenesis. In some cases there appears near the nucleus of the interstitial cells a Nebenkern. It is from the interstitial cells that the spermatogonia are derived.

Derivation of the spermatogonia.

In a preliminary report (in: Science, V. 12, p. 228) it was stated that the spermatogonia were derived from the ectoderm. In very many cases the ectoderm cells along the margin of the spermary are seen to be dividing amitotically, which led to this conception. But careful measurement negatived such a conclusion, demonstrating that the interstitial cells were the immediate progenitors of the spermatogonia. The interstitial cells themselves may arise from the ectoderm cells. They do embryologically and it would not be surprising if they should so arise during spermatogenesis when they are needed in large numbers. Still, the ectoderm cells are usually too large to give the interstitial cells by a single equal division. Cells of a size intermediate between ectoderm cells and interstitials have very rarely been seen, and then doubtfully. The division of ectoderm cells has always been observed as equal or nearly so. It is not probable therefore that the interstitial cell is budded off from the large ectoderm cell. What evidence has been obtained would then tend to disprove the possible derivation of the interstitial cells during adult life, from the ectoderm cells.

Early stage of spermary.

In the preliminary steps of the formation of a spermary an interstitial cell divides mitotically (Fig. 12). The daughter cells grow to the parent's size and then divide. Adjacent interstitial cells begin to divide, grow and multiply rapidly. They fill the space between the ectoderm cells and begin to distend the ectoderm, the cells of which elongate peripherally. The multiplication of interstitial cells increases at the initial point and the process spreads to a wider area. This increase in interstitial cells forms the early stage of the testis, — a conical elevation, covered externally with polygonal plates, the expanded outer ends of the ectoderm cells, the

elongated bodies of which extend through the mass of interstitial cells to the mesogloea, thus forming supporting strands of protoplasm.

Migration of interstitial cells.

There might be another means conceived for the multiplication of the interstitial cells at the point of formation of the spermary, i. e. migration. This method of formation of genital organs is quite common in other of the coelenterates. Thus Hardy (29) finds in *Myriothela phrygia* that sex cells are modified interstitial cells which migrate to the point where the gonophore develops. In the medusae of *Millepora murrayi* he finds that the „sperm cells originate in the ectoderm of the coenosarc, wander into the ectoderm of the zooids where they fuse into aggregations to form a spermarium". Ishikawa (42) says sex cells in *Eudendrium racemosum* originate in the ectoderm and migrate to the endoderm. Examples might be multiplied. The sex cells are usually recognized by their amoeboid-like form and undifferentiated structure. Weismann (89) figures such cells in actual transit from ectoderm to endoderm. No such cells have been observed in *Hydra*. The interstitial cells never appear amoeboid. Thallwitz (80) says the sex cells may be recognized in transit in *Sertularella polyzonias* by the fact that they frequently display mitotic figures. Such a criterion will not apply in *Hydra* as other cells besides the interstitial display mitotic figures, and even if this were not so we could not tell that a certain sex cell in mitosis was migrating to an already formed spermary rather than initiating in situ a new spermary.

To determine whether migration of the interstitial cells does occur in *Hydra* to form the accumulation that marks the spermary both longitudinal and cross sections of the *Hydra* with spermary were taken, and the number of interstitial cells noted in every 36.5 μ (20 divisions of the eye-piece micrometer) of the body wall. If there be a migration of the cells toward the spermary from other points on the hydra one ought to find the interstitial cells more numerous as the spermary is approached and less so the greater the distance from the spermary. This is not so. Taken then with the fact that no cells with amoeboid appearance are found, this seems sufficiently conclusive that the spermary is formed by multiplication of the interstitial cells in situ rather than their migration to the spermary from distant parts of the *Hydra*.

It is to be noted that the basal portion of the *Hydra* is free

from interstitial cells and a very narrow zone immediately under the tentacles is also free from them. This agrees with observations of previous authors, yet I think that from the statements previously made one would expect to find a much wider zone under the tentacles free from the interstitial cells than is found. They reach almost to the peristome and during multiplication in the formation of the spermary may be crowded up to this region (Fig. 7).

Cell generations.

The interstitial cell has a diameter of about 12 μ. After repeated divisions, necessarily repeated to form the large mass of the young spermary, there comes a time when the daughter cells formed do not grow to the size of the parent interstitial cell but remain of about half its volume. These cells are the first generation of spermatogonia. They divide to form the second generation of spermatogonia. During this division the chromosomes are reduced to half the somatic number and the spermatogonia of the second generation are transformed with little change to spermatocytes. The cell generations then from an interstitial cell or primordial germ cell to the sperm are: —

(1) Primordial germ cell — an interstitial cell. From this by mitosis are formed (2) spermatogonia of the first generation. These divide mitotically and form (3a) spermatogonia of the second generation, which are transformed without mitosis into (3b) spermatocytes of the first order. Mitosis occurs forming (4) spermatocytes of the second order. These divide indirectly into (5) spermatids which transform to the spermatozoa.

Spermatogonia.

The spermatogonia of the first generation are at first nearly spherical cells about 10 μ in diameter. Later as the spermary grows in size by the increase in the number of cells, they become dodecahedra or polyhedra with a flattening in at least one direction, frequently with three dimensions (Fig. 29). Calling the protruding tip of the spermary the pole, then the long diameter of the spermatogonia will usually be meridional, the shorter diameter in the plane of a parallel and the shortest diameter radial. The dimensions being on an average $10 \times 9 \times 7.5$ μ. But this is variable within narrow limits. The nucleus of the spermatogonia is about 7 μ in diameter. It is usually, possibly always, eccentrically placed, but whether the eccentricity is always in the same direction I have not been able to deter-

mine, as there have not yet been discovered any constant landmarks in the cell. In the flattened dodecahedral cells the nuclei partake of the change in shape of the cell measuring on an average $7.2 \times 6 \times 5.4$ μ. The structure of the spermatogonia is like that of the ectoderm cells, except that the protoplasm is not vacuolate.

Division of spermatogonia. Prophase.

As division approaches the nucleolus fragments into two or more parts and disappears (Fig. 31). The microsomes at the intersection of the yet indistinct karyoplasmic threads become quite prominent, giving to the nucleus a very granular appearance. This phase lasts for a comparatively long time and at the base of the spermary the majority of the spermatogonia of the first generation will there be found in this condition. Then the thread connecting the microsomes become more apparent and constantly coarser, while the microsomes themselves are reducing in number (Fig. 32, 33). This process of reduction continues until forty-eight are to be noted, joined by coarse strands of the karyoplasm (Fig. 34). The chromatin seems to be almost entirely collected in these forty-eight microsomes and since they persist to form the chromosomes we may cease speaking of them as microsomes and designate them the chromomeres. All but one of the connecting linin bands between any two chromomeres dissapear giving thus a continuous thread on which the chromomeres appear bead-like. During the changes mentioned above the nucleus seems to hold no constant shape. It now however usually becomes an ellipsoid of revolution with the long axis at right angles to the line connecting the poles at which the centrosomes will appear.

Spireme.

The thread of chromomeres now becomes a spiral on the nuclear wall, the axis of the spire coinciding with the major axis of the nucleus (Fig. 35). It is difficult to count so large a number of chromomeres with absolute accuracy, and I cannot be absolutely sure of their number or the arrangement during this prophase of the spermatogonic division. There is no doubt, however, that during the division of the spermatocytes there are twenty-four chromomeres usually, and that the spiral thread makes three complete turns of the nucleus. So far as it has been possible to count accurately, there have been found forty-eight chromomeres in the spermatogonia, and the thread makes six complete turns of the nucleus The nu-

cleus next changes its shape and becomes a flattened spheroid; the centrosomes appear over the poles. The spiral thread of chromomeres is no longer a spiral, but the halves of each turn have come to lie meridionally with reference to the poles, each half containing four chromomeres. The thread now divides at the poles, forming twelve meridional segments.

Equatorial plate.

Each segment contracts, the chromomeres fuse more or less completely and twelve approximately spherical chromosomes are formed in an equatorial position (Fig. 36).

Metaphase. Anaphase. Telephase; reduction in number of chromosomes.

An interesting feature of the spermatogenesis of *Hydra* is the frequent distinctness and persistence of these chromomeres. Fig. 37 shows a nearly spherical spermatogonium in the metaphase. Two chromomeres are to be made out in most of the daughter chromosomes. The karyokinetic figure is simple. A single fibre runs from each chromosome to the centrosome. Sometimes polar radiations appear; when they do they seem like continuations of the spindle fibres through the centrosome to the cell wall. In the spermatocyte division traces of central spindle fibres are to be found. They have not been noticed in the spermatogonic divisions. As the chromosomes pull apart they are connected by interzonal fibres and we often get, especially in a region where the cells are not very crowded, such a typical division figure as 39 where the ‚Zwischenkörper' are visible. As the twelve daughter chromosomes approach the poles in the separating daughter cells there is visible a tendency toward reduction of their number by the fusion of adjacent chromosomes. Fig. 40 shows this process plainly. Usually this process is delayed until the chromosomes are densely massed in the new daughter nucleus. In the upper nucleus of Fig. 38 nearly all trace of the individual chromosomes is lost. In the lower nucleus the chromosomes are still apparent and fusion of the adjacent ones is progressing. This fusion begins apparently at the tips nearest the poles which are naturally approximating as the chromosomes converge and there is temporarily produced a somewhat V-shaped figure.

Spermatogonia of second generation. Synapsis.

As the daughter cells are separated new nuclear membranes form and the daughter cells are the spermatogonia of the second generation. At first the nucleus of the spermatogonium of the second generation stained with iron haematoxylin appears uniformly deep blue black. It would seem as if the chromatin were diffused throughout the nucleus. Later the deeply stained area may be crescentic or localized in several rings. With the transparent safranin stain at this stage, however, while the deep stain of the nucleus shows much diffused chromatin the chromomeres can yet be distinguished persisting as twenty-four distinct bodies. This is the resting stage or synapsis. In the early part of this stage the centrosomes disappear, at least no stain has been tried which will make them apparent.

Spermatocyte of the first order.

This cell (Fig. 42) must now be considered the spermatocyte of the first order, since from it are derived the four spermatids and since the reduction in the number of chromosomes occurs in it. However the growth period which in higher animals marks the transition of the last generation of spermatogonia to spermatocytes seems wanting in *Hydra*. There is however a period of protracted rest in which the nucleus remains granular from the prominence of the chromomeres (Fig. 41). By using the extreme rather than the average cell measurements, there could be introduced another generation of spermatogonia followed by the typical growth period. But the evidence seems to be against such a third generation of spermatogonia and it is the exceptional measurements rather than the average which support such a possibility. The measurements will be given later.

It is interesting to note a difference in the method of karyokinesis in those spermatogonia which are found occasionally dividing at the very margin of the spermary, for they mark a transition to the type of division by which the interstitial cells usually divide. The preparation for division up to the appearance of the spireme stage is as already described. Then gradually the mesh work of strands is replaced as one connecting thread after another disappears until only a single continuous thread remains with the chromomeres beadlike on it. This thread takes the stain more and more intensely as the chromomeres disappear. Finally the thread is smooth, showing

no indications of chromomere swellings (Fig. 16). Whether it segments by the same method of formation of the spiral whose half turns became meridional I have not been able to determine, but I think not, for I have searched diligently for such stage without finding it, while the spireme of the spermatogonia within the spermary as given in Fig. 35 is very conspicuous. After the thread had fragmented, however, we find twelve V-shaped chromosomes arranging themselves in a equatorial plate (Fig. 17). In Fig. 18 the upper part of the cell has been partly cut away and one is looking down into the lower half, the pole of which is tipped toward the observer. Each V-shaped chromosome contains four chromomeres. Now the division is a longitudinal division, beginning at the tip of the V's where the mantle fibres attach. The ends of the daughter V's however often seem to hang to each other with tenacity, so that the chromosomes are changed to rod-shaped bodies during the metaphase. Moreover the half-chromomeres produced by the longitudinal splitting of the chromosomes seem to fuse now so that each rod-shaped chromosome contains four chromomeres. This process is not universal, however, for sometimes we get in the spermatogonia (recognized by their size) a persistence of the open V's until their fusion in the daughter nuclei (Fig. 21). And this seems to be the almost universal method of division in the interstitial cells.

Mitosis in interstitial and ectoderm cells.

The division of the interstitial cells and as far as observed of the ectoderm cells when they divide mitotically is as follows: — The cell consists of an indistinct reticulum in the meshes of which is the cytolymph. Similarly the nucleus is made up of a mesh work of fibres containing in their interstices the karyolymph and supporting one or more, usually one nucleolus.

Prophase.

The first indication of approaching division is the increased intensity of stain which the reticulum assumes, especially in the nucleus. The nucleolus then fragments into two or more spherical granules, which gradually disappear. Contemporaneously the fibres of the reticulum become coarser, and at their intersections the microsomes are prominent. This process goes on, the fibres and microsomes becoming fewer until the linin fibres are very coarse and the microsomes large and deeply staining. At last all the linin

strands disappear except one continuous strand which connects all the microsomes, or better now termed the chromomeres. Of these there are probably forty-eight. They gradually disappear and the entire thread becomes smooth and takes nuclear stain with great intensity. It fragments into twelve segments, which come to lie in the equatorial plane, the point of the V directed toward the cell's center. Centrosomes appear at the poles. Spindle fibres stretch from the tips of the V's to the centrosomes.

Metaphase.

The division of the chromosomes is longitudinal beginning with the tip. The open arms of the V's remain united temporarily and as they separate they are connected by fibres.

Anaphase.

The daughter chromosomes are V-shaped. As they approach the pole their tips fuse as they come into contact and we have often a deeply staining mass with the ends of the chromosomes still unfused and protruding. The interzonal fibres dissolve apparently. If they persist sufficiently long the zwischen Körper may appear as the cell contracts to make the daughter cells.

Telephase.

The spindle fibres also disappear and the centrosomes. The chromosomes fuse ultimately to make the daughter nuclei. The nuclear membrane reforms. The chromomeres reappear and become connected again by linin threads. These threads decrease in thickness as they increase in number. The chromomeres are replaced by numerous microsomes. The reticulum is re-established by a process the reverse of that by which it disappeared in the parent cell. The reticulum becomes gradually finely meshed and indistinct. The nucleolus reappears, small at first but gradually larger, and the daughter cells are in the resting stage. They may grow to the size of their parent becoming interstitial cells or remain without growth as the spermatogonia of the first generation. Similar telephase has been reported by Flemming and Rabl in the epithelial cells of amphibia.

Reduction in the number of chromosomes in marginal spermatogonia.

In the marginal spermatogonia whose division approximates that of the interstitial or ectoderm cells as just described, reduction is foreshadowed as in those well within the spermary by the fusion of adjacent chromosomes as they converge toward the poles (Fig. 20). We then have six bivalent chromosomes, each containing four bivalent chromomeres. Diagrams 22—28 present a synopsis of this process. Fig. 22 gives a diagram of the equatorial ring of V-shaped chromosomes. These divide longitudinally beginning with the tip of the V (Fig. 23). Their open arms resist separation (24) and the chromosomes become rod-shaped while their four half chromomeres fuse and form two. Thus what appears like a reduction division is not such at all (25). When separation of the daughter chromosomes does occur, each one contains two chromomeres (26). As the chromosomes converge toward the poles, adjacent ones fuse (27) to form the bivalent chromosomes of the spermatocytes.

The recognition of this process came slowly. At first attention was confined to the spermary and the process of division as first outlined was worked out. Mitosis outside of the spermary was not encountered for a long time. Then there were found interstitial cells with the V-shaped chromosomes — cells of the size of the spermatogonia of the first generation with twelve V-shaped chromosomes and most perplexing, cells of the size of spermatogonia of the first generation with apparently six V-shaped chromosomes seen in the late anaphase. When it was discovered that these latter chromosomes were made up of two rod-shaped chromosomes fusing and when cells like Fig. 19 showed how the V-shaped chromosomes became rod-like, the interpretation given was apparently the only one tenable. Other possibilities were considered however, and eliminated; for instance that the germ cells were distinguished from the somatic interstitial cells by having only six chromosomes and that this number was doubled when the spermatogonia were formed; o again that only the germ cells showed the type of mitosis given in Fig. 36, while that of Fig. 21 was confined to somatic interstitial cells. But the facts cited disproved these and other similar hypotheses.

The cell generations.

It has been exceedingly difficult to make out the cell generations. The growth of the spermary is so rapid and the spermary like the other tissues of *Hydra* is so mobile that the cells are of a great variety of shapes. Moreover there is not a succession of zones in the spermary corresponding to the cell generations as in many of the higher animals, but a bunch of spermatogonia may be retarded in their development until they get well out toward the tip, or they may develop precociously so that there will be spermatocytes and spermatids down among the spermatogonia. It was a few fortunate cases of the former character where spermatogonia of the first generation had been retarded in development until reaching the outer zone of the spermary that enabled me to determine with definiteness the number of cell generations, for but here they were released from pressure, assumed consequently a spherical form, and this makes measurement of the successive stages easy. From the first measurements of cells, in the deeper parts of the spermary, I was persuaded that only one generation of spermatocytes was present, and so reported the matter. It was hoped that some such simplification of the process of spermatogenesis would be found in this lowly form; but later and more favorable measurements have convinced me that there are really two generations of spermatocytes. Measurements will be tabulated later.

Mitosis in the spermatocytes of the first order.

When the spermatogonia divide, the daughter nuclei are spheroids with the centrosome above one pole (Fig. 39). In the early stages of the spermatocyte of the first order, — that is, after division of the spermatogonia is completed, — the shape of both cell and nucleus changes, becoming elongated in an axis, parallel to the plane of division of the spermatogonia. The centrosome then lies at one end of the cell's long diameter instead of in a short diameter. The chromomeres of the nucleus persist and without going into a reticulate condition they become connected into a beaded thread, at first irregularly twisted, but finally spirally coiled on the nuclear walls. The cell is now a flattended polyhedron. The nucleus is an ellipsoid of revolution with its major axis coinciding in direction with the major axis of the cell. The axis about which the spireme is coiled is the major axis of the nucleus (Fig. 43). The persistent centrosome

which lay at one end of the nucleus has disappeared. Again the nucleus changes its shape, becoming spheroidal. The half turns of the spiral thread of chromomeres become meridians. Segmentation of the thread occurs at the poles, and the nuclear membrane disappears. The segments of the spireme thread contract forming an equatorial plate of nearly spherical chromosomes. Two centrosomes now appear over the poles and therefore 90° from the point at which the centrosome disappeared. A thread from each chromosome grows to each centrosome, or through it to the cell wall. Division is an equation division for each daughter chromosome contains four chromomeres, the same number as the parent chromosomes (Fig. 44 and 45). The late anaphase and the telephase do not differ from those already noted for the spermatogonia. In the late telephase the nucleus stained with iron haematoxylin takes a deep blue black color and frequently has a collapsed appearance (Fig. 46). Occasionally the color settles in spots and rings (Fig. 47). While the nucleus stains similarly with safranin, yet being a transparent stain the chromomeres can still be seen. In the majority of cases 24 are visible. Yet in some cases where it seemed hardly possible that any were hidden, fewer than twenty-four could be counted.

Mitosis in the spermatocytes of the second order.

It is in this division of the spermatocytes of the second order that the details of division have been most carefully worked out. These cells are sufficiently small to be usually all included within the thickness of a single section, whereas the spermatogonia were much more likely to have part of the mitotic figure cut away in the next section. The chromomeres during the late telephase of the first spermatocyte division become connected by linin strands, but do not form the fine meshed reticulum of the resting nucleus (Fig. 49).

Prophase.

The early prophase of the division of the spermatocytes of the second order is marked by the disappearance of all these connecting strands except one between each two adjacent chromomeres, thus forming a continuous beaded thread irregularly coiled (50).

Spireme.

As in the spermatogonic division the daughter cells after the division of the spermatocytes are polyhedra with the centrosome

lying in a short axis. The shape then changes, this minor axis becoming the major axis (Fig. 49). The irregularly beaded thread becomes a spiral coiled about the major nuclear axis on the nuclear wall, and making there three complete turns (Fig. 51). Again the nucleus changes shape, becoming a flattened sphere and the spireme thread so shifts that its segments become meridional. Fig. 53 and 54 show pole views. The nuclear membrane now disappears. The spireme fragments at the poles, forming six segments, each containing four chromomeres (Fig. 55).

Equatorial plate.

These segments contract, forming six spherical chromosomes, in the equatorial plate. Meantime the centrosomes have appeared at the poles and each chromosome is connected with the centrosome or through it with the cell wall by a fibre (Fig. 56).

Metaphase.

As the metaphase begins the spherical chromosomes are elongated (Fig. 57). At division two chromomeres go into each daughter chromosome. It seems probable therefore that this is a reduction division. Interzonal fibres connect the chromosomes as they pull apart and during this anaphase the central spindle fibres were detected (Fig. 58). The usual appearance of the chromomeres in the late telephase is evident here.

The spermatid.

The spermatid immediately after the separation of the daughter cells of the spermatocyte division is spherical, but there is a projecting point of protoplasm on one side frequently, marking its connection with the sister cell (Fig. 60). Let us call the diameter passing through this projection and through the centrosome the polar diameter. The nucleus is now biscuit-shaped (Fig. 59 and 60). In pole view it appears circular in outline, in side view plano-convex. There follows the same change of shape in the spermatid that was noted in the spermatocytes at this stage. The cell becomes an elongate polyhedron, the nucleus an ellipsoid of revolution. Their long axes coincide and lie in the direction of the polar axis which becomes therefore the major axis of the spermatid. It would of course be possible for the cell to elongate at right angles to the polar axis; the centrosome would lie in a minor axis. It might then

migrate to a position at one end of the nucleus as figured in Fig. 61 and 49. But it seems more likely that it retains its place, the cell elongating in the polar axis. For the customary division of the centrosome would then bring the daughter centrosomes to the poles of the new cell. (Compare Fig. 49 and 61.)

Formation of the spermatozoon.

The spermatozoon is formed out of the nucleus of the spermatid by the absorption of the cell body by the nucleus and formation of the various parts of the spermatozoon by a process of growth. Immediately about the nucleus appears a transparent film which increases in breadth. Apparently the cell body next to the nucleus is becoming liquified. At one end of the nucleus, the future tail end of the spermatozoon, there appears a tiny droplet of clear substance, the nucleus becomes cylindrical with the growing droplet at the posterior end and a conical point at the anterior end. When about one half the cell body has been liquified, the droplet has nearly attained its maximum size and has a diameter about one-third that of the minor axis of the cell. The nucleus has now increased in length until it is as long as the cell, the pointed tip of the nucleus is the anterior end of the spermatozoon. The posterior edge of the dome-shaped droplet which is the middle piece, touches the cell wall. The unliquified cell body remains now about the forming spermatozoon as a cylinder, the longitudinal section of which will give on either side a concavo-convex section, the convexity (exterior) corresponding to the surface of the cell (spermatid). During this process the nucleus lying free in a fluid often shifts its position. Thus in Fig. 66 its long axis is at right angles to the position it first held in the polar diameter of the cell.

At about this time the tail rudiment appears as a growing point at the posterior end of the middle piece. At the outset the tip of the tail is somewhat enlarged, but it soon tapers to a point. I am unable to determine whether the cell wall is carried out as a membrane over the growing tip or not, but I have no evidence that it is and believe that at the point where the limiting membrane of the middle piece and the cell wall touch there is a fusion. It is from this point that the tail outgrowth proceeds. As the tail grows the middle piece decreases in size. The liquefaction and absorption of the cell body and wall continues until all has

disappeared. When the tail attains its full growth it is about three times the length of the head.

The mature sperm.

The mature sperm (Fig. 68), (when at last all vestige of the surrounding cell wall has disappeared) consists of a cylindrical head tapering to a point at the anterior end, a somewhat hemispherical middle piece at the posterior end of the head, and attached to this the long tapering tail. The head stains deeply with any nuclear stain. But the extreme tip bears an acrosome. The middle piece is clear, taking the stain slightly, even a plasma stain. The lash-like tail is about three times the length of the head and middle piece. Within the head may be seen six bodies each consisting of two more or less connected spherical masses. These apparently are the persistent chromosomes, each consisting of two bivalent chromomeres. These are not easily detected, and I think mark the early stage of the mature sperm, disappearing shortly after complete absorption of the enclosing spermatid. Near the posterior end of the middle piece, the centrosome can be detected. Through it the axial fibre extends into the tail and anteriorly toward the tip of the head. During the development of the spermatozoa the centrosome could not be detected after the stage represented in Fig. 61 until the surrounding cell was nearly absorbed (Fig. 67). It reappears in about the same position as it disappeared. That it persists seems probable.

What this droplet forming the middle piece really is can only be judged from analogy. In many of the higher animals the centrosome of the spermatid divides. One part forms or helps form the middle piece; the other gives rise to the axial thread. Paulmier (110) has shown in *Anasa* that the axial thread grows out from the centrosome. Hermann (100), Benda (95) and Meves (106) have found in *Salamandra* that one of the centrosomes gives rise to the middle piece or part of it at least, and the other is connected with the axial filament. Similarly the results of Moore (108), Suzuki (113), Korff (101), Lenhossek (103) and others agree that the axial thread comes from one part of the divided spermatid centrosome, while the other often greatly enlarged, forms or helps form the middle piece. We find in the adult sperm of *Hydra* that the axial filament passes through the centrosome and likely originates as an outgrowth from it. Possibly the centrosome divides when the other part may form

the middle piece. If this droplet of nutritive material derived largely from the liquid and absorbed cell body is to be regarded as a greatly enlarged centrosome, as seems likely from the facts given, then we may justly assign to the centrosome the function of liquefaction and assimilation of the cell body. The centrosome then becomes a digestive ferment. This can only be a suggestion rendered possible in *Hydra* by what we know of the centrosome in animals where it can be plainly followed.

The acrosome seems to be derived from no special body as in many higher forms for no Nebenkern or idiosome has been found in the spermatid. It arises apparently from that portion of the cell protoplasm which adheres to the exterior tip of the elongating nucleus. This spermatozoon is small. From tip of head to base of tail it measures 4.2 μ. Of this the acrosome is 0.2 μ, the head proper 3.2 μ, and the middle piece 0.8 μ. The diameter of the head at its widest point is 1.2 μ, of the middle piece 1.3 μ.

Summary of the phenomena of mitosis.

It will not be amiss to recapitulate the phenomena of cell division, classified under several headings.

The centrosome. This structure is followed with difficulty. It is apparently absent in the spermatogonia until just prior to the equatorial plate stage and disappears usually shortly after division. This is true also for the spermatocytes. Occasionally however it persists until the early prophase of the succeeding division (Fig. 49). That its disappearance is apparent rather than real seems likely, for it is occasionally found dividing before its disappearance (Fig. 20). And then too a central spindle is not infrequently seen (Fig. 57 and 58). It is probable then that during or after cell division of the centrosome occurs, the new daughter centrosomes separating and moving each 90 degrees to the new poles of the daughter cells. In the spermatid then it remains (Fig. 61) at the pole, as no new daughter cells are to be formed, and is there incorporated in the middle piece.

Occasionally some detail could be made out for the centrosome, when it appears as a small sphere with a more deeply staining central granule (Fig. 40).

The mitotic figure. This is extremely simple as asters are wanting. It was noticed by Brauer in the formation of the polar bodies that the polar spindle is barrel-shaped and

has no polar radiations. Yet the cells of the developing embryo do have such polar radiations. The nearest approach to such asters in any mitosis observed either of interstitial or ectoderm cells is an occasional extension of the spindle fibres through the centrosome to the cell walls. Since the linin mesh work has been found during the prophase to form the thread on which the chromomeres are suspended, and since the entire structure goes into the chromosomes, the fibres of the achromatic spindle cannot arise from it unless they are outgrowths of the chromosomes. This is apparently their origin as already noted. They originate at the periphery of the chromosome, one for each chromosome, as a thread which elongates to the centrosome or beyond it to the cell wall. In disappearing however, the fibre seems to dissolve first at the end attached to the chromosome and then progressively toward the centrosome. As the anaphase of mitosis occurs the previously attached ends of the separating chromosomes are connected by fibres.

On these interzonal fibres appear the Zwischenkörper (Fig. 39). They seem to disappear by absorption.

The nuclear wall. This disappears in division shortly after the polar segmentation of the spireme into the individual chromosomes. It reappears immediately after the complete fusion of the chromosomes in the daughter nuclei.

The chromosomes. The marked difference in shape of the chromosomes in the spermatogonia inside of the spermary and those at its border or without it is a point of interest demanding an explanation. The difference may be supposed to be due either to altered physical conditions, difference in pressure perhaps, or to altered chemical constitution, produced by some substance existing in appreciable quantity in the spermary. The phenomenon is quite common in spermatogenesis as we frequently find the chromosomes in the spermatocytes assuming a spherical form, or at least departing widely from the somatic form of a V or a rod. It may be due to a greater plasticity of the chromatin during reduction phenomena.

The chromomeres. Whether these are actual parts of the living cell or are merely coagulation phenomena, they form our best criteria for judging the relation of certain apparently unlike chromosomes. To homologize the V-shaped chromosomes with four distinct chromomeres of Fig. 21 and the spherical daughter chromosomes of Fig. 37 with two chromomeres was at first not deemed possible. It

was only when the transition stages of Fig. 22—26 were discovered that it seemed they could be identical. This longitudinal splitting of the V-shaped chromosomes and the subsequent fusion of the approximated arms produces an apparent transverse division and makes an equation division appear very like a reduction division. Since we are sure that no reduction division occurs in some spermatogonia of the first order, we conclude that it does not occur in any in spite of the apparent reduction in Fig. 36.

Reduction. The reduction in the number of chromosomes occurs in the telephase of the first spermatogonic division. The chromosomes of the spermatocyte of the first order, though showing no signs of tetrad structure are the equivalents of the tetrads: Their first division is a longitudinal division as each daughter chromosome contains four chromomeres and the spireme prior to the division of the spermatocytes of the second order with spermatids contains twenty-four chromomeres. Since the chromomeres from which each of these was derived were bivalent and the division is not a reduction division, the chromomeres of the spermatocytes of the second order are bivalent.

In the division of the spermatocytes of the second order there probably occurs a reduction division for each daughter chromosome contains two chromomeres (Fig. 18). This might be explained as an equation division however, similar to that of the spermatogonia (Fig. 36). But the contrast to the division of the spermatocyte of the first order where there are four daughter chromomeres in the daughter chromosomes would seem to indicate that the division of the chromosomes of the spermatocytes of the second order is at right angles to the division in the first order of spermatocytes, so that the latter being an equation division the former is a reduction division. The chromomeres which appear in the head of the sperm are at any rate bivalent.

Changes in volume during mitosis.

Throughout the division stages of spermatogonia and spermatocytes there are constant changes in both absolute and relative sizes of cells and nuclei. There is a slight decrease in the size of the cell throughout the prophase up to the formation of the spireme. The nucleus meantime is increasing in size. After the spireme forms the cell begins to grow and attains its maximum size during the early anaphase. Immediately after division the nucleus is larger,

the cells also are larger. Both then decrease in size during the synapsis, the nucleus having frequently a collapsed appearance. These changes in volume produce a difference between maximum and minimum of about one third the least volume of the cell nucleus. These facts came out in making measurements to determine the number of cell generations. No uniformity could be obtained in relative volumes of spermatogonia, spermatocytes etc., until measurements were confined to the same phase. The equatorial plate stage was selected and herewith are given the tables of average measurements, made up from the measurement of fifty of each kind of cells:

	Spermatogonia	Spermatocyte 1st order	Spermatocyte 2nd order	Spermatid stage of Fig. 61
Length of cell	10 μ	82 μ	7 μ	5,1 μ
Breadth	9 „	6.5 „	4.9 „	3,8 „
Length of spindle	8.2 „	6.7 „	5.2 „	
Breadth	5.4 „	4 „	3.5 „	

Probably these measurements are approximately correct, yet we cannot be sure of the third dimension of these irregularly shaped cells since only two dimensions can be seen in the sections. It was consequently found more satisfactory to rely on the measurements of those cells which had been largely released from pressure of adjacent cells and so had assumed the spherical form. The average of these measurements gives the following:

Diameter of the cells.

	Diameter	Cube of diam. (relative volumes)
Interstitial cells (approximate)	12 μ	1728
Spermatogonia 1st generation	9.6 „	885
„ 2nd „ / Spermatocytes 1st order	7.4 „	405
„ 2nd „	5.4 „	157
Spermatids	4.1 „	69

It will be seen that in spite of the growth of the cell mentioned as occuring during the prophase, the changes which the cell undergoes during division produces a net decrease, at every division.

The variations in size of the various cells taken at the equatorial plate stage and including only those spherial or nearly so is as follows:

Spermatogonia	10.2 μ	to 9 μ	in diameter
Spermatocytes 1st order	7.7 „	to 6.7 „	„
„ 2nd „	5.9 „	to 5.1 „	„
Spermatids	4.5 „	to 3.8 „	„

As before mentioned, by considering extreme sizes we might introduce another generation of spermatogonia which would allow of a growth period in the transition from spermatogonia to spermatocytes of the first order, thus:

	Diameter	Relative volume
Interstitial cells	13 μ	2197
Spermatogonia 1st generation	10.2 „	1061
„ 2nd „	7.7 „	457
„ 3rd „	5.9 „	206
which would grow to		
Spermatocytes of 1st order	7.4 „	405

The other generations would remain as before. But the complete absence of transition sizes from 5.9μ to 7μ except a few exceptional cases between 6.7 and 7μ seems to nullify this hypothesis. The entire process of spermatogenesis is so hasty in *Hydra* that it seems impossible moreover that there should be a prolonged pause and grown at any stage. The series of measurements for the cell generations as given seems to be marked by a regularity that commends its probability.

Nutrition of the spermary.

We should naturally connect these changes in size with metabolic changes. That such are going on cannot be doubted. During the formation of the spermary there is a great increase of the volume of tissue. Cells are dividing and the daughter cells growing at a rapid rate. Especially is nucleinic material used during the process, for the nuclei of the interstitial cells are relatively very large, and it will be remembered, during the prophase they are rapidly growing, while the cell body diminishes in size. As already noted a marked character of the cells of *Hydra* during spermatogenesis is the vacuolate condition. Fig. 14 and 15 have already been contrasted, —

the former a well-nourished budding *Hydra*, the latter an animal in the early stages of testicular development. Fig. 12 gives a still earlier stage in which the interstitial cell is dividing, no spermary having yet formed on the animal. Fig. 15 was from an animal having testes, although this particular section was through a region where the testis was just beginning to form. Fig. 13 however is a section at the base of a well developed testis. The endoderm cells, it will be noticed, are highly vacuolate. When *Hydra* is well fed, nutritive material is ingested and digested by the endoderm cells and passed on to the ectoderm cells, where it is stored in granules in the peripheral portion. These granules are a golden brown in color and are likely in the nature of fat as they stain very dark with osmic acid (Fig. 14). All observers have noted that brown *Hydra* when starved lose their color. This change is due, it seems probable, to the use of this stored food reserve during starvation. For such pale emaciated *Hydra* I find are always without the peripheral layer of granules that stain intensely with osmic acid. The *Hydra* that are bearing many testes are in the same condition. But in addition to absorbing this stored food material during the reproductive season there is also passed to the ectoderm nutritive material from the endoderm.

Many of the endoderm nuclei contain spherical particles which do not stain with the osmic acid but do take a deep stain with iron haematoxylin or any other nuclear stain. There are also in the endoderm cells masses of protoplasm containing such spheres from particles very minute in size up to droplets 2 or 3 μ in diameter. Moreover among the cells at the base of this spermary are to be found accumulations of exactly similar droplets. The correct interpretation of this is that the nuclei of the endoderm cells elaborate a substance akin to nucleinic acid, which is passed out into the cell protoplasm and thence on into the ectoderm. No case of actual transition through the mesogleea has been seen, so that it cannot be stated whether the droplets are passed through it intact or whether they are first dissolved and reappear after transfiltration. The negative evidence would indicate the latter process. During life these streams of nutritive material between the spermatogonia give to the testes a striated appearance, the droplets appearing as lines of orange yellow material, which is the color during life of the nuclei of the cells at the base of the spermary. In stained section it is to be noted too that the nuclei at the very base of the

spermary next to the mesogloea are often pale. As division occurs and some of the interstitial cells move out and become spermatogonia their nuclei stain very intensely, while the granules before mentioned decrease in number and gradually disappear. The conclusion seems to be that this nuclear nutrition material secreted by the endoderm nuclei and passed out to the ectoderm is absorbed by the rapidly dividing and growing nuclei of the primordial germ cells as the spermatogonia are formed.

It will be noted that there is a marked contrast in color in the female and male of *Hydra dioecia* given in Fig. 6 and 7, — a contrast which has been found constant. The female shows the presence of much of the yellow-brown oily nutritive material, while the male shows very little. It will be remembered also that in the hermaphroditic forms such as *H. fusca*, the spermaries appear after the ovaries have been formed. The egg moreover is simply a cell containing a vast amount of nutritive material. It seems likely therefore that the yolk material which is used up in egg formation helps to produce that impoverished condition which seems invariably to accompany the spermatogenesis. This explanation would not account for the impoverished condition of the male of the unisexual species, but would lend itself readily to the hypothesis that when the season of sexual reproduction began these animals of *H. dioecia* which were fortuitously poorly nourished develope testes while those well nourished become females. Disregarding theoretical considerations however, this much is established: That spermatogenesis is a process always accompanied by dearth of reserve nutritive material and that the necessary nutrition for the rapid cell multiplication of the interstitial cells is largely supplied by the endoderm, the nuclei of which cells elaborate it as a substance with the staining reaction of nucleinic acid. When passed to the ectoderm it lies among the interstitial cells, but decreases in amount as their nuclei increase in size and density of stain due to absorption of the nutrition.

Mitosis occurs in what cells?

In all the sections studied mitosis has been the universal mode of division in the interstitial cells, the exceptional mode in the ectoderm cells and amitosis the constant rule in the endoderm cells Fig. 5 shows an ectoderm cell in process of amitotic division. At first it was thought this was the only mode of division of the ecto-

derm cells. But a few cases have been observed, only two or three in hundreds of sections, however, where mitosis occurred in an undoubted ectoderm cell. SCHNEIDER had observed and figured mitosis in an ectoderm cell. But cell multiplication, except of the interstitials seems to be almost invariably amitotic. THALLWITZ (80) noticed that the nuclear division of the germ cells was indirect among hydroids in contrast to the other cells of the body. So constant is the indirect division among the germ cells and the direct among somatic cells that he feels safe in accepting mitosis as a distinguishing characteristic of the germ cells. NUSSBAUM (70) also notices that the sex cells are derived from the interstitial cells by mitotic division in *Hydra*. What the cause of this difference may be seems difficult to determine: whether it means an inherent difference in the interstitial cells from the other body cells or whether the fact that they are crowded into the interstices of the other cells produces altered physiological conditions experiment alone can determine. When living *Hydra* is teased up in water so that clusters of interstitial cells are set free, mitosis in them is still indirect. The cells do not live long enough to continue their division processes so that the condition of pressure anteceding the release may have determined the style of this first division.

The mesoderm.

At any rate both structurally and physiologically this interstitial layer seems a distinct cell layer. BRAUER finds in the embryology of *Hydra* that the endoderm and ectoderm are separated by a process of delamination. Some time after these layers have become distinct there is seen between the ectoderm and endoderm the interstitial cells — a third layer. This is formed from the ectoderm cells. BRAUER does not think this third layer should be considered mesoderm (p. 198). Certain it is that it has characters that are not mesodermal for from it are derived the nerve cells and the nematoblasts as well as the sex cells. That the primitive interstitial cells do develop differently may be evidence that there are differences, though they are not histologically apparent. The recognizable nematoblasts are distinguished by some stage of the developing nematocyst; the ganglion cells by their smaller size, lack of granulation and processes; the sex cells by their very large nuclei, extremely granular, and often by the presence of a Nebenkern (Fig. 15). SCHNEIDER says however that these three types are all derived from

the same primitive interstitial cells, and that he has observed all stages in the transition from interstitial cells to ganglion cell, nematoblast and sex cell. My own observations are contradictory on this point, however. The characters of the sex cells as given above seem constant, and my conclusion would be 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. Nor does it seem that SCHNEIDER's statement without demonstration of the so-called intermediate stages ought to be accepted. Certainly the 'intermediate stages' between interstitial cells and the sex cells which he figures are nothing but stages of development of the spermatids from the spermatagonia. The latter and I believe the interstitials from which they are derived are clearly distinct from the other cell elements.

Continuity of the germ plasm.

I believe therefore that the sex cells are a distinct group of cells and that the germ plasm is then continuous in *Hydra*. But yet there are included in this interstitial layer cells of very diverse fates. It would certainly be a mixing of characters to designate that layer mesoderm from which are derived ganglion and sex cells. But evidently *Hydra* is on the border land between the animals with two layers and those with three. The higher protozoa undoubtedly and some of the coelenterates probably have two germ layers. If the higher animals are derived phylogenetically from these we would expect to find some animals in which the mesodermic characters were mixed. Then the interstitial layer of *Hydra* would represent mesoderm, in nascendi, with certain cells having distinct mesodermal characters, others still bearing ectodermal characters.

The mesoderm in 'nascendi'.

It was not intended however to revive discussion of this antiquated germ layer theory, but merely to point out that the interstitial layer of *Hydra* might represent a transition phase from a two-layered condition to the three-layered of the higher animals. And even in those forms in which the three layers are distinct and well defined, it is known that they do not always retain their true function, but mesoderm may give rise to nerve cells, especially in regeneration.

Relation of the bud to the spermary.

LANG (57) gives the initiation of the budding process in *Hydra* as follows: The beginning of the bud is marked by the karyokinetic division of the interstitial cells which thus increase until the ectoderm is double its normal thickness. Then the mesogloea disappears and cells wander from the ectoderm into the endoderm until the former is reduced to its normal thickness. The mesogloea now reforms and in the thickened endoderm the new body cavity appears. The point particulary to be noted is that it is the increase of interstitial cells by karyokinesis and their accumulation in the ectoderm that gives rise to the early stages of the bud. The similarity of this process to the initiation of the spermary is apparent, and lends support to the attempts made before to homologize buds and spermaries. Since both processes are dependent on the interstitial cells, it is evident that the budding zone and the zone of reproductive organs must coincide. It is to be recalled that in *Hydra*, so far as observed, when sexual organs appear on a budding individual they appear on the bud and not on the parent stock. If this expresses the rule, sexual organs appearing on the vigorous bud rather than on the parent partially exhausted by budding, then we need only conceive of the process carried to the point where the bud always produces the sexual organs, and at an increasingly early stage, to see how from a primitive form like *Hydra*, a complex colonial form might be established, with buds specialized as gonophores. This does not imply that *Hydra* is necessarily the primitive form. It may be a degenerate. The homology in the early stages of budding and formation of the spermary would seem to permit of such an interpretation. The spermary would represent a degenerate bud. The line of degeneration would pass from a hydroid with free swimming sexually mature medusae like *Pennaria*, though hydroids with attached medusae (*Tubularia*) with attached gonophores evidently medusa buds (*Clava*) with attached gonophores that are evidently degenerate medusae (*Campanularia*), with gonophores that are scarcely recognized medusae (*Cordylophora*) to *Hydra* in which the gonophore can only be recognized as a bud in the very earliest stage. Since the process of budding departs from that of spermary formation at so early a stage and seems to occur under such different physiological conditions, and since *Hydra* has no other marks of degeneration either in its embryolgy or

structure, it seems more likely a primitive than a degenerate type. The absence of migration in the sex cells of *Hydra* while such migration is universal in the medusoid polypes would seem to add to the probability. The sex cells originally existing in each simple polyp would naturally in a complex colonial form migrate to those individuals which had assumed the reproductive function.

I desire to express my appreciation of the help and inspiration given me by Dr. C. O. WHITMAN in the pursuance of these studies.

Note. Since the completion of this paper there have appeared two papers by KONRAD GUENTHER on this subject; „Die Samenreifung bei Hydra viridis", in: Zool. Anz., V. 26, No. 705, p. 628—630 and „Keimfleck und Synapsis, Studien an der Samenreifung von Hydra viridis", in: Zool. Jahrb., Festschrift f. WEISMANN, 1904. GUENTHER's research was undertaken to derive evidence on a previously conceived theory regarding the function of the nucleolus. This is not an appropriate place to discuss that theory but there are some important matters of observation on which we differ that need comment.

GUENTHER maintains that the spermatogonia are derived from the ectoderm cells, but gives no proofs other than his figures. In these the so called ectoderm cell, fig. 6*a*, is smaller than the spermatogonium fig. 1—6. The difference in size of ectoderm cells and interstitial cells shown in my Figs. 12 and 15 and the fate of the ectoderm cells of the spermary shown in Fig. 9 and described in the text seem to me to disprove this hypothesis.

He points out the importance of the disappearance of the cell wall. In my preparations, the cell walls are perfectly distinct. *Hydra viridis* was found more difficult to fix than the other species and was not used as extensively in consequence.

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Explanation of plates.

Plate 22.

Fig. 1. The earliest figure of *Hydra* with testes, after BAKER, 1743.

Fig. 2. Section at margin of a spermary (*H. fusca*); shows multiplication of the primordial germ cells. 1342 : 1.

Fig. 3. An ectoderm cell. 1342 : 1.

Fig. 4. An ectoderm cell dividing amitotically. 1342 : 1.

Fig. 5. Section through a mature spermary, showing general arrangement of the generations of cells during spermatogenesis. 580 : 1.

Plate 23.

Fig. 6. Female of *H. dioecia*. 22 : 1.

Fig. 7. Male of *H. dioecia*. 22 : 1.

Fig. 8. *H. viridis* with mature ovary and testes just appearing. 22 : 1.

Fig. 9. Section of *H. dioecia* taken through spermary to show elongated ectoderm cells. 415 : 1.

Fig. 10. *H. dioecia*, male, copied from BRAUER.

Fig. 11. Cross section of *H. dioecia*, through spermary, to show relative proportions of parts. 220 : 1.

Fig. 12. Section through ectoderm of *H. dioecia*; a primordial germ cell dividing. 1342 : 1.

Fig. 13. Section at base of spermary (*H. fusca*); endoderm cells vacuolate, but elaborating nutritive material for the spermatogonia. 1342 : 1.

Fig. 14. Section of *H. dioecia*; a budding individual. 1342 : 1.

Plate 24.

Fig. 15. Section of *H. dioecia*; early stage of spermary. 1342 : 1. Interstitial cells in early prophase of mitosis.

(Fig. 16—67, 2684 : 1.)

Fig. 16. Spireme in spermatogonia outside the spermary.

Fig. 17. Late prophase of same.

Fig. 18. Equatorial plate of same. Top of cell cut away.

Fig. 19. Metaphase of same.

Fig. 20. Anaphase, reduction in number of chromosomes foreshadowed by fusion of adjacent ones.

Fig. 21. Telephase of same.

Fig. 22. Schematic equatorial plate of same, showing chromomeres.

Fig. 23. Two of the V-shaped chromosomes dividing (heterotypic mitosis).

Fig. 24. A later phase of same.

Fig. 25. The V-shaped chromosomes appear rod shaped.

Fig. 26. The anaphase: real longitudinal division appears transverse.

Fig. 27. The late anaphase, reduction in number of chromosomes foreshadowed.

Fig. 28. Fusion of adjacent chromosomes complete.

Fig. 29—39. Division of a spermatogonium within the spermary.

Fig. 40. A spermatogonium of the second order. This becomes the spermatocyte of the first order, Fig. 41.

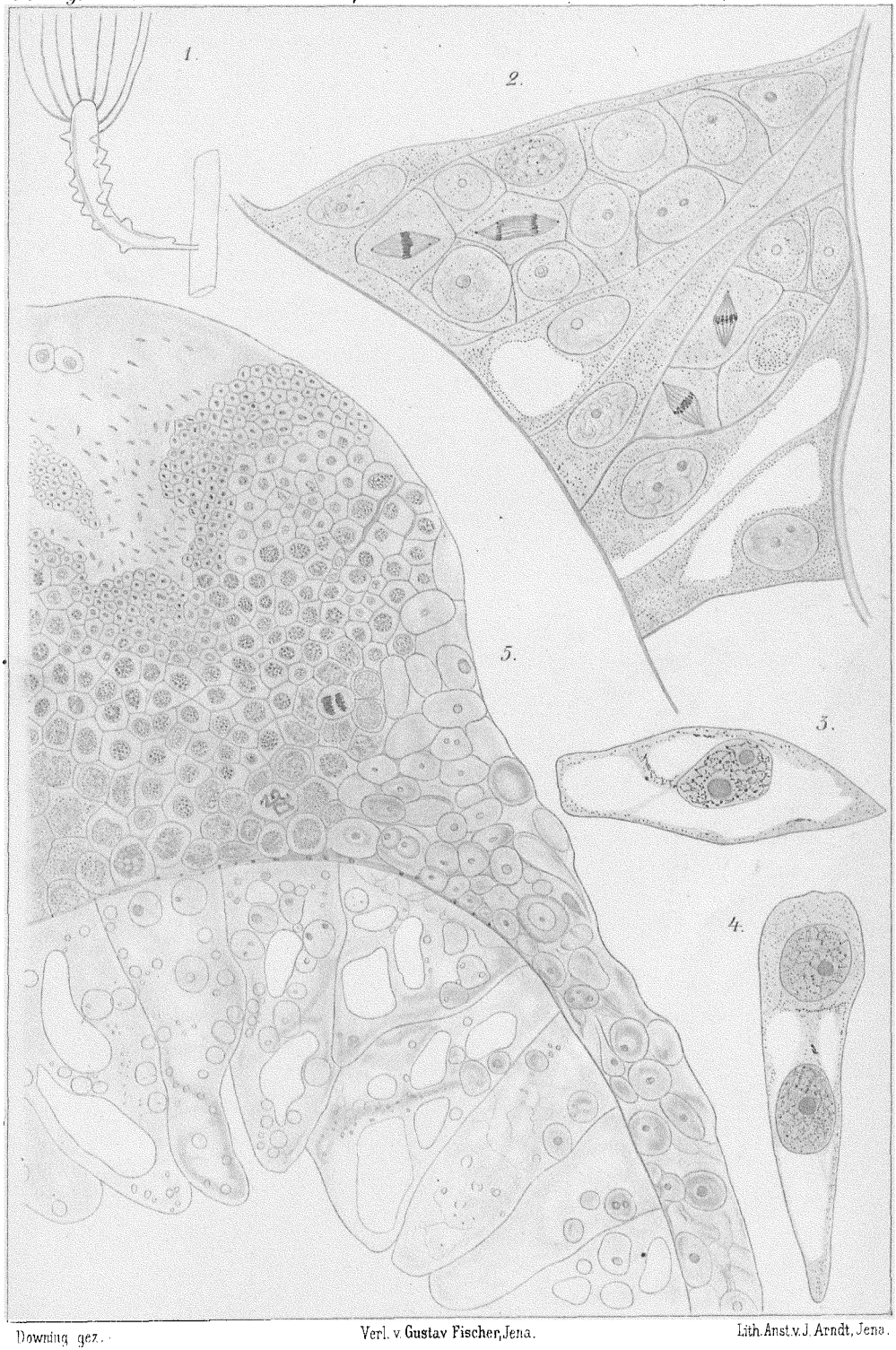
Fig. 41—45. Division of the spermatocyte of the first order.

Fig. 46—48. Synapsis stages of spermatocyte, second order.

Fig. 49—59. Division of spermatocyte of second order.

Fig. 60—67. Transformation of spermatid to spermatozoon.

Fig. 68. Adult sperm. 10736 : 1.



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