STUDIES ON GERM CELLS

I. THE HISTORY OF THE GERM CELLS IN INSECTS WITH SPECIAL REFERENCE TO THE KEIMBAHN-DETERMINANTS.

II. THE ORIGIN AND SIGNIFICANCE OF THE KEIMBAHN-DETERMINANTS IN ANIMALS.

ROBERT W. HEGNER

From the Zoological Laboratory of the University of Michigan, Ann Arbor, Michigan, U.S.A.[1]

SEVENTY-FOUR FIGURES

CONTENTS

[1] Contributions from the Zoological Laboratory of the University of Michigan, and the Marine Biological Laboratory, Woods Hole, Mass.

GENERAL INTRODUCTION

Studies of the history of the germ cells in animals have proven that in many cases these cells originate in a perfectly definite way and at such an early embryonic period as to represent the first cellular differentiation that takes place in ontogeny. In certain animals such a determinate segregation of the germ cells cannot be established with the data available without certain assumptions to which objections may be made. Limiting ourselves, therefore, to the instances where the germ cell cycle is completely known, it is possible to divide the history of the germ cells from one generation to the next into the following periods:

1. Primary cellular differentiation, i.e., the formation of one or more primordial germ cells during the segmentation of the egg;

2. A short period during which in some cases the primordial germ cells increase slightly in numbers by mitosis;

3. A long period of *rest* characterized by cessation of cell division, either active or passive change of position, separation of the germ cells into two groups which become the definitive germ glands, accompanied by the general growth of the embryo until the larval stage is almost attained;

4. Multiplication by mitosis of the primitive oogonia or spermatogonia to form a definite number (Miastor and perhaps

others) or indefinite number (so far as we know) of oogonia or spermatogonia;

5. In some cases the differentiation of oogonia into nurse cells and ultimate oogonia, and the spermatogonia into Sertoli cells and ultimate spermatogonia;

6. The growth of the ultimate oogonia and spermatogonia to form primary oocytes and primary spermatocytes;

7. Maturation;

8. Fertilization (if not parthenogenetic).

This list of periods differs from the series usually recognized in that it starts with the *beginning* of the germ cell cycle instead of at a comparatively late stage, i.e., with oogonia and spermatogonia. Certain of these periods, especially those of maturation and fertilization, have been emphasized by investigators much more than others. Many of the fundamental problems of heredity and development are, however, concerned with the events which take place during the less known stages.

For a number of years the writer has been particularly interested in the segregation of germ cells during embryonic development, and has studied especially certain visible substances which are present in the egg before cleavage begins, and later become part of the material contained in the primordial germ cells. In the eggs of certain Chrysomelid beetles this substance was termed the 'pole-disc' (fig. 7, A, *g.c.d.*, p. 403), and the granules of which the pole-disc is composed were called 'germ-cell determinants' because they enable us to determine which cells will become germ cells. Since this term is likely to be misinterpreted, the granules of the pole-disc and other similar substances that have been found in the eggs of animals are, in this paper, called 'Keimbahn-determinants,' since they furnish the means of recognizing the germ-cell material in the undivided egg or in cleavage stages, and thus make it possible for us to determine the 'Keimbahn' from one generation to another.

The following events may be listed in the history of the Keimbahn-determinants:

1. Localization of the Keimbahn-determinants in the oocyte or mature egg;

2. Association of one or more cleavage nuclei with part or all of the Keimbahn-determinants to form one or more primordial germ cells;

3. The apparently equal distribution of the Keimbahn-determinants between the daughter germ cells at each mitotic division (Sagitta possibly excepted);

4. The disappearance of the Keimbahn-determinants in the oogonia and spermatogonia;

5. The reappearance of the Keimbahn-determinants in the oocyte or mature egg.

In the general history of the germ cells there may be two periods of differentiation:

1. The segregation of the primordial germ cells during cleavage stages.

2. The differentiation of nurse cells and ultimate oogonia, or Sertoli cells and ultimate spermatogonia in the germ glands. This second differentiation does not occur in Miastor and certain other animals, and even when it does occur it is doubtful whether the nurse cells and Sertoli cells should be considered as true somatic cells or simply as abortive oogonia or spermatogonia which have been unsuccessful in the struggle for development. A casual examination is liable to delude one into thinking that the differentiations mentioned above are widely separated in the germ cell cycle, but a little closer study shows that they really occur during a relatively short period in the entire history. For example, in certain insects, where nurse cells arise from oogonia, this process takes place just before the growth period during which the Keimbahn-determinants became localized in preparation for the primary cellular differentiation.

It is evident from the general outline as stated above that the most important period in the germ cell cycle is that extending from the formation of the ultimate oogonia and spermatogonia to the complete segregation of the primordial germ cells. Our knowledge of events during the latter part of this period is comparatively great, whereas we know practically nothing about the early stages involving the genesis of the Keimbahn-determinants and their localization in the oocyte or mature egg.

Embryological investigation has gradually progressed from the study of germ layers back to the study of the segmentation of the egg, and from this to the organization of the ovum, and from here to the genesis and localization of organ forming substances in the oocyte.

In the following pages the results of some investigations made by the writer are described, and a discussion of the results obtained by other investigators is given, in an attempt to determine the origin, nature, and significance of the Keimbahn-determinants.

I. THE HISTORY OF THE GERM CELLS IN INSECTS WITH SPECIAL REFERENCE TO THE KEIMBAHN-DETERMINANTS

1. INTRODUCTION

The Keimbahn in animals was first described by Metschnikoff ('65, '66) in the paedogenetic larvae of the fly, Miastor. Since that time various investigators have been able to trace the germ cells in many other species of insects, belonging to several different orders, from early cleavage stages to the definitive germ glands, and have discovered that a complete Keimbahn can also be demonstrated in species belonging to other classes and phyla, notably the Crustacea, the Nematoda, and the Chaetognatha. The writer ('09) has published an account of our knowledge of the origin and early development of the germ cells in insects up to the year 1908, but no complete account of the Keimbahn in other groups of animals has ever appeared.

The data regarding this phase of the germ cell cycle are widely scattered in the literature; frequently buried in treatises on general embryology, and less often contained in contributions devoted to this subject alone. The accounts found in current reference books and text books are for the most part obsolete or inaccurate. In the following account statements, with figures, of the more important discoveries of other investigators have been included in order to allow a general consideration of our entire knowledge regarding the Keimbahn-determinants.

2. DIPTERA

A. Historical account

The segregation of the germ cells in the early embryonic stages of animal development was first discovered in certain Dipterous insects. In 1862 Robin described, in the nearly transparent eggs of Tipulides culiciformes the appearance of four to eight buds at one pole just previous to the formation of the blastoderm. He called these buds 'globules polaires' and thought that they were protruded at the anterior end. Weismann ('63) likewise discovered bud-like protrusions at a corresponding stage in the development of the egg of Chironomus nigroviridis and Musca vomitoria. He corrected Robin regarding their orientation by proving that they arise at the posterior end and not at the anterior end of the egg. Because of their position he applied to them the term 'Polzellen,' a term that has persisted until the present time. Weismann was unable to follow the history of the pole cells and so did not succeed in determining their true significance.

Metschnikoff ('65, '66) and Leuckart ('65) were the first to announce that the pole cells are really primordial germ cells, and the first to trace them from their initial appearance until they entered into the constitution of the definitive germ glands. Their results, obtained from the study of the eggs of Miastor and Simula, were confirmed by Grimm ('70) and Balbiani ('82, '85) in Chironomus.

Pole cells have also been described among the Diptera, in Musca by Kowalevsky ('86), Voeltzkow ('89), and Escherich ('00); in Calliphora by Graber ('89), and Noack ('01); in Chironomus by Ritter ('90), and Hasper ('11); in Lucilia by Escherich ('00); and in Miastor by Kahle ('08) and Hegner ('12).

Among insects belonging to other orders typical pole cells have been found only in parasitic Hymenoptera (Silvestri, '08), and in Chrysomelid beetles (Lecaillon, '98; Hegner, '08, '09; Wieman, '10a) although germ cells have been described at an early stage in the development of the butterflies, Euvanessa antiopa (Woodworth, '89) and Endromis versicolora (Schwan-

gart, '05); in the aphids (Metschnikoff, '66; Balbiani, '66–'72; Witlaczil, '84; Will, '88); in the honey-bee (Petrunkewitsch, '01–'03); and in Forficula auricularia (Heymons, '95).

Since the original work contained in this paper was undertaken in order to determine the origin and significance of certain pecular inclusions in the primordial germ cells of various animals the writer has been particularly interested in any extra-nuclear substances visibly different from the general cytoplasm. One of the principal characteristics used for the purpose of identifying germ cells in the embryos of animals is the presence within their cytoplasm of yolk substance. Many of the authors cited above noticed yolk globules in the pole-cells. For example, in Chironomus, Weismann ('63) states that each pole-cell possesses "ein oder zwei Dotterkörnchen;" and Metschnikoff ('66) described dark yolk masses in the pole-cells of Simula and Miastor. These examples indicate the general presence of yolk-like substances in the primordial germ cells of the Diptera, but it remained for later more detailed investigations with finer methods to determine the origin and fate of these cytoplasmic inclusions. Five papers have appeared which contain information bearing on these problems; (1) Ritter ('90) on Chironomus, (2) Noack ('01) on Calliphora, (3) Kahle ('08) on Miastor, (4) Hasper ('11) on Chironomus, and (5) Hegner ('12) on Miastor.

Chironomus. As stated above, the pole-cells of Chironomus, were first described by Weismann ('63) who, however, did not recognize them as germ-cells. Grimm ('70) succeeded in tracing the pole-cells in Chironomus until they became surrounded by other cells, forming two germ-glands, thus confirming Metschnikoff's ('66) account in Miastor. Chironomus was later studied again by Weismann ('82), by Balbiani ('82, '85), by Jaworowski ('82), by Ritter ('90) and by Hasper ('11). Only the work of the last two needs to be considered here since that of the other writers mentioned was not carried on with modern methods nor in such great detail.

