ON THE DEVELOPMENT OF PARASITIC COPEPODS.[1]

PART I.

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CONTENTS.

The work herein presented was begun at Wood's Hole, 1904, with a view of determining the effects of pressure on the eggs of parasitic copepods. Owing to technical difficulties, three summers have passed with few experimental results, and in consequence the present paper is chiefly of a morphological nature.

I obtained parasitic copepods from fish caught in traps of the Marine Biological Laboratory and the U. S. Fish Commission, at Woods Hole. It was usually necessary to go to the trap to get them fresh, or in the case of some Caligidæ to catch them before they left the fish.

Most of the work was done on *Pandarus sinuatus* Verrill, *Læmargus muricatus* Kröyer (collected by Dr. Conklin), and a new species (text Fig. 3) of the family Dichelesteidæ and of a new

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genus near *Kröyeria* (according to Prof. Charles B. Wilson) which I will call throughout this paper the dichelestid. The eggs of *Pandarus sinuatus* are the most difficult to handle, being very flat and thin and pigmented, but I used them because this is the most common species at Woods Hole. I used the eggs of the following for comparison (arranged according to the classification of Claus):

Caligidæ:

Caligus bonito Wilson (and several undetermined species of *Caligus*).
Caligus rapax Milne Edwards.
Lepioptherius edwardsi Wilson.
Perissopus communis Rathbun.
Nesippus alatus Wilson.
Cecrops latreillii Leach.
Læmargus[1] *muricatus* Kröyer (and an undetermined species of *Læmargus*).
Philorthagoriscus serratus Kröyer.
Pandarus sinuatus Verrill.

Dichelesthiidæ:

Anthosoma crassum Abilguard.
Kröyeria ?
Eudactylina nigra Wilson and *E.* sp.?

Lernæidæ:

Penella.

Chondracanthidæ:

Chondracanthus.
Sphyrion ?

For the determination of species I am indebted to Prof. Charles Branch Wilson, of Massachusetts State Normal School, Westfield, Mass., who has been very kind in his interest in my work.

After my work was nearly finished I received the material collected at Woods Hole in 1899 by Prof. Edw. Rynearson, of Pittsburgh, with a view of working on this same subject, and which he kindly turned over to me. I wish to express my thanks for this abundance of material, which allowed me to confirm

[1] The word *Læmargus* was applied to a copepod and a shark in the year 1837 and as yet it is disputed which should claim priority.

many points on which my material was scanty and to add some new ones.

I am indebted to Dr. Conklin for constant guidance and assistance besides the general direction of my work, and to Mr. Kribs for the use of Zeiss apochromatic lenses.

Dr. Formad and Dr. Fischelis rendered me invaluable service in translating Russian.

I am under obligations to the Carnegie Institute for a table in the Marine Biological Laboratory, Wood's Hole, 1904, and to the U. S. Fish Commission for a table in the U. S. F. C. Laboratory, Wood's Hole, Mass., 1905–6.

The material was fixed in Flemming's fluid, picro-acetic, or corrosive-sublimate-acetic. The first gave the best fixation but the second was the most convenient. Heidenhain's iron hæmatoxylin followed by various counter-stains, and Hermann's safranin-gentian violet were used most frequently.

II. Historical.

The ground covered in this paper was almost entirely untouched by earlier workers as regards the particular genera treated, and I will consider here only some papers on related forms.

The free living copepods have long been favorite objects for study. Gruber ('79) described their reproductive organs. Weismann and Ischikawa ('88 *a*, *b*) and Ischikawa ('92) used them in studying the question of reduction in number of chromosomes. Haecker ('91, '92 *a*, *b*, '94 *a*, *b*, '95 *a*, *b*, '97, '02) found them favorable objects for study of oögenesis, maturation, reduction, and unsymmetrical mitoses. Later, Rückert ('94, '95 *a*, *b*, *c*) studied oögenesis, maturation, and reduction in these.

Of the parasitic and half parasitic copepods, Heider ('79) notes the spermatogenesis of *Lernanthropus*, and Giesbrecht ('82) the oögenesis of the Notodelphidæ.

Charles Branch Wilson in his monograph on the Caligidæ ('05) describes the anatomy of the reproductive organs and the life history.

III. Anatomy of the Reproductive Organs.

In *Pandarus*, *Caligus* and allied forms the two ovaries lie in the head (Fig. 1) and the two oviducts run backwards to the

genital segment, where they are much convoluted and increase greatly in diameter and each communicates by an opening, the os uteri, with an egg string that trails behind the animal. The ovary is formed of a much convoluted cord of cells in a single linear series, small at the oögonial end and gradually increasing in diameter until it passes into the oviduct. As these cells (oöcytes) grow in the ovary they become much compressed in the direction of the long axis of the cord and the cell boundaries almost completely disappear. Passing into the oviduct, the oöcytes continue to grow and soon their boundaries reappear, and they become more flattened as they grow larger. There are at least two broods in the oviduct at once, caused by the periodic activity of the ovary. The younger brood extends some distance into the genital segment and is sharply marked off from the older brood, which occupies the last coils of the oviduct and consists of oöcytes that have nearly or quite completed their growth. As the oöcytes pass out through the os uteri they are fertilized from the seminal receptacles (Fig. 2, *sr*) and surrounded with secretion from the cement gland (*c.g.*) which forms the wall of the egg string. The eggs are distorted when passing through the thorax, but in most cases regain their symmetrical form. Embryos in the egg strings have their heads turned latero-ventrally and their ventral surfaces anteriorly, in relation to the mother. Occasionally one finds an embryo reversed, or partially rotated.

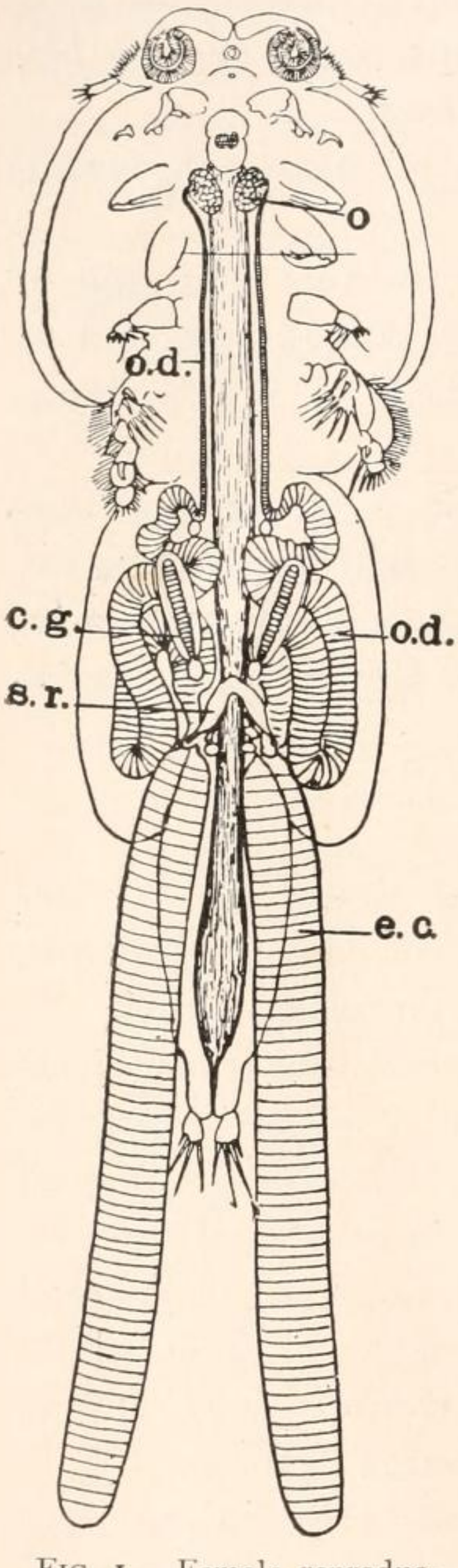

FIG. 1. Female reproductive organs of Caligus bonito. (Drawn by Emerton from Wilson's *Caligidæ*.) *c. g.*, cement gland; *e. c.*, external egg cases; *o*, ovary; *o. d.*, oviduct; *s. r.*, semen receptacle.

In the male the same general arrangement of reproductive organs exists, save that the seminal receptacles are absent and the distal ends of the vasa deferentia are enlarged and become the spermatophore receptacles. The cement glands secrete the material for the walls of the spermatophores.

In the dichelestid (FIG. 3) the genital segment is very much elongated and the oviducts are not convoluted. The ovaries have been carried backward until they lie in the anterior end of the genital

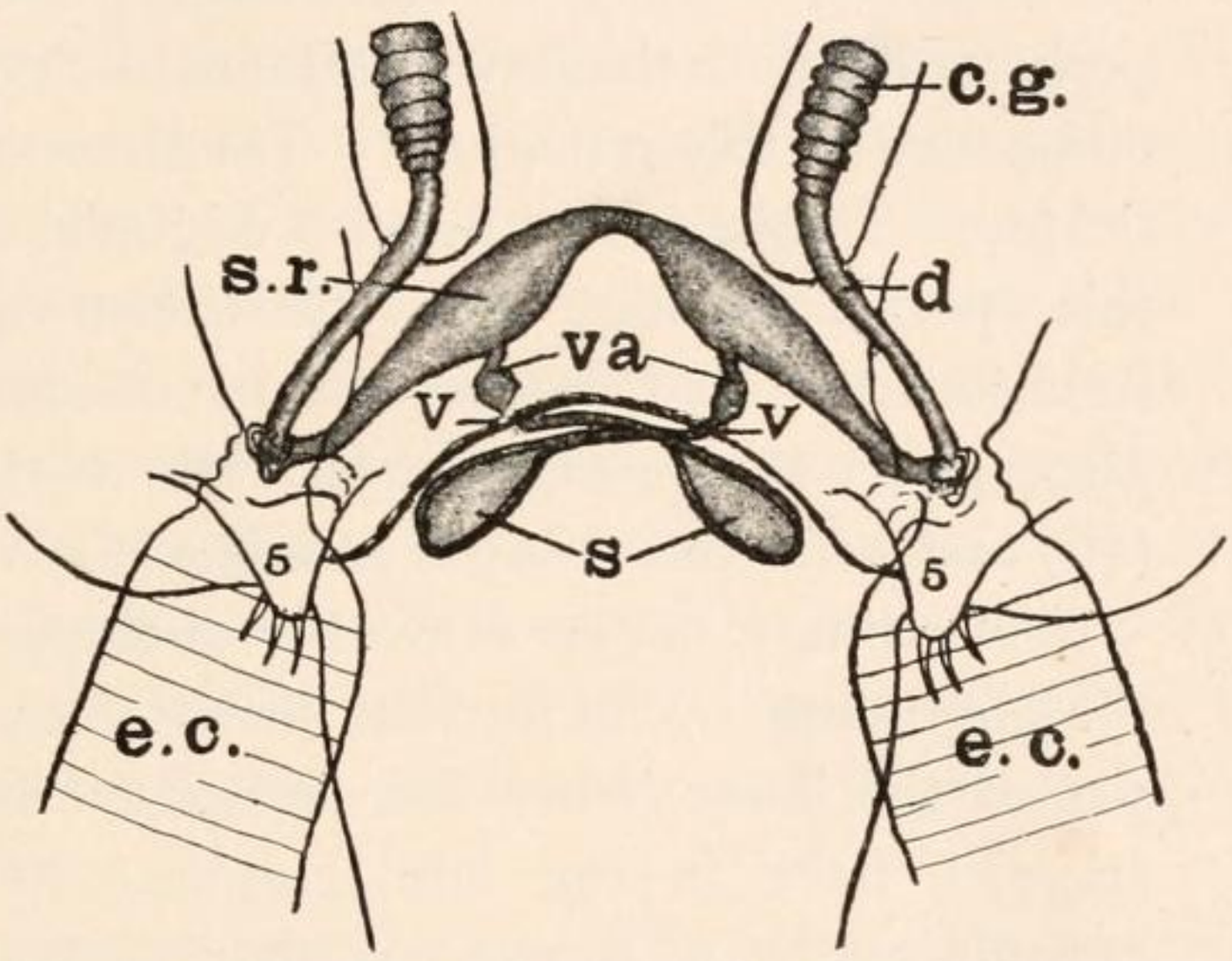

FIG. 2. Semen receptacles and vagina of a female *Lepeophtheirus*. (Partly after Claus.) *c. g.*, cement glands; *d.*, cement gland duct; *e. c.*, egg cases; *s.*, spermatophores; *s. r.*, spermaries; *v.*, vulva; *va.*, vagina; 5, fifth legs. (From Wilson's *Caligidæ*.)

segment and the oviducts pass forward to the anterior end of the thorax, then backward to the attachment of the egg strings. In other respects the description given for *Caligus* will hold for this species. The older brood of oöcytes occupies the posterior two-thirds of the oviduct. When one brood passes into the egg string the distal eggs of the remaining brood have nothing to press against and tend to round up and become distorted. The thickness of the oviduct at its posterior end varies somewhat, depending on the number of eggs produced in one brood, and the size of the eggs. When fewer, the eggs are thicker, and when more numerous, thinner. The egg string is more slender than the posterior portion of the oviduct, and the contained eggs are, therefore, thicker.

In *Læmargus* the egg strings are packed in loops under broad lamellar coverings.

IV. Oögenesis and Maturation.

1. *Oögenesis.*

A. The Dichelestid. — The oögonia (Fig. 1) are very small isodiametrical cells. The nucleus is spherical and contains chromatin granules in a peripheral linin reticulum. Minute nucleoli are also embedded in this reticulum. The cytoplasm stains deeply. I have found but few oögonial mitoses in this species although I have sectioned over a hundred females; the number of chromosomes is the same as in the primary germ cells of the embryo (16) and *twice* the reduced number (8).

The primary oöcyte is readily distinguished from the oögonium by its nucleus being about half the size of the latter (when first formed). The earliest stage I have is the late telophase, in which the chromosomes are drawn together as though at the pole of the spindle, yet the whole mass is surrounded by a nuclear membrane (Fig. 2). The nuclear membrane is therefore formed of the fused linin sheaths of the chromosomes (or from cytoplasm?). This mass is placed excentrically in the nucleus and the individual chromosomes are so pressed together or fused that only their ends sticking out can be distinguished separately, so that it resembles a "synapsis." The cytoplasm stains deeply. The oöcytes are arranged in single linear series as stated above and are pressed and flattened one against the other; the nuclei are close to the free surfaces of the cells, and that edge of an oöcyte containing the nucleus is thicker than the opposite edge. Soon the chromosomes swell and the chromatin becomes

FIG. 3. The *Dichelestid*, ventral view of ♀. The ovaries and oviducts are solid black. Portions of the egg strings are still altached.

dispersed as granules in a peripheral achromatic reticulum, as in the oögonia (Fig. 3). Minute nucleoli appear in this reticulum. The boundaries between the oöcytes become so faint as to be no longer everywhere perceptible. At the same time the cytoplasm begins to increase in volume and the diameter of the egg cord to increase. This process continues until the egg cord passes into the oviduct, and for some time thereafter. When the oöcytes have traversed about a fifth of the oviduct, the cell boundaries reappear and the nucleus migrates to very near the center, there forming an enlargement in the oöcyte (Fig. 4). The reticulum and the three to four nucleoli have grown considerably with the growth of the nucleus. A few yolk spherules and fewer oil globules appear and grow to considerable size. For some time growth of the oöcyte consists almost solely in the addition of yolk spherules and oil globules and this continues until the yolk almost obliterates the cytoplasm and closely surrounds the nucleus. I have seen these spherules and oil globules separated from the protoplasm with a high speed centrifuge (kindness of Dr. Lyon), the former are heavier and the latter lighter than the protoplasm.

B. Læmargus muricatus. — The oögenesis resembles that of the dichelestid. In the oögonial divisions there appear to be sixteen chromosomes, but these are set so close together that I cannot be sure by actual count.

In the growth period the reserve materials, yolk and oil globules, are laid down in ways that show them to be of quite different consistency. The oil globules (dissolved out in sections) appear as minute points that grow in size but always retain a spherical shape. The yolk substances, at least some of them, are laid down as thin discs. A disc receives new substance on its flat surfaces only, and the layers of substances are alternately chromatic and achromatic in staining qualities (when much destained after certain stains). Some of these piles of discs become spherical, others oblong by addition of more discs. The constituent layers do not mix but remain separate until dissoved in the segmenting egg or developing embryo. The oil globules are relatively few in number and large in size. The yolk bodies vary greatly in size, none of them reaching the size of the oil globules.

C. Pandarus sinuatus. — The oöcyte of *Pandarus sinuatus* differs from that of the dichelestid in that a single nucleolus is formed, about equal in bulk to that of the several nucleoli of the latter species together. The oöcyte of the former is much more compressed (thinner) than that of the latter.

2. *Maturation.*

A. Pandarus sinuatus. — At the end of the growth period the nucleus becomes irregular in outline which gives it a shrunken appearance. The nucleolus becomes vacuolated and the nuclear sap intensely staining. The location of chromatin cannot be made out very well, but in thin sections chromatic threads can be seen radiating from the nucleolus. Soon the nuclear sap fades enough to show that the chromatic threads have split (Fig. 6). The nuclear wall is dissolved. Sometimes the vacuoles in the nucleolus increase very much in size and fuse into one. In preparations of the entire oöcyte the chromatic thread can be seen to be divided into eight double chromosomes (Fig. 5). By shortening of these double-rod-shaped chromosomes, ring-shaped chromosomes are formed (Fig. 7). A transverse constriction transforms the diad into a tetrad, that is a ring constricted at four equidistant points, the two opposite constrictions, representing the divisions between the original rods, being deeper than the other two. The spindle when first formed is longer than the shortest diameter of the egg. It is similar to that found in free living copepods, having no polar rays. The dense protoplasm at each end of the spindle (Figs. 7 and 9) may possibly be derived from archoplasm of preceding divisions, and it shows a striking resemblance to the pole plates of the dividing nucleus in Protozoa. The spindle is at first parallel to the flat surfaces of the egg and rotates to an almost vertical position (Fig. 8). The first polar body is very small and is extruded between the egg and its neighbor at about the center of the flat surface that is posterior (in relation to the mother) (Figs. 9 and 10). The second polar spindle is smaller than the first. It is at first parallel to the flat surfaces of the egg and rotates nearly to a perpendicular position under the first polar body (Figs. 9 and 10). I have not seen the second polar body being cut off and

vacuolated and irregular in outline. The reticulum containing chromatin begins to break up and the nuclear sap stains intensely, probably due to the solution of chromatin. The nucleus becomes irregular in outline, probably due to pressure of yolk. From one to three spheres of protoplasm (Fig. 11) are found in some eggs near the wall of the oviduct, which appear to be abnormal structures. With the breaking up of the reticulum, the chromosomes are formed, but the manner in which this occurs is obscured by the intense staining of the nuclear sap. (This process shows better in *Pandarus*.) In order to see any structures in the nucleus it must be de-stained until it is very faint. The nuclear membrane disappears and the karyoplasm increases in volume and soon some of the chromosomes are distinctly double. The nucleoli disappear suddenly. The karyoplasm fades somewhat and becomes filled with alveoles, some of which are very large (Fig. 12). Each chromosome, of which there are eight (Fig. 13), is surrounded by a sphere of homogeneous protoplasm that stains slightly deeper than the surrounding cytoplasm. Each chromosome is a tetrad and often opens out into a ring constricted at four equidistant points. Two opposite constrictions are deeper than the other two and are to be regarded (from comparison with *Læmargus* and *Pandarus*) as the first divisions formed, and probably divisions between whole chromosomes. When seen on edge the tetrad usually appears dumb-bell shaped. There is some variation in the shape of the chromosomes in early prophases (Fig. 12) but in later stages they appear quite uniform in size and shape (Fig. 14). The spheres of homogeneous protoplasm surrounding the chromosomes fuse into one mass. A colorless area appears around each chromosome (Fig. 13) which makes it appear as though each chromosome was enclosed in a linin sac.

These linin "sacs" are each drawn out in the form of a spindle, and these spindles, lying parallel, form the first maturation spindle. Spindle fibers develop (Figs. 13 and 14) and some of them become attached to the chromosomes. The spindle is elongated, and at each pole the protoplasm stains more intensely resembling the pole plate in protozoön mitosis (Fig. 16). The paler protoplasm forms a sphere around each pole of the spindle,

(Fig. 16). From these spheres radiate strands of protoplasm simulating astral rays, but not so dense. The spindle is formed parallel to the flat surfaces of the egg and then begins to rotate to a perpendicular position, at the same time shortening (Fig. 17). In this state it remains until fertilization, and further than this I have not followed it. The behavior of the linin sheath of the chromosome is very peculiar, and it seems to be more conspicuous than in any other instance I know of. The spindle, in general appearance, resembles the first cleavage spindle of *Cyclops* (Häcker).

V. Spermatogenesis.

Læmargus muricatus. (*Preliminary Notice.*)

The spermatogonia are small cells with comparatively large spherical nuclei, and are arranged in single linear series. The nucleus contains chromatin granules in a peripherical linin reticulum. When preparing for mitosis these granules become arranged into looped rows, but I have not ascertained whether these rows form a continuous spireme or not. There is a single large nucleolus. In the last spermatogonic divisions there are 16 chromosomes (twice the number of the spermatocytic divisions). The preliminary spermatocytes are half the size of the spermatogonia when first formed, and are arranged in single linear series. Whether there is a single chain or cord of cells in the testes as in the ovaries, I cannot tell, owing to the many convolutions, but think there are at least several such chains or cords. The regular growth of these causes the testis to be divided into zones. During the growth period the cells grow to the size of the spermatogonia and cannot be distinguished from them save by their position in the testis (growth zone). When preparing for division the cells lose their linear arrangement; the chromatin forms eight double rods which lie close together (synapsis stage). Each double rod becomes ring shaped and the rings contract until only a small lumen is left. An additional pair of constrictions converts the ring into a tetrad. The division forms two secondary spermatocytes, each with eight diads, and another division immediately following forms four spermatids, each with eight chromosomes. The spermatids are grouped in fours. In

some of these groups, each of the cells becomes filled with an achromatic substance which presses the chromatin and protoplasm against the cell wall. This substance increases greatly in quantity and becomes more and more like yolk (called Austreibestoff by C. Heider, '79). In very rare cases in some very young spermatids I have seen a small achromatic sphere in the cytoplasm and have found a still smaller sphere in the cytoplasm of some primary spermatocytes. On the other hand the substance in question at first appears to lie within the nucleus and is closely surrounded by chromatin. A plausible explanation might be that the small globule seen in some very young spermatids moved up to and indented the nucleus, there growing until it became larger than the original nucleus yet I have been unable to find steps in such a process, in fact I believe the substance arises in the nucleus.* The cells containing these spheres nourish the spermatozoa, and may be called nutritive cells.

Going back to the spermatids, many groups degenerate, becoming much shrunken, while those that will form spermatozoa collect into larger groups, the cells of which begin to elongate radially. As the spermatids become longer they come to lie nearly parallel. The nucleus elongates into a spindle shaped deeply staining fiber, covered by a thin layer of achromatic substance. The remaining protoplasm fuses with that of adjacent spermatozoa and forms a mass in the center of the group. These groups sometimes lie with one end against a nutritive cell, and when the groups break up the spermatozoa collect around nutritive cells until the cytoplasm of the latter disintegrates and the chromatin collects into rounded masses that float about, leaving only a sphere of a yolk-like substance, the achromatic substance, which has now developed an affinity for plasma stains. As the elements pass down the vas deferens the nutritive spheres lie near its walls and on reaching the spermatophore the nutritive spheres (Austreibekörperchen) form a layer several spheres deep, and become pressed together into polyhedrons. Most of the chromatic spherules form a layer within the nutritive layer but some chromatic spherules caught between the nutritive spheres,

*Compare formation of "Glanzkörper" from the plasmosomes of Pelomyxa: Goldschmidt, Arch. f. Protistenkunde 5, p. 130.

become elongate curved bodies that remain for a long time. The spermatozoa fill the center of the vas deferens and lie parallel to one another. In the spermatophores they extend radially from the nutritive walls. In spermatophores that have been attached to the female a long time, the nutritive material has disappeared, leaving a mass resembling evacuated cell walls.

The same description of the spermatogenesis holds in general for *Pandarus sinuatus*, though there are many minor differences. As regards the behavior of these nutritive bodies I have observed one similar instance, in *Peripatus*, but in *Peripatus* the nutritive bodies are nuclei of degenerating cells. In these copepods the nutritive body appears to form in or in close relation to the nucleus, but too little is known both of the nutritive bodies in *Peripatus* and copepods to suppose them genetically related.

VI. Polarity of the Egg.

The fertilized egg and embryo in all cases in which the egg string extends straight behind the animal and contains a single linear series of eggs, is definitely oriented in relation to the mother. The animal pole of the egg is posterior and the first protoplasmic cell and resulting head end of the embryo is latero-ventral. The chief axis of the egg is manifested in the ovary in the flattening of the egg (being the shortest axis). In any stage in which the primary oöcyte is considerably thicker than the diameter of the nucleus, the latter lies nearer the animal pole. The head end of the embryo coincides with the region in which the first protoplasmic cell is formed, and this is probably determined by the point of entrance of the sperm. The seminal receptical opens into the oviduct by a small orifice which would lead sperm to the egg at or near the position of the future head of the embryo. Cases of rotation of the long axis of the embryo, which sometimes occur, might be due to the spermatozoön getting around the egg before entering.

The first polar body is extruded in a slightly eccentric position on that flat side of the egg directed toward the free end of the egg string. Thus the chief axis of the egg does not exactly coincide with the shortest axis, but is a little inclined toward the anterior end, yet not enough to cause the first polar body to lie

in the first cleavage furrow. This is probably due to the great inequality in the first cleavage. Furthermore, the blastopore does not close at a point diametrically opposite to the first polar body, but considerably posterior to such a point. From a study of the literature on gastrulation in crustacea, I am led to believe that the blastopore in the majority of cases in this group closes posterior to the vegetal pole and such a character would be accentuated by flattening of the egg. As the eggs occur in single linear series in an egg string surrounded on all sides by sea water, there is probably nothing in the surroundings that could determine the axes of the egg, and we should regard them as probably determined by the structure of the protoplasm.

VII. Summary.

1. In the maturation of the eggs of parasitic copepods the behavior of the chromosomes in regard to the question of *reduction* is very similar to the same process in the free living copepods, yet I differ from Haecker as to the reducing division, considering the first maturation division most probably to be the reducing division.

2. In the spermatogenesis only a small proportion of the spermatids become spermatozoa. Many spermatids degenerate, others become metamorphosed into peculiar nutritive cells. The protoplasm of the nutritive cells degenerates leaving only a sphere of deutoplasm "Austreibekörperchen," which C. Heider ('79) thought was a glandular secretion.

IX. Abbreviations.

n = Nucleolus.
p = First polar body.
s = Sphere of protoplasm, probably an abnormal structure
x = Darkly staining cell in yolk.
y = Yolk spherule.

Explanation of Plate I.

(*Figs. 1–14, The Dichelestid.*)

Fig. 1. An Oögonium.

Fig. 2. Three consecutive primary oöcytes in the synapsis stage. Optical section of the cord of ovarian cells.

Fig. 3. Early growth stage of same, partial disappearance of cell walls.

Fig. 4. Middle portion of oöcyte containing the nucleus, from longitudinal section of oviduct. Commencement of formation of yolk spherules (*y*). Two nucleoli are seen (*n*).

(*Figs. 5–10, Pandarus sinuatus Verrill.*)

Fig. 5. A little later stage. Nucleus viewed from the animal pole. Two of the eight double rods, or diads, are joined together end to end (to the right), so that it is difficult to distinguish them separately.

Fig. 6. The same in a section through the short axis containing six of the diads. Above at *n*, the nucleolus of another egg is represented to show a later stage in the disintegration of the nucleolus.

Fig. 7. The first maturation spindle.

Fig. 8. Later stage of same, in which it has rotated through almost a right angle and is pushing through the egg membrane to extrude the first polar body.

Fig. 9. Late metaphase of the second polar spindle. The first polar body is represented at *p*. (Constructed from two sections of the series.)

Fig. 10. The same stage from a surface preparation of the egg. The wall of the oviduct was torn off and the eggs allowed to separate in sea water (two hours). In separation the first polar body is pulled out into a stalked structure.

(*Figs. 11–17, The Dichelestid.*)

Fig. 11. Less magnified figure of prophase showing sphere of cytoplasm (*s*) near periphery of egg.

Fig. 12. A later stage. The nuclear sap has faded, it is vacuolated, and around each chromosome stains darker (*f*) than elsewhere. The two chromosomes that are stippled are out of focus. The chromosomes are ring-shaped tetrads.

Fig. 13. The chromosomes are each enclosed in a linin sac, and these sacs have begun to elongate.

Fig. 14. The same or a little later stage. The elongated linin sacs lie parallel, with spindle fibers developed between them. The incipient spindle is formed in dense (dark) protoplasm while this latter is surrounded by looser (paler) protoplasm.

Fig. 15. Equatorial plate, same stage as 14.

Fig. 16. The spindle has elongated perpendicular to the short axis of the egg. The dense protoplasm forms the two poles of the spindle. While the loose protoplasm forms astrosphere-like structures enclosing the poles of the spindle.

Fig. 17. The spindle is half rotated toward the short axis of the egg and has considerably shortened in doing so.

Fig. 18. *Læmargus muricatus* Kröyer. Central portion of the egg containing the first maturation spindle. Metaphase. The spindle fibers do not show in this preparation. Three of the eight chromosomes are in focus, one being seen from the end and appearing smaller than the others.

1

2

3

4 n y

n

5

7

8

9 p

10

11 s

12 f

13

14

16

17

15

18