Ritter ('90) used the section method and was thus able to study the structure of the germ cells more carefully and to trace them more accurately during embryonic development. He found

that the first pole-cell appeared at the posterior end of the egg when there were a large number of nuclei scattered about in the yolk. A second pole-cell was protruded close behind the first. Each carried out of the egg part of a flat mass of granules which, in section, formed a wreath around the nucleus. The two original pole-cells increased by division to four and then to eight. Two divisions of each pole-cell nucleus now occurred, resulting in eight quadrinucleated cells; these seemed to move of their own accord through the blastoderm which closed after them. They now lay at the posterior end of the germ-band from whence they were possibly moved anteriorly by the growing forward of the entomesoderm. The mass of pole-cells finally divided into two groups which occupied a position on either side of and dorsal to the hind-intestine; there they remained until after the larva hatched, when they became the definitive sex-organs.

Ritter was the first to determine the fact that the 'yolk masses' contained in the pole-cells of Chironomus are derived from a definite structure and are not chance acquisitions from the yolk granules in the egg. After giving a brief sketch of the polar bodies and male and female pronuclei, he says:

> In dem nächsten Stadium sind in dem Dotter keine Zellen mehr zu sehen; dagegen tritt an demjenigen Pol, an welchem später die Polzellen erscheinen, also an dem hinteren, ein eigenthümlicher wulstartiger Körper auf, welcher durch das Hämatoxylin sehr dunkel gefärbt wird. Er erscheint auf mehreren Schnitten und stellt eine etwas nach oben vorgewölbte Platte dar, welche vielfach runde Fortsätze zeigt und aus feinkörnigem Protoplasma besteht. Er bleibt bis zum Austritt der Polzellen an derselben Stelle.

Ritter then gives a fragmentary account of the early divisions of the cleavage nucleus, at the end of which, the two first pole-cells appear each containing a "grossen Kern und um denselben herum kranzförmig einen Theil des obengenannten dunklen wulstförmigen Körpers." This darkly staining body he called 'Keimwulst.'

That the 'Keimwulst' played an important rôle in the segregation of the germ cells was quite obvious to Ritter, but he was in error when he stated that this body contained the first cleavage

nucleus and "dass nach der Theilung des Furchungskernes die Theilprodukte theils in dem dunklen wulstförmigen Körper verbleiben, theils aus demselben herausrücken."

In 1911 Chironomus was again studied by Hasper, who published a complete description of the Keimbahn in Chironomus confinis and C. riparius. At the posterior pole of the eggs at the time of deposition is a disc-shaped mass of granules (fig. 1 A, kbpl) called by Hasper the 'Keimbahnplasma,' which is identical with the Keimwulst' of Ritter. Hasper characterizes this 'Keimbahnplasma'

> . . . . als dichte, scharf konturierte, wurst- oder flaschenförmige, gerundete oder auch in 2 Klumpen getrennte, mit wenigen Vacuolen versehene Masse präsentiert, die am hintern, im Ovarium nach hinten gekehrten Ende des Eies etwas unter der Oberfläche leigt, in schaumigem Protoplasma eingebettet, zuweilen aber auch noch ganz von Dotter umgeben. Es ist diese wichtige Differenzierung des Ooplasmas nichts anderes als jene spezifische Substanz, die bei der Determinierung des ersten von der Entwicklung dargebotenen embryonalen Materials eine entscheidende Rolle spielt und die daher im Folgenden als Keimbahnplasma noch mehrfach Erwähnung finden wird (pp. 549–550).

Ritter ('90) advanced the idea that the cleavage nucleus of Chironomus divides within the 'Keimwulst' and that here the first cleavage division occurs, one daughter nucleus remaining in the 'Keimwulst' and becoming the center of the primordial germ-cell, the other giving rise to somatic nuclei. This is probably the basis for Weismann's ('04) statement regarding his conception of the germ-plasm that

> If we could assume that the ovum, just beginning to develop, divides at its first cleavage into two cells, one of which gives rise to the whole body (soma) and the other only to the germ-cells lying in this body, the matter would be theoretically simple. . . . . As yet, however, only one group of animals is known to behave demonstrably in this manner, the Diptera among insects . . . .

There is, however, nothing in the literature to warrant the above statement, since Ritter's hypothesis has been disproved by Hasper.

The primordial germ-cell is really recognizable as such in Chironomus at the four-cell stage (fig. 1, B, *p.g.c.*). One of the

first four cleavage nuclei migrates to the posterior end, and, separating from the rest of the egg together with the 'Keimbahnplasma' and the cytoplasm in which this substance lies, forms the 'Urgeschlechtszelle.' The primordial germ cell is undergoing division by mitosis at the time when it is protruded from the egg and during this process the 'Keimbahnplasma' is apparently equally divided between the daughter cells.

Während die ersten Teilungen rasch aufeinander folgen, kommt die letzte gar nicht mehr sur Vollendung, d.h. sie erstreckt sich nur auf die Kerne, so dass schliesslich 8 zweikernige Genitalzellen im hintern Polraum liegen. Und damit ist die Entwicklung der Keimbahn für lange Zeit überhaupt abgeschlossen; denn während der nun folgenden Embryonalperiode ist sie durch ein durchaus passives Verhalten ausgezeichnet (p. 553).

One of these binucleated germ-cells is shown in figure 1, C.

It is unnecessary to trace the history of these primordial germ-cells (pole-cells) since it has been shown repeatedly that they give rise to the oogonia or spermatogonia in the definitive germ-glands. Portions of the 'Keimbahnplasm' persist at least until the larva hatches (fig. 1, D). The origin and nature of the 'Keimbahnplasm' was not discovered by Hasper but the name applied to it and the fact that the author adopts my term 'germ-cell determinants' (Keimzelldeterminanten) in discussing it, indicate that he considers it of fundamental importance in the segregation of the germ-cells.

The possibility of determining the origin of the 'Keimbahnplasma' of Chironomus led the writer to study the oogonia in the terminal chamber and the various stages in their growth up to the time of deposition. Larvae were collected and allowed to develop in the laboratory and the ovaries were dissected out of the adults which were obtained from these larvae. However, the material procured has been found lacking in both the earlier stages of the development of the oocytes and the late stages in the formation of the ovum. It has been considered best, therefore, to reserve a study of this material until a complete series can be secured.

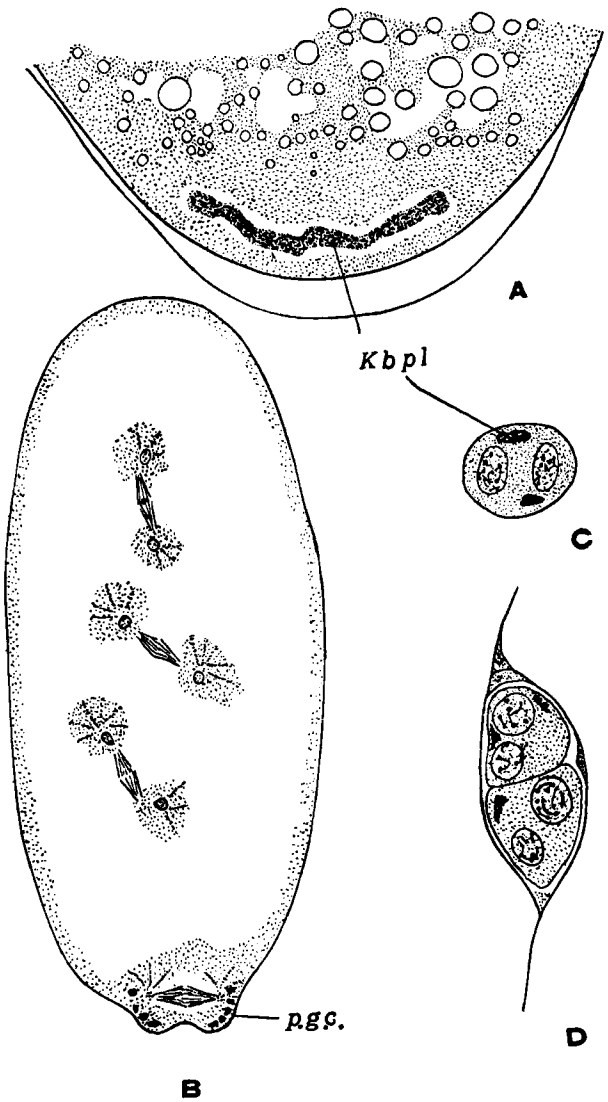


Fig. 1 Chironomus (redrawn from Hasper, '11). A, longitudinal section through the posterior end of a freshly laid egg. B, longitudinal section through egg during division of first four cleavage nuclei; at posterior end primordial germ cell is just being formed. C, one of primordial germ cells containing two nuclei and remains of 'Keimbahnplasma.' D, germ gland of the larva in which remains of 'Keimbahnplasma' still appear. *Kbpl*, 'Keimbahnplasma'; *p.g.c*, primordial germ cell.

Calliphora. Noack ('01) found a dark granular layer, which he called the 'Dotterplatte' (fig. 2, *Dpl*) at the posterior end of the egg of Calliphora erythrocephala similar to the 'Keimwulst' discovered by Ritter in Chironomus. Each pole-cell took part of this layer of granules with it as it passed through the 'Keimhautblastem.' Concerning this process Noack says, "Im nächsten Stadium haben die Kerne eine runde Gestalt angenommen, die Platte hat sich in so viel Theile getrennt, als Kerne in ihren

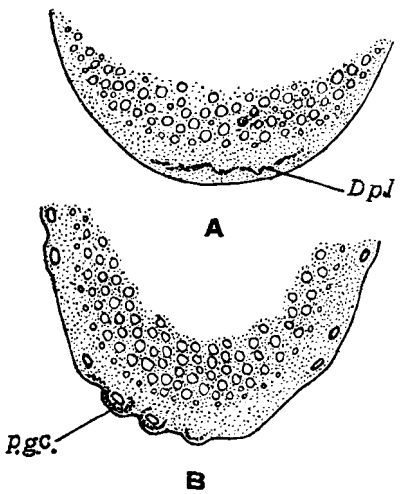


Fig. 2 Calliphora (redrawn from Noack, '01). A, longitudinal section through posterior end of freshly laid egg showing 'Dotterplatte' (*Dpl*). B, longitudinal section through posterior end of egg at time of blastoderm formation showing protrusion of primordial germ cells (*p.g.c*).

Bereich eingetreten sind, und bildet nun um jeden dieser Kerne einen peripher gelegenen feinkörnigen Halbmond. Hiermit ist die erste Zelldifferenzirung eingeleitet." Those cells which now contain granules from the "Dotterplatte' are recognized as pole-cells, while the remaining cells which have reached the periphery of the egg constitute the blastoderm. The 'Halbmond' of granules which surrounds the nucleus of each pole-cell now

. . . . schliesst sich allmählich zu einem Kreise, welcher um so mehr auffällt, weil die von ihm eingeschlossene und den Kern einbet-

tende Protoplasmamasse fast farblos erscheint (fig. 2, B, *p.g.c*). Bei der Fortentwicklung der Polzellen schwindet allmählich die scharfe Grenze zwischen Zellprotoplasma und Polplatte. Letztere löst sich auf und es entsteht eine gleichmässige Pigmentirung, welche den Polzellen noch auf lange Zeit ein ganz charakteristisches Aussehen verleiht.

Concerning the nature of this 'Dotterplatte' Noack says:

Dass die Platte am hinteren Pole des Musciden-Eies sich aus Dotterelementen zusammensetzt. Sie scheint den Zweck zu haben, das Wachsthum am hinteren Pol zu beschleunigen, ferner durch Eintritt in die Polzellen es diesen zu ermöglichen, sich auch weiterhin lebhaft zu vermehren, obgleich sie vom Dotter her keine Nahrung mehr erhalten. Schliesslich verursacht sie die charakteristische Pigmentirung dieser Zellen.

B. The Keimbahn in Miastor americana Felt

The paedogenetic flies of the genus Miastor furnish especially favorable material for the study of the germ-cell cycle. The process of paedogenesis was discovered by Wagner, and a short statement was published by him in 1862; three years later a more detailed account appeared by Wagner ('65) whose extraordinary discovery was confirmed by Meinert ('64), Pagenstecher ('64) and Ganin ('65); but of the early authors Leuckart ('65) and Metschinkoff ('65, '66) have given the best descriptions of the developmental stages. In 1870 Grimm announced the occurrence of paedogenesis in a species of Chironomus. From that date until 1907 nothing new concerning this peculiar method of reproduction in insects was learned. Zavrel ('07) then reported paedogenesis in the genus Tanytarsus, and this has been confirmed for T. dissimilis by Johannsen ('10). In the meantime Kahle ('08) published perhaps the best account that has ever appeared on the 'Keimbahn' of any animal, using Miastor metraloas for this purpose. He was able to trace the germ cells from one generation to the next with remarkable clearness. Many of Kahle's results have been confirmed (Hegner, '12) for Miastor americana Felt and reference will be made to them more in detail in the following pages. Finally a short study of the life history of Miastor metraloas has been made by G. W. Müller ('12).

Metschnikoff's ('66) studies on Miastor indicated that the germ-cells of this fly are set aside very early in embryonic development, and led me in 1907 to attempt to obtain material of this genus. I was informed at that time by Prof. Samuel W. Williston that no paedogenetic Diptera were known to occur in this country. On October 5, 1910, however, Dr. E. P. Felt discovered great numbers of the larvae of Miastor americana Felt under the partially decayed inner bark and in the sapwood of a chestnut rail near Highland, New York, and kindly sent me an abundant supply of material.

Habitat and life history. Dr. Felt found the larvae of Miastor americana living

> . . . . in the moist, partly rotten inner bark and punky sapwood which has not been invaded to any considerable extent by other Dipterous larvae or Coleopterous borers. They exhibit a tendency to occur in segregated masses, frequently between loose flakes of bark or in rather broad crevices. These colonies contain in autumn old empty skins of mother larvae; a number of yellowish mother larvae with approximately five to fifteen young within; very numerous, small yellowish larvae showing no trace of embryos; a number of white, various sized larvae, frequently white, sometimes semi-transparent; and a few quiescent white larvae containing young embryos. . . . . The mouth parts of the larvae, though the anterior portion of the head is strongly chitinized, appear to be comparatively weak. . . . . The alimentary canal contains little that can be discerned with the aid of a compound microscope, and we are inclined to believe that a considerable portion of their nourishment is absorbed by osmosis after escaping from the mother larva, as well as before. It would appear as though the several types of larvae occurring in a colony are possibly only modifications due to the relative amount of nourishment obtained by the individual.
>
> Normally, reproduction by paedogenesis occurs throughout the warm months of the year and even into late fall, and commences in early spring, the cold weather of winter simply causing a suspension of activities. . . . . The adults of Miastor and Oligarces occur in midsummer, a season when midges of most of these forms are probably abroad (Felt, '11).

Methods. A number of fixing and staining methods have been employed in an endeavor to determine the origin and history of the 'polares Plasma' which plays such an important rôle in the differentiation of the germ cells of Miastor. Perhaps the easiest

and most successful methods for studying the Keimbahn are fixing in Gilson and staining in Mayer's acid hemalum followed by Bordeaux red. Entire larvae may be fixed, sectioned and stained in this way. To bring out cytoplasmic details other methods were resorted to. The anterior and posterior ends of larvae were cut off and the middle part of the body containing the ovaries, and eggs and young were fixed in Meves' fluid. In other cases the eggs and young were dissected out and fixed in Meves' fluid. Still other larvae were fixed in Carnoy's solution. The best fixation was obtained with Gilson's Mercuro-nitric fluid. Besides acid hemalum the following stains were used: Heidenhain's iron hematoxylin followed by Bordeaux red or eosin, the iron hematoxylin method used by Rubaschkin ('12) in his studies of the mitochondria in the embryonic cells of the guinea pig, safranin followed by light green, Altmann's acid fuchsin differentiated in picric acid, Benda's method for the study of mitochondria, and the Erhlich-Biondi triple stain.

The morphological continuity of the germ cells. The ovaries of Miastor lie on either side of the body in the tenth or eleventh segment. They appear yellowish green in living white larvae, but are whitish transparent in the young yellowish larvae. Each ovary, when in the stage shown in figure 27, is surrounded by a thin cellular envelope (*en*) and contains typically thirty-two oocytes (*ooc.n*) each with an accompanying group of mesodermal nurse cells (*n.c.*), which are enclosed with it in the follicular epithelium (*f.ep*). The oocytes grow at the expense of the nurse cells, separate from the ovary, and are distributed throughout the body of the larva. Usually from five to seventeen embryos develop in one larva, but sometimes only one or two larvae are produced by a single mother-larva.

The nucleus of the oocyte (fig. 27, *ooc.n*) is large and clear, and the chromatin within it forms slender threads, rather evenly scattered about in the nuclear sap.

Figure 28 is a longitudinal section of an oocyte just before the maturation division. The germinal vesicle (*g.v.*) is large and clear and contains a great number of small scattered chromatin granules. It lies near one side of the oocyte in preparation for

the formation of the first polar spindle. The nurse chamber (*n.c.*) contains a syncytium with about eighteen large nuclei, each of which possesses a large, centrally placed nucleolus surrounded by irregular chromatin granules. At the posterior pole of the egg is an accumulation of material (*pPl*) which stains deeply, and, as will be shown later, is intimately associated with the origin of the primordial germ-cell. This mass of material has been termed by Kahle 'polares Plasma'—a term adopted in the following description. A discussion of the origin and significance of the 'polares Plasma' will be reserved until later (p. 396).

There is nothing unusual in the process of maturation. The egg nucleus preparing for division is shown in figure 29, *m.s.* One polar body is formed in Miastor metraloas and the number of chromosomes could not be determined by Kahle but is probably from twenty to twenty-four. The number of chromosomes appears to be similar in M. americana but an accurate count could not be made. The first polar body divides by mitosis (fig. 30, *p.b.*) and the pronucleus (*f.n.*) moves over into the mass of cytoplasm (*c*), which is apparently elaborated by the nuclei in the nurse chamber, and becomes the cleavage nucleus. Here in this mass of cytoplasm the first cleavage takes place resulting in two apparently similar daughter nuclei (fig. 31, *c.n.*).

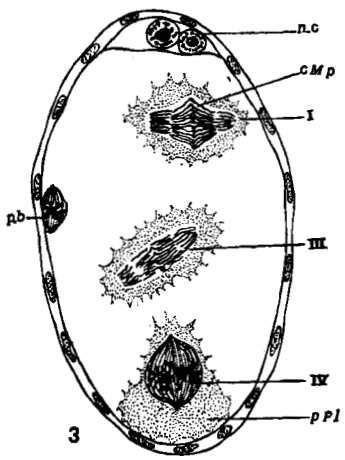
Reference must be made to Kahle's figures and description for most of the events of early cleavage. The two nuclei of the two-cell stage divide by mitosis and the four daughter nuclei are apparently similar. They have been numbered I, II, III, IV, beginning at the anterior pole (fig. 3). The succeeding nuclear division is important, since the advance from the four-cell to the eight-cell stage witnesses the origin of the primordial germ-cell as well as a casting out (diminution) of chromatin by nuclei I, II, and III. In figure 3 nuclei I, III and IV are shown in mitosis. Spindle IV is undergoing the ordinary process of mitosis, but spindles I and III (and also spindle II, which is not shown in the figure) are long and slender and a large portion of their chromatin (fig. 3, *cMp*) does not take part in the formation of the daughter nuclei but remains behind in the cytoplasm as a 'Chromosomenmittelplatte.' These masses of cast-out chromatin are never

found in stages earlier than the four-cell stage, but are present in many of the later stages (figs. 32, 33, 34, 35, 36, *cR*) and are called by Kahle 'Chromatinreste.' One daughter nucleus resulting from the division of nucleus IV (fig. 3) becomes imbedded in the 'polares Plasma' and, with this substance, is cut off from the rest of the egg as the primordial germ-cell (fig. 32, *p.g.c.*). The other daughter nucleus of spindle IV remains in the egg. These two nuclei are the only ones at this stage which contain a complete amount of chromatin.

During the next stage (VIII-XV) the daughter nucleus of cleavage cell IV, which remains in the egg, undergoes a diminution process whereby it loses part of its chromatin, and the other six nuclei within the egg pass through a second diminution process during which a second 'Chromosomenmittelplatte' is formed (figs. 32 and 4, *cMp*). At the fifteen-cell stage, therefore, one nucleus (that of the primordial germ-cell, fig. 32, *p.g.c.*) contains the full amount of chromatin; whereas all of the others (somatic nuclei) have lost a large portion of their chromatin. After the second diminution process, according to Kahle, the somatic nuclei possess only half the number of chromosomes present in the germ cells, that is "der Diminutionsprocess und der Reductionsprocess in derselben Karyokinese verenigt sind." My material did not contain enough of the early cleavage stages to enable me to confirm in detail Kahle's investigations, but one egg contained well marked mitotic figures which represent stages in the second diminution process (fig. 32) and a large number of sections were obtained which contained chromatin masses ('Chromatinreste,' figs. 32, 33, 34, 35, 36, *cR*). The details of the second diminution process are shown in figure 4.

The history of the germ-cells, from the time of the formation of the primordial germ-cell to the production of the sixty-four oocytes contained in the two ovaries, thirty-two in each, will now be described briefly.

The somatic nuclei divide rapidly, forming the blastoderm as shown in figures 33 and 34. Chromatin masses (*cR.*) representing chromatin cast out during the diminution processes are present in these early stages. The primordial germ-cell (fig. 32,



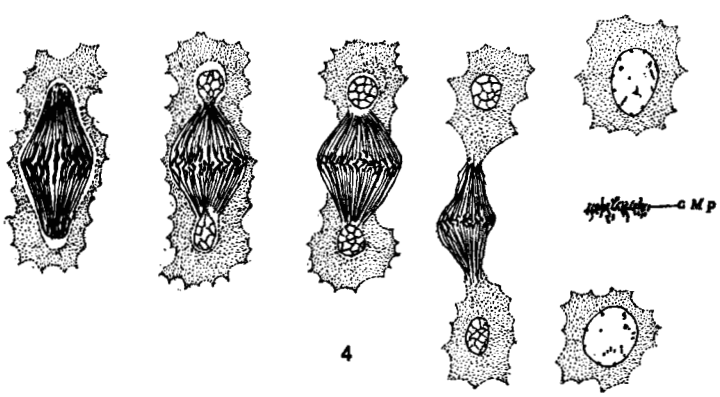


Fig. 3 Miastor metraloas (redrawn from Kahle, '08). A section through an egg showing a dividing polar body (*p.b*); cleavage nuclei I and III undergoing the chromatin-diminution process; and cleavage nucleus IV dividing normally. The daughter nucleus of the latter which enters the 'polares Plasma' (*p. Pl*) becomes the nucleus of the primordial germ cell.

Fig. 4 Miastor metraloas (redrawn from Kahle, '08). Five stages in the second chromatin-diminution process. *cMp*, 'Chromosomenmittelplatte.'

p.g.c.) divides by mitosis resulting in two oogonia (fig. 33, oog_1) which lie at the posterior end of the egg. Each of these divides again about the time when the blastoderm cells are cut off by cell walls. Four oogonia of the second order (fig. 34, oog_2) are formed in this way. A third division results in the production of eight oogonia of the third order (fig. 35, oog_3). The germ-band then forms and segments, and the eight oogonia are passively carried around by the growth of the tail fold as shown in figure 36, oog_3). The embryo then grows broader and shorter until it entirely surrounds the yolk and the end of the tail fold coincides with the posterior end of the egg. During these developmental stages the oogonia remain undivided, but become separated into two groups of four each, which lie in two rows, one on either side of the embryo in the region of the eleventh segment (fig. 37, oog_3). Soon each row of four oogonia becomes enclosed by mesoderm cells, forming an ovary. The germ-glands then become almost spherical and soon the oogonia undergo a division by mitosis, thus forming eight oogonia of the fourth order in each germ-gland (fig. 38, oog_4). These divide again by mitosis (fig. 38, *a*) producing sixteen oogonia of the fifth order (fig. 39, oog_5) in each germ-gland. The final division of the oogonia takes place shortly before the larva hatches.

Typically, there are then thirty-two oogonia of the sixth order in each germ-gland, but in some cases certain of the oogonia of the fifth order are prevented from dividing. All of the oogonia do not produce embryos, since, as a rule, only from five to seventeen larvae are produced by a single mother-larva. The oogonia of the sixth order grow into oocytes (fig. 40, *ooc.*); each of these, together with a syncytium containing about twenty-four nurse-cells of mesodermal origin (fig. 27, *n.c.*), becomes surrounded by follicular epithelium (fig. 27, *f.ep.*) also of mesoderm cells. During this process the nucleolus of the germ-cells disappears and the chromatin forms long slender threads (fig. 27, *ooc.n*). This completes the history of the germ-cells from one generation to the next. The accompanying diagram (fig. 5) shows graphically the germ-cell cycle in this animal.

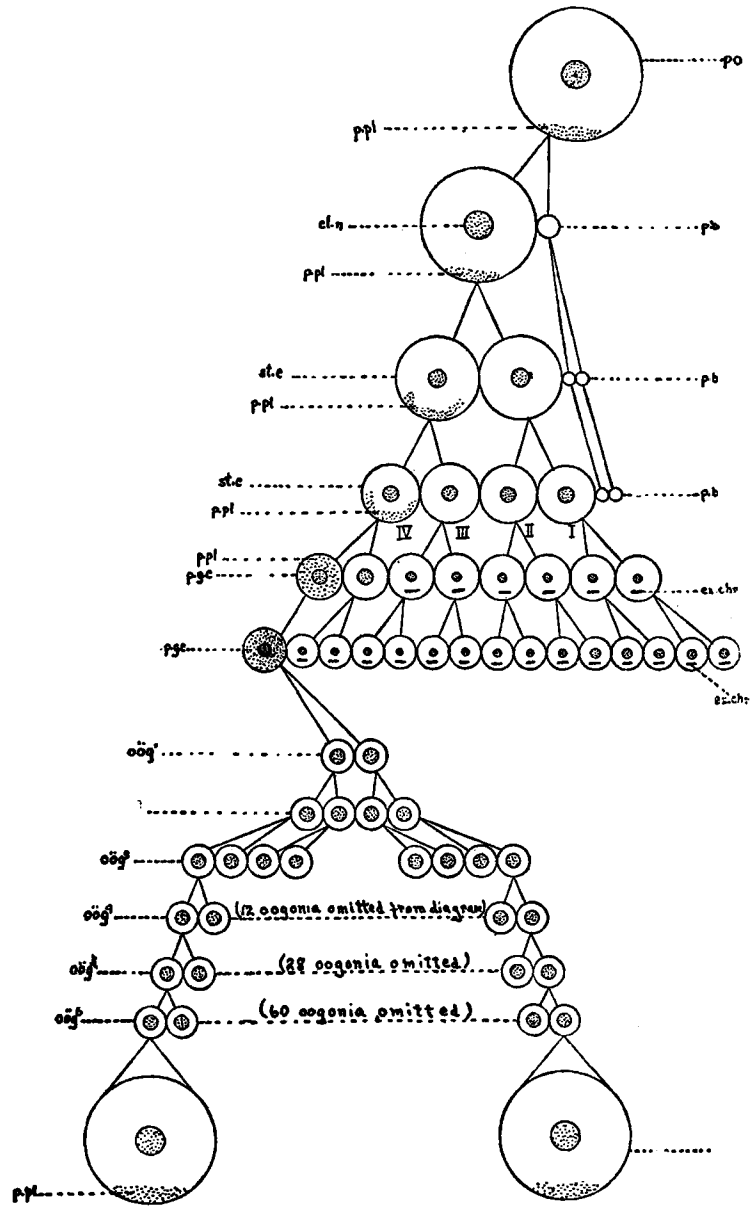


Fig. 5 Miastor americana; diagram showing the entire germ cell cycle. *cl.n.*, cleavage nucleus; *ex.chr.*, extruded chromatin; *oog*, oogonium; *p.b*, polar body; *p.g.c*, primordial germ cell; *p.o*, primary oocyte; *p.pl*, 'polares Plasma'; *st.c*, stem-cell.

Summary. The principal points that should be emphasized are as follows:

1. Miastor americana Felt is truly paedogenetic and agrees with M. metraloas in regard to its reproduction as described by Kahle ('08).

2. One polar body, which divides by mitosis, is produced (fig. 30).

3. A diminution process takes place during the division of the first four cleavage nuclei in which a large part of the chromatin of three of these cleavage nuclei is cast out into the cytoplasm (fig. 3).

4. One daughter nucleus of the fourth cleavage nucleus, which does not lose any of its chromatin, passes into a deeply staining mass of material ('polares Plasma') situated at the posterior end of the egg, and is cut off from the egg by a cell wall. This cell which thus contains the 'polares Plasma' and a nucleus with the full amount of chromatin is the primordial germ cell (figs. 32, *p.g.c*).

5. A second diminution process takes place, during which each of the seven somatic nuclei loses part of its chromatin and emerges with one-half of the number of chromosomes. The primordial germ-cell does not undergo a diminution process (fig. 32).

6. The primordial germ-cell divides by mitosis until eight oogonia are produced. These separate to form two rows of four oogonia each. After a long period of rest further divisions result in the production of thirty-two oogonia in each germ gland.

7. The nurse cells are of mesodermal origin.

8. We have here for the first time a definite number of oogonial divisions, namely six, a definite and equal number of oogonia in each germ-gland, and a definite number of oocytes (sixty-four) produced by the primordial germ-cell. It is no longer necessary, therefore, to express our ignorance by saying that there are n divisions during the period of multiplication of the oogonia, since in Miastor the number (n) is known positively to be six.

The differentiation of the germ cells. We have seen from the foregoing account that in Miastor there are two features which distinguish the germ cells from the somatic cells; (1) the nucleus of the primordial germ cell is the only one in the egg which retains the full amount of chromatin and the complete number of chromosomes; and (2) the primordial germ cell contains, in addition to this nucleus, *all* of the 'polares Plasma' and apparently no other kind of material. In considering the differentiation of the germ cells we must therefore examine more in detail these two features.

Kahle does not discuss the origin of the 'polares Plasma.' In describing this structure in an oocyte just before the formation of the polar body, he says, "Ganz auffällig ist eine Ansammlung von Protoplasma am hinterer Eipol. Sie wird durch Anilin- und Karminfarben tiefer tingiert als das übrige Plasma und macht den Eindruck einer ausserordentlich verdichteten Substanz" (p. 12). Further on the following statement is made:

> Wie wir sahen, stammt der Kern der Urgeschlechtszelle in direkter Folge vom Furchungskern ab, das Protoplasma der Urgeschlechtsze le aber war schon lange vor ihm da. Es ist dasselbe, das ich als polares Plasma bezeichnet habe, das sich durch besonders intensive Färbung auszeichnet, dessen Vorhandensein in unveränderter Lage in allen aufeinanderfolgenden Stadien nachweisbar ist das im Fortgang der Entwicklung ein immer stärkeres Wachstum zeigt und sich bis in die ungereifte Eizelle zurüchverfolgen lässt. Das polare Plasma ist infolge dessen also Keimplasma aufzufassen, das wahrscheinlich besondere Qualitäten enthält und bereits in der ungereiften Eizelle präformiert wird, um später seine Aufgabe als Geschlechtsplasma zu erfüllen. Erscheint uns also die Bildung der Urgeschlechtskerns schon als eine sehr frühe, so ist die Differenzierung des Urgeschlechtsplasmas auf ein noch viel jüngeres Stadium verlegt. Dieses Plasma erwartet gewissermassen seinen Kern, um sich dann sofort mit ihm als Urgeschlechtszelle zu isolieren (p. 21).

If, as Kahle says, the 'polares Plasma' represents the 'Keimplasma,' it is of the greatest importance to determine its origin and fate. For this reason hundreds of young were preserved, sectioned and stained by the methods most likely to enable one to trace the history of this substance (p. 388). It must be confessed, however, that notwithstanding the efforts made with

this end in view, the problem is still unsolved. It is evident from the preparations that the oocyte is nourished by and grows at the expense of the nurse cells (figs. 28–32). It is also absolutely certain that these nurse cells are not derived from the oogonia, as is true in so many insects, but, are modified mesoderm cells (figs. 38, 39, 27). At first, the growth of the oocyte takes place so slowly as to make almost no perceptible difference in the character of the cytoplasm contained within it. The oogonia are remarkably easy to distinguish during the embryonic development because (1) of their comparatively enormous nuclei, filled with large chromatin granules; and (2) the deeply staining quality of their cytoplasm, consisting of the corresponding deeply staining substance of the 'polares Plasma' of the mature egg (figs. 31–32). As the oocyte grows its cytoplasm becomes less deeply colored and presents a uniform appearance not distinguishably different from the cytoplasm of the other cells (fig. 40). Sometime before the oocyte is ready for maturation, however, deeply staining cytoplasm appears in the neighborhood of the nurse chamber and a substance begins to accumulate at the posterior pole which has a strong affinity for various dyes (fig. 28). The former is evidently elaborated under the influence of the nurse cells; the latter, which represents the 'polares Plasma,' may be derived from the nurse cells, but if it is, the process is so slow and its mass compared with the mass of the remaining egg contents so small that its passage from the nurse chamber to the posterior end of the oocyte is indistinguishable. We must conclude, therefore, that the 'polares Plasma' may originate from or under the influence of the nurse cells, but that this has not been demonstrated and probably never can be established.

A second hypothesis which may account for the presence of the 'polares Plasma' in succeeding generations is that of continuity and growth. Each oogonium is supplied with a portion (typically one sixty-fourth) of the 'polares Plasma' of the mature egg. Hence a certain amount of this substance, as well as a like amount of nuclear material, is passed on from one generation to the next. What is more probable than that this part, although

minute when compared with the enormous contents of the mature egg, may become segregated at the posterior end of the egg and there bring about the development of a greater volume of similar substance, either by the division or budding of preexisting particles, or from the yolk or cytoplasm under its influence. A full discussion of this subject will reserved until the Keimbahn-determinants of other animals have been described (p. 460).

C. The Keimbahn in Compsilura concinnata Meig

Compsilura is a tachinid fly, introduced into this country in 1906 for the purpose of destroying gypsy and brown-tail moths. "Its eggs hatch in the uterus of the mother, and the tiny maggots are deposited beneath the skin of the host caterpillar by means of a sharp, curved 'larvipositor,' which is situated beneath the abdomen" (Howard and Fiske, 1912, p. 219). The maggot is ready for pupation in about two weeks; the pupal period is about one week; and the females require only about three or four days after their emergence to become sexually mature. I wish here to acknowledge my indebtedness to Dr. John N. Summers of the Gipsy Moth Parasite Laboratory, Melrose Highlands, Massachusetts, for an abundance of material.

The internal reproductive organs of a sexually mature female are shown in figure 6. Oocytes of various sizes are present within the ovarian tubules (*o.*). At a point near the union of the two oviducts (*od*), the uterus is connected with two accessory glands (*a.g.*), and three seminal receptacles (*s.r.*). The mature eggs, which make their way down the oviduct and into the uterus, are here fertilized. They then gradually move down the uterus and are present to the number of about one hundred in a sexually mature individual. All stages from the maturation of the egg to the condition when the larva is ready to be deposited are passed through within the uterus of the mother, and most of these may be observed in a single specimen. Those eggs nearest the ovaries are of course the youngest. An attempt to trace the origin of the pole-disc granules in this species was unsuccessful, so only two illustrations are presented here to show that in this species

there is a primary cellular differentiation similar to that already described in other Diptera. Figures 41 and 42 represent two stages in the formation of the primordial germ cells at the posterior end of the egg. The granules of the pole-disc are encountered by the cleavage nuclei which chance to reach the posterior pole; they surround these and are distributed about

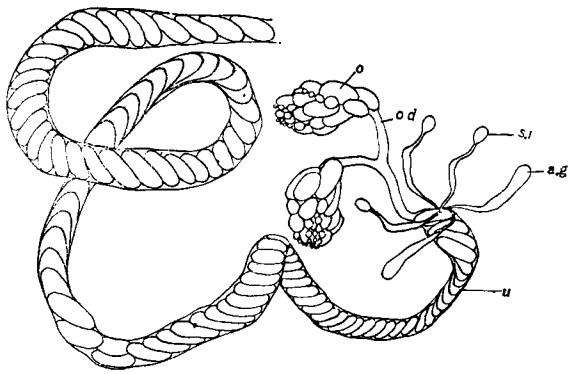


Fig. 6 Compsilura concinnata; reproductive organs of a female. *a.g*, accessory gland; *o*, ovary; *od*, eviduct; *s.r*, seminal receptacle; *u*, uterus full of eggs in various stages of development.

within the cytoplasm of the germ cells when they are cut off by cell walls. The further history of the germ cells does not seem to differ in any way from that described in other Diptera.

Cecidomyia strobiloides. A pole-disc was also found in the eggs of the willow-cone gall just before deposition (fig. 43), and an attempt was made, as in Miastor, to trace this structure to its place of origin. The growth of the egg of this species resembles that of Miastor and all efforts to connect the pole-disc with substances within the oocyte previous to its appearance at the posterior end were futile.

3. COLEOPTERA

A. Historical account

The primordial germ cells of beetles have not received as much attention from investigators as have those of the Diptera, probably because they are not so conspicuous. The germ glands have been described in the embryo of Hydrophilus piceus by Heider ('89), in Leptinotarsa (Doryphora) decemlineata by Wheeler ('89), in Melolontha vulgaris by Voeltzkow ('89b), in Hydrophilus piceus, Melolontha vulgaris, and Lina tremulae by Graber ('91), in a number of Chrysomelid beetles by Lecaillon ('98), and by Friederichs ('06), in Tenebrio molitor by Saling ('07), and in Chrysomelid beetles by Hegner ('08, '09, '09b, '11a, '11b) and Wieman (10a', 10b', '10c). Of these only the work of Wheeler, Lecaillon, Hegner, and Wieman needs to be considered. Although Wheeler ('89) failed to find the pole-cells in the very early stages of Leptinotarsa, he figures several of them (his fig. 82) in a sagittal section of an egg carrying a segmented germ-band. Here are shown three cells "which are on the surface of the embryo in the amniotic cavity. They are very large and clear and the more anterior is apparently creeping in the manner of an Amoeba, along the surface of the abdominal ectoderm. These cells, the ultimate fate of which I have been unable to determine, probably escape from the anal orifice of the gastrula before it closes." This author also shows in transverse section (his fig. 87) a cell which, he says, is "about to wander through the blastopore into the amniotic cavity." He suggests that this may be the homologue of the 'Polzellen.' That the cells thus described by Wheeler are really pole-cells was proved by my investigations on the same species.

The embryological development of the following species of Chrysomelidae was studied by Lecaillon ('98); Clytra laeviuscula, Gastrophysa raphani, Chrysomela menthastri, Lina populi, L. tremulae, Agelastica alni. In Clytra, the principal form examined, Lecaillon found that the first nuclei to arrive at the posterior pole of the egg became the centers of the primitive germ-cells; these could be distinguished from neighboring cells by their

large size, larger nuclei, and more deeply staining cytoplasm. The germ-cells did not stop when they reached the surface of the egg, but passed outside and became separated from it; their number increased . . . . peu à peu par suite de l'arrivée de nouvelles cellules périphériques et aussi sans doute de la division des premières cellules détachées du pôle de l'oeuf." The germ-cells then started to re-enter the egg, retarding, by this migration, the formation of the blastoderm at this point. "Finalement, le blastoderme achève de se former au pôle posterieur de l'oeuf, et alors les cellules sexuelles se trouvent groupées. . . . . entre le vitellus et l'enveloppe blastodermique."

Several species of Chrysomelid beetles were also studied by Friederichs ('06), who discovered that the cleavage nuclei in Donacia crassipes reach the posterior later than the anterior end of the egg; the reverse is the rule in species of allied genera. After the blastoderm is formed "an der Ventralseite unmittelbar seitlich vor dem Pol, findet eine besonders lebhafte Zellvermehrung statt, so dass einzelne Zellen aus dem Blastodermverband heraus und ins Innere gedrängt werden." These, the primitive germ-cells, were not very different in Donacia from blastoderm-cells, but in Timarcha nicoeensis and Chrysomela marginata they could be distinguished by the larger size and darker color of their nuclei.

In a series of papers published within the last six years, the writer has given the results of morphological and experimental studies on the primordial germ cells of Chrysomelid beetles, particularly Calligrapha bigsbyana, C. multipunctata, C. lunata, and Leptinotarsa decemlineata. It has been possible to trace the entire Keimbahn in these insects, and to carry on experiments with the eggs and embryos without preventing further development. The reader is referred to the original papers for details, but a general account will be given here as an introduction to the original work to be presented in the succeeding pages.

At the time of deposition, the eggs of the Chrysomelid beetles studied are not always in the same stage of development, although usually polar body formation is taking place. The egg

figured (fig. 7, A) was fixed four hours after deposition. The polar bodies have already been produced and the male and female nuclei are in the act of conjugation. The egg consists of a large central mass of yolk and a comparatively thin peripheral layer of cytoplasm, the 'Keimhautblastem' of Weismann. The interdeutoplasmic spaces are filled with cytoplasm which is connected with the 'Keimhautblastem' by delicate strands of the same material. The enormous amount of yolk contained in these eggs makes the identification of other substances extremely difficult. The yolk-globules range in size from large deutoplasmic spheres to small granules, and, as the dissolution of some of them is continually taking place, one is unable to determine where yolk ends and cytoplasm begins. The only accumulations of cytoplasm large enough for examination are those surrounding the nuclei within the yolk mass, and the peripheral layer, the 'Keimhautblastem.' No differences in composition or staining qualities were observed between the cytoplasm of these two regions. The 'Keimhautblastem' consists of a fluid ground substance in which are suspended very fine granules. It is a homogeneous layer of cytoplasm everywhere except at the posterior end of the egg. At this point there is a disc-shaped mass of larger granules imbedded within the inner portion of it. These granules stain deeply with haematoxylin. They are easily seen, not only in sections but also in eggs that have been properly stained in toto. Because of their ultimate fate I have called these granules the germ-cell determinants (fig. 7, *A*, *g.c.d.*).

The first cleavage divisions take place where the pronuclei fuse. The daughter nuclei move away from each other and as cleavage progresses a separation of the nuclei into two sections occurs. The nuclei of one group form a more or less regular layer equidistant from the periphery; these preblastodermic nuclei (fig. 7, *B*, *pbl.n*) move outward and fuse with the Keimhautblastem. Cell walls now appear for the first time and a blastoderm is formed of a single layer of regularly arranged cells.

The genesis of the pole-cell is as follows: (1) four nuclei lying near the posterior end of the egg are recognized by their

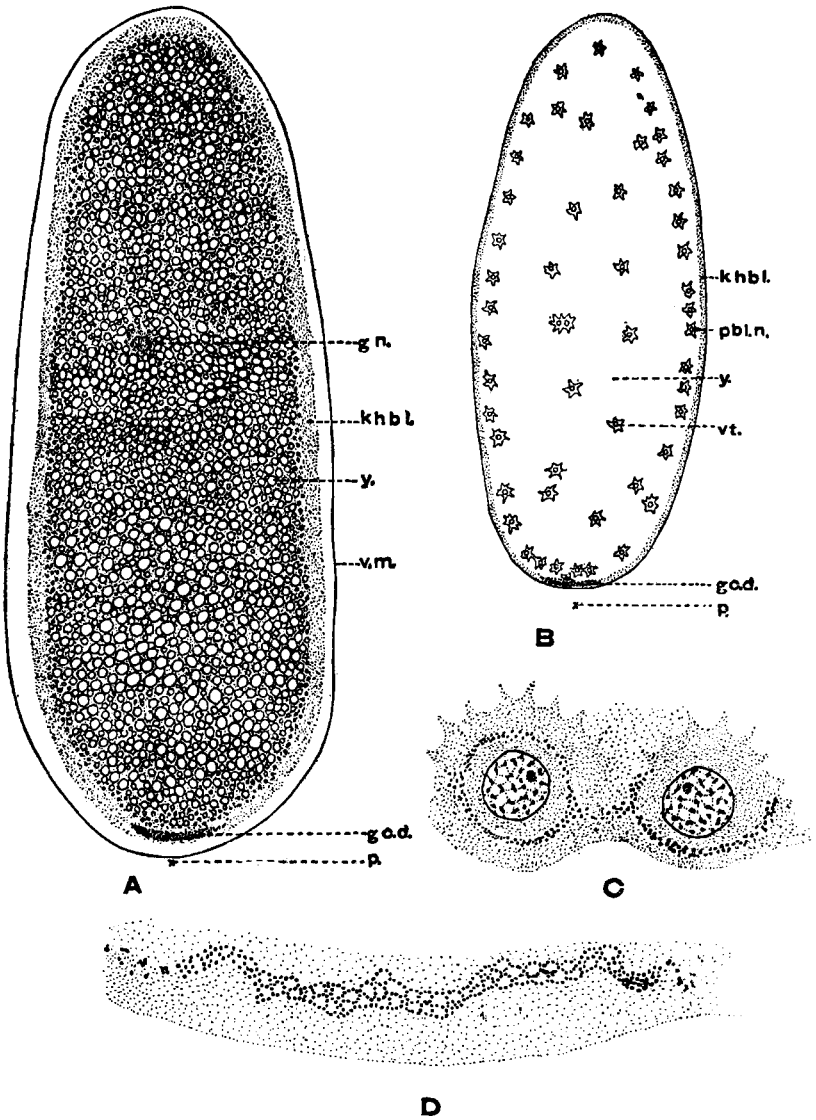


Fig. 7 Calligrapha (from Hegner, '09a and '09b). A, longitudinal section through an egg of C. bigsbyana, four hours after deposition. B, longitudinal section through an egg of C. bigsbyana 14 hours after deposition. C, two germ cells just protruding from posterior end of egg of C. multipunctata. D, the pole-disc in an egg of C. multipunctata. *g.c.d*, pole-disc; *g.n*, germ nuclei fusing; *khbl*, keimhautblastem; *p*, posterior end of egg; *pbl.n*, pŗeblastodermic nuclei; *v.m*, vitelline membrane; *vt*, vitellophags; *y*, yolk.

position as pole-cell antecedents; (2) these four nuclei divide producing eight daughter nuclei which move closer to the periphery of the egg; (3) these in turn divide resulting in sixteen nuclei, arranged in pairs, each of which separates entirely from the egg, carrying with it a portion of the Keimhautblastem containing pole-disc granules (fig. 7, *C*); (4) the sixteen primary pole-cells divide to form thirty-two secondary pole-cells; these divide resulting in sixty-four tertiary pole-cells which do not increase in number until a late period of embryonic life; (5) in mitosis the pole-disc granules are approximately equally distributed between the two daughter cells (fig. 8, *B*). After separation from the egg the pole-cells are (1) carried slightly forward on the ventral surface of the egg by the contraction of the ventral plate; (2) they sink into the posterior depression of the ventral groove, which is the beginning of the posterior amniotic cavity; (3) they are carried along by the developing tail-fold, which penetrates dorso-anteriorly into the yolk; (4) they migrate through a pole-cell canal into the embryo by means of amoeboid movements; (5) upon reaching the interior of the embryo they separate into two groups, which come to lie, one on either side of the body, in the last two abdominal segments; (6) these two strands become shorter by a crowding together of the germ-cells; (7) each of the two germ-glands thus produced acquires an epithelial covering of mesoderm-cells; (8) the germ-glands, situated as before in the last two abdominal segments, are carried, by the shortening of the embryo, to a ventral position on either side of the body; (9) by its lateral growth around the yolk, the embryo carries the germ-glands to a point near the dorsal surface on either side of the mid-gut; (10) the sexes can be distinguished at this time by the shape of the germ-glands, that of the male being dumb-bell shaped, while the female reproductive organ is pear-shaped, and shows the development of terminal filaments.

In all stages the germ cells may be distinguished easily from the surrounding somatic cells. Figure 8, *A* shows a pole cell shortly after separation from the egg. The pole-disc granules are quite conspicuous, and pseudopodia-like projections are

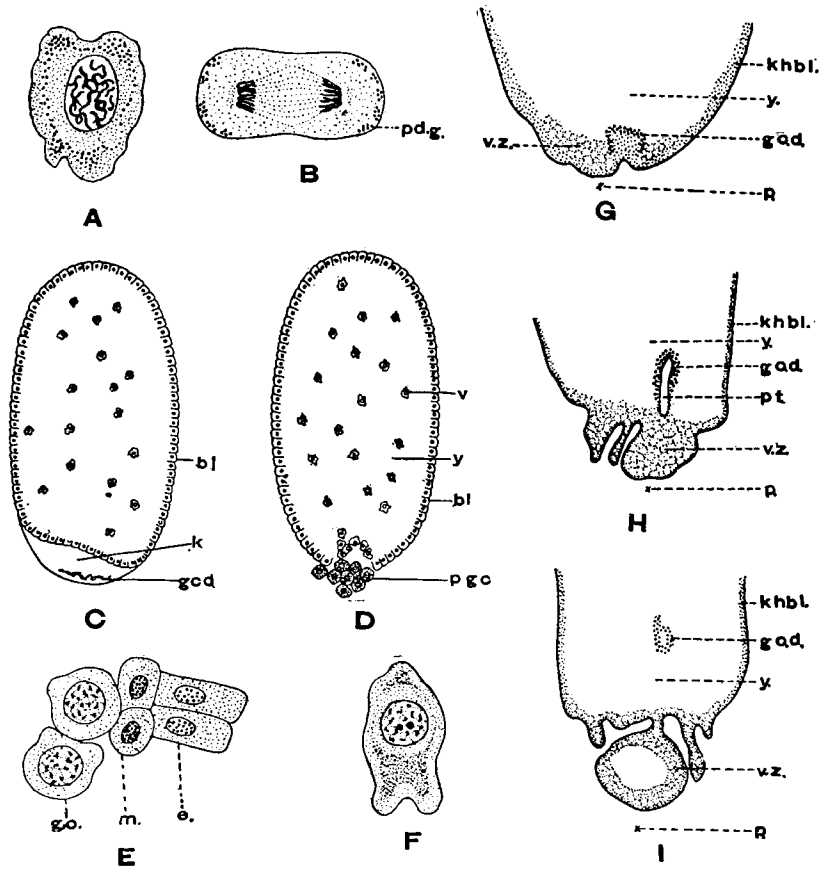


Fig. 8 Calligrapha (from Hegner, '09a, '09b, '11a). A, a germ cell of C. multipunctata shortly after being cut off from the egg. B, division of a primordial germ cell. C, longitudinal section through egg of C. bigsbyana at blastoderm stage; the posterior end was killed with a hot needle just after deposition. D, longitudinal section through uninjured egg at same stage. E, two ectoderm cells (*e*), two mesoderm cells (*m*), and two germ cells (*g.c.*) from an egg three days old. F, germ cell during migration into the embryo (three days old). G.H.I, longitudinal sections through eggs centrifuged for one hour, two hours and four hours respectively. *bl*, blastoderm; *g.c.d*, granules of pole-disc; *k*, killed portion of egg; *khbl*, keimhautblastem; *p*, posterior; *pgc*, primordial germ cells; *v*, vitellophags; *v.z*, vesicular zone; *y*, yolk.

plainly evident. Sixteen pole-cells were present at this time. After a mitotic division, during which the pole-disc granules are apparently approximately equally divided between the daughter cells (fig. 8, *B*), the pole cells are smaller, but, although no larger than the neighboring blastoderm cells, they may still be distinguished by the presence of the pole-disc granules and also by the larger nucleus containing a lesser number of chromatin granules. Pole-disc granules are still faintly visible at a later period when the germ cells are migrating into the embryo through the pole-cell canal, and still later, as figure 8, *E* shows, the germ cells can be distinguished easily from the ectoderm and mesoderm cells although the pole-disc granules have entirely disappeared.

The pole-disc. The pole-disc varies somewhat in compactness but in most cases appears in section as shown in figure 7, *D*. Nothing resembling it occurs in other parts of the egg. Its granules are very susceptible to stains and can be made visible by means of a number of different dyes. The 'Keimwulst' of Chironomus (Ritter '90), the 'Dotterplatte' of Calliphora (Noack, '01; fig. 2) and the 'Keimbahnplasma' of Chironomus (Hasper '11; fig. 1) all present a similar appearance. In these forms, as well as in the Chrysomelid beetles I have studied, all or nearly all of the granules (fig. 7, *C*) are taken out of the egg by the pole-cells. Wieman ('10a) however, gives a figure showing a section of the posterior end of the egg of Leptinotarsa signaticollis *after* the protrusion of the pole cells, in which there is still represented what he calls the pole-disc. The fact that the mass of granules described by Wieman does not resemble the pole-disc as I have found it, nor other similar accumulations in insect eggs (Keimwulst, Dotterplatte, Keimbahnplasma) and the statement that "the granules are not all taken up by the cells in their migration and the greater part of them remains behind after the cells have passed through" (Wieman, '10a, p. 186), a condition contrary to that described by every one of the writers cited above, lead to the conclusion that Wieman has confused something else for the pole-disc. This seems all the more probable, since the species studied by Wieman, namely Leptinotarsa signaticollis, is very

closely allied to one of the species that I investigated (L. decemlineata) in which a typical pole-disc like that shown in figure 7, *D* occurs. Furthermore the cells which Wieman designates as pole cells have none of the characteristics of the pole cells described by other writers.

Several important results have been obtained by experiments that I have performed with the object of determining the character and significance of the pole-disc. When the freshly laid eggs of Leptinotarsa decemlineata are centrifuged with the posterior end toward the center of revolution the pole-disc is moved gradually toward the outer anterior end as shown in figure 8, *G,H,I, g.c.d.* The movement *en masse* of the pole-disc granules proves that they are heavier than the oil globules of the vesicular zone (*v.z.*) and indicates that they do not form an adventitious accumulation but constitute a definite structure of sufficient rigidity to withstand the dispersing effects of a strong pull exerted during a period of at least four hours. It was hoped that by means of centrifugal force the pole-disc could be located in a part of the egg different from that normally occupied and that experimental proof of the necessity of their presence for the formation of germ cells might thus be obtained, but the abnormal development of the eggs prevented an accurate determination of the various cells in sections of these centrifuged eggs.

Two sets of experiments were undertaken in an attempt to deprive the eggs of the pole-disc. If the embryo developing from an egg without the pole disc failed to possess germ cells the obvious conclusion would be that the pole-disc consists of real germ cell determinants, necessary for the differentiation of germ cells. In the first set of experiments the freshly laid eggs were oriented and then pricked with a sharp needle in the center of the posterior end. Since the egg is turgid a small drop of the contents was forced out. Eggs treated in this manner continue to develop producing embryos and larvae apparently normal. Sections of these seemed to show that less than the characteristic number of germ cells were present. My inability, however, to determine whether all of the pole-disc had been

removed constituted a source of error which made the results uncertain.

A second set of experiments (Hegner '11a) were therefore devised and a method employed which made it absolutely certain that the pole-disc could not take part in the development. In these experiments the posterior end of the egg was touched with a hot needle and that portion containing the pole-disc was killed. In every instance the development continued and the blastoderm formed normally over all of the surface except at the posterior end; here it was built at the end of the living substance as shown in figure 8, *C*, *bl*. No germ cells were produced, as in the normally developed egg at this stage (fig. 8, *D*). I conclude from this that the pole-disc granules or the substances in which they are imbedded *are* necessary for the formation of germ cells.

B. Nuclear division and differentiation in the eggs of Chrysomelid beetles

This work was undertaken in order (1) to determine whether or not a chromatin diminution process takes place in the cleavage nuclei of Chrysomelid eggs similar to that described in Ascaris by Boveri ('92) and in Miastor by Kahle ('08); (2) to study the differentiation of the nuclei which take part in the formation of the primordial germ cells, blastoderm cells, and vitellophags; and (3) to record what appears to be amitotic nuclear division among the vitellophags.

General account of early cleavage. Eggs of Calligrapha multipunctata, C. bigsbyana, and Leptinotarsa decemlineata have been used for this purpose. Beetles were kept in the laboratory in Stender dishes and closely watched, so that the exact interval between the time of deposition and time of fixation could be determined. Eggs were killed in Tower's second solution heated to the boiling point. The chorion was dissected off after a few days and no difficulty was encountered in imbedding in paraffin and sectioning. Because of the abundance of yolk, Mayer's acid hemalum was used principally for staining pur-

poses although iron hemotoxylin was also employed. The hemalum does not stain the yolk if properly used. It is frequently difficult to stain the early cleavage nuclei, but several hundred preparations have furnished all the necessary stages required. Division figures are comparatively rare and one can only conclude that mitosis takes place very rapidly and is followed by a long period of rest.

The stage of development is not the same in all eggs at the time of deposition, but usually the maturation divisions are in progress or the first cleavage has begun. As shown in figure 44, the polar bodies are produced about half way between the anterior and posterior ends of the egg. The nucleus of the egg lies in an accumulation of cytoplasm at the periphery. Two polar bodies are formed; then the female nucleus with its surrounding cytoplasm moves out toward the center of the egg and copulates with the male nucleus (figs. 7, *A*, and 45). The first cleavage occurs at this point. The cleavage nuclei, each with a small portion of cytoplasm, then move apart and divide almost synchronously. Figures 44 to 51 were made from longitudinal sections of eggs of various stages and all of the nuclei are here represented in a single plane, but their relative positions are indicated as determined by superimposing sketches made with a camera lucida. It will be seen that the nuclei are more numerous near the anterior end of the egg and that they become rather evenly scattered throughout the yolk. There are of course no cell walls and the thin strands of cytoplasm between the yolk globules connect the cleavage nuclei. Each nucleus lies in a sort of ameboid island of cytoplasm, forming a body equivalent to a cell, but probably better designated by the term energid (Sachs, '92).

In figure 46 two energids are shown just after the first cleavage division. Figure 47 represents the division of the first four energids to form eight; those lettered *a* and *c* are in the anaphase of mitosis, whereas *b* and *d* have reached the telophase. The relative positions of the first four energids is indicated in figure 48 which was reconstructed from a series of transverse sections. Here a condition exists quite similar to that shown in figure 47;

the nuclei *c* and *d* are in the anaphase and *a* and *b* in the telophase stages of mitosis. The egg from which figure 49 was made contained eight energids; that represented by figure 50 possessed sixteen. So far as could be determined the nuclei of these energids are all alike. One of them is shown in figure 52. One hundred and thirty-three nuclei were counted in the egg shown in figure 51; these are still alike. Soon after this stage is reached part of the energids move out to the periphery and fuse with the 'Keimhautblastem' to form the blastoderm (fig. 7, *B*), whereas the rest remain behind among the yolk globules and become vitellophags. Those energids which encounter the pole-disc do not take part in the blastoderm formation but become the primordial germ cells.

Does a chromatin-diminution process occur in Chrysomelid eggs? The fact that part of each chromosome is cast out into the cytoplasm in all except the 'stem-cell' during the early cleavage of Ascaris is well known (fig. 19, p. 442). A similar process was described by Kahle ('08) in Miastor metraloas and confirmed by me (Hegner, '12) in Miastor americana (fig. 3, p. 392). This chromatin-diminution process results in the formation of a single primordial germ cell containing the complete amount of chromatin and a number of somatic cells with a reduced amount of chromatin. The origin of the germ cells has been carefully studied in a number of forms which in other respects resemble Ascaris and Miastor, but in none of them has such a process been discovered. Hasper ('11) was unable to establish it for Chironomus which is very similar to Miastor in early development, nor has such a phenomenon been found in Sagitta (Elpatiewsky, '09, '10; Stevens, '10; Buchner, '10a, '10b) and the copepods (Haecker, '97, Amma, '11) and Cladocera (Kuhn, '11, '13) which undergo total cleavage and are otherwise similar to Ascaris.

The nuclear divisions in the eggs of Chrysomelid beetles have been examined by the writer with considerable care, but nothing resembling a diminution process was found. Furthermore, there are no evidences of chromatin bodies in the cytoplasm or yolk as in Ascaris (fig. 19) and Miastor (figs. 33, 34, *cR*) where the cast-out chromatin does not disintegrate immediately, but can

be distinguished for a considerable period during early embryonic development. It seems necessary to conclude therefore that in Chrysomelid eggs both germ cells and somatic cells possess the full amount of chromatin, or else the elimination of this substance takes place in some other way. This point will be more fully considered later (p. 465).

The differentiation of the nuclei of the blastoderm cells, primordial germ cells, and vitellophags. The conclusion that no chromatin-diminution process occurs during the early cleavage divisions in the eggs of Chrysomelid beetles necessitates the search for some other method of differentiation among the cleavage nuclei. The insect egg is particularly advantageous for testing Roux's hypothesis of qualitative nuclear divisions, since we have here the production of an enormous number of nuclei before any cell walls are formed, and an egg that is remarkably definitely organized, as indicated by my experiments (Hegner, '09b, '11a), before the blastoderm is formed.

I have been unable to find any differences in the nuclei before the energids fuse with the keimhautblastem, but as soon as this does occur, a gradual change takes place, and at the time when the blastoderm is completed three sorts of nuclei are distinguishable: (1) The nuclei of the primordial germ cells (fig. 53) are larger than the others and contain comparatively few spherical chromatin granules evenly distributed. The cytoplasm of these cells is distinguishable from that of all other cells because of the presence of granules from the pole-disc; (2) The nuclei of the blastoderm cells (fig. 54) are small and completely filled with large spherical chromatin granules; (3) The nuclei of the vitellophags (fig. 55) resemble the early cleavage nuclei; they are midway between the other two kinds in size, and their chromatin is more diffuse.

Whether these three kinds of nuclei were all potentially alike before their differentiation is an important question. Visibly they are all similar until they become localized in definite regions of the egg, and associated with particular cytoplasmic elements. One can but conclude that they were all potentially alike and that their differentiation was brought about through the influence

of the cytoplasm in which they happened to become imbedded. The writer has shown (Hegner, '11a) that if the posterior end of a freshly laid egg of Leptinotarsa decemlineata is killed with a hot needle, thus preventing the pole-disc granules and surrounding cytoplasm from taking part in development, no primordial germ cells will be produced. A large series of similar experiments have also proved that at the time of deposition, "The areas of the peripheral layer of cytoplasm (fig. 7, A, *khbl.*) are already set aside for the production of particular parts of the embryo, and if the areas are killed, the parts of the embryo to which they were destined to give rise will not appear. Likewise, areas of the blastoderm are destined to produce certain particular parts of the embryo" (Hegner '11a, p. 251). What becomes of the nuclei which are prevented from entering the injured region of the egg? No evidence has been discovered to indicate that they disintegrate, so they probably take part in development after becoming associated with some other part of the egg. If these nuclei were qualitatively different they should produce germ cells and other varieties of cells in whatever region they chance to reach. It is evident that they are not potentially different and that their 'prospective potency' and 'prospective significance' do not coincide. The cytoplasm is, therefore, the controlling factor at this stage in the germ-cell cycle, although cytoplasmic differentiations are for the most part invisible and probably the result of nuclear activity during earlier stages.

Amitotic nuclear division in vitellophags. The cleavage nuclei in Chrysomelid eggs all divide by mitosis, but, after the blastoderm has become established, the vitellophag nuclei, which remain behind in the yolk, show evidences of amitotic division. At this stage of development the vitellophags are more or less evenly distributed within the yolk mass. Here and there groups of from two to four or more nuclei are present, indicating that several divisions have occurred in quick succession. All of the stages characteristic of amitotic nuclear division are abundant. Three of them are shown in figure 56; nucleus *a* is just becoming dumb-bell-shaped; nucleus *b* has almost become constricted into

two; and nucleus *c* is apparently undergoing a second amitotic division before the first division is actually completed—the result would probably have been a row of four nuclei. As recorded by Child ('07), Patterson ('08), Maximow ('08), Wieman ('10b) and others, amitosis here likewise occurs in rapidly dividing nuclei and is probably due to some physiological condition.

A type of amitosis differing from that just described was discovered in eggs that had been subjected to the action of centrifugal force. The three nuclei shown in figure 57 are from vitellophags of an egg of Leptinotarsa decemlineata which was centrifuged for sixteen hours after deposition and was then fixed immediately. The chromatin in nuclei *a* and *b* forms rather condensed clumps in the center of the nucleus and the nuclear membrane appears to force its way through the center of this clump thus bringing about the formation of two daughter nuclei. This membrane cannot be a cell wall since vitellophags do not possess cell walls, and this appearance cannot be due to poor fixation because other nuclei in this egg were perfectly preserved. This sort of division must therefore be a normal process or else due to some unknown influence of the centrifugal force. Various authors have contended that there are within the nuclear membrane all the elements necessary for an equal division of chromatin. This view is supported by the discovery of part or all of the mitotic figure within the nuclear membrane in certain cells, e.g., during the maturation division of Canthocamptus (Hegner, '08). The case just described and figured in this paper contributes to the support of this hypothesis.

C. The growth of the oocytes and development of testicular cysts in Chrysomelid beetles

The investigations on Chrysomelid beetles described in the succeeding pages were undertaken for the following purposes: (1) To study the differentiation of the nurse cells and oocytes from the oogonia; (2) to determine the origin of the pole-disc granules; (3) to discover, if possible, stages in the cycle of the

male germ cells corresponding to the formation of nurse cells in the cycle of the female germ cells; and (4) to test Wieman's ('10b) statements regarding the occurrence of amitosis in the oogonia and nurse cells, and in the spermatogonia during the formation of testicular cysts.

(*1*) *The differentiation of nurse cells and oocytes in the ovary of Leptinotarsa decemlineata.* So far as we know, in the majority of cases the cells which are segregated as primordial germ cells in the embryo do not produce eggs or spermatozoa. Many of them degenerate, although they probably once possessed the potentiality of true germ cells; others undergo modifications, becoming nurse cells, Sertoli cells, etc.

Among the most interesting cases of the differentiation of nurse cells from germ cells is that of Dytiscus marginalis. We owe detailed accounts of the process in this species to Giardina ('01), Debaisieux ('09) and Günthert ('10), but as long ago as 1886 Korschelt figured what was evidently one stage in this differentiation. Giardina ('01) established the fact for Dytiscus that the mitoses which result in the formation of nurse cells are differentiating, as theoretically postulated by Paulcke ('00). During the four divisions preceding the formation of the oocyte, a single oogonium gives rise to one oocyte and fifteen nurse cells. A differentiation takes place in the chromatin of the oogonial nucleus, one half consisting of a condensed mass, the other half of large granules which correspond to the forty chromosomes of the oogonium. During mitosis the chromosomes become arranged as an equatorial plate, and the chromatic mass forms a ring about it—the 'anello cromatico.' This ring passes intact to one of the daughter cells, whereas the chromosomes are equally divided. During the succeeding mitoses similar differential divisions occur, resulting in one oocyte containing the chromatic ring, and fifteen nurse cells lacking this nuclear substance. Thus, as Paulcke's theory demands, the difference between the nurse cells and the oocytes is the result of internal and not external causes.

Giardina ('01) considered the formation of the chromatic ring as a sort of synapsis, and later ('02) distinguished between

a complete synapsis, such as ordinarily occurs in the germ-cell cycle, and a partial synapsis as exhibited by Dytiscus. Regarding the significance of this differential mitosis, he maintains that this phenomenon is the cause of the differentiation into nurse cells and oocytes, resulting in a complete amount of chromatin in the keimbahn cells and perhaps also an unequal distribution of cytoplasmic substances. As in the cases of Ascaris and Miastor, it might better be regarded as a means of depriving the nurse cells (somatic cells or abortive germ cells?) of part of their chromatin. Moreover, Boveri ('04) has compared the chromatin diminution in Ascaris with Giardina's differential mitoses. Debaisieux ('09) and Günthert ('10) have confirmed Giardina's results, and the latter studied two other Dytiscidae (Acilius and Colymbetes) which also exhibit differential mitoses similar except in certain details. Günthert found that the chromatic ring is composed of fine granules which may split off from the surface of the chromosomes (compare with Ascaris and Miastor) and stain like cytoplasm. He interprets this as 'Zerfallsprodukte' of the chromosomes. Debaisieux, on the other hand, claims that this cast-out nuclear material is nucleolar rather than chromatic in nature.

It seems highly probable that the 'anello cromatico' of Giardina consists of chromatin, and Goldschmidt ('04) and others do not hesitate to class it as an example of a 'Chromidialapparat.' Furthermore it is apparently the result of a chromatin-diminution, as Boveri ('04) maintains, differing from the similar process in Ascaris and Miastor in details, but not in the ultimate result. Finally, the discovery of this peculiar body in Dytiscus adds one more argument to the hypothesis that the chromatin content of the germ cells differs from that of the somatic cells quantitatively, at least in some cases, and perhaps also qualitatively.

Many are the bodies that have been homologized with the 'anello cromatico' of Dytiscus. Buchner ('09, '10) claims that the nucleolar-like structure in the oogonia and young oocytes of Gryllus is homologous to both the accessory chromosomes of spermatogenesis and to this chromatin ring in Dytiscus. This 'accessorische Körper' passes intact into one half of the oocytes

where it disintegrates into granules of a 'trophische Natur.' Foot and Strobell ('11) have also compared it with the chromatin nucleolus in the oogonia of Protenor with which it has certain characteristics in common, but no such differential divisions occur as in Dytiscus.

Govaerts ('13) has recently reported upon the differentiation of the oocytes in Carabus, Cicindela, and Trichiosoma. He was unable to find anything resembling the chromatic ring of Giardina, and concludes

> . . . que la formation d'une masse chromatique, extériorisant la differenciation entre l'ovocyte et les cellules nourricieres, est jusqu'à present un fait isolé, observé uniquement chez les Dystiscides. Mes recherches demontrent que ce phénomène n'est pas applicable à tous les Insectes, et qu'il faut chercher au fait de la differenciation, de ces elements une cause plus large que la repartition inegale de certains elements chromatiques.

If no differential divisions are present, as in Dytiscus, what is the cause of the formation of oocytes and nurse cells? Govaerts decides that, since the ultimate oogonium possesses a definite polarity marked by the localization of the 'residu fusorial,' and the two kinds of daughter cells arise from opposite ends of the mother cell, the cause of the differentiation resides in the polarization of the oogonium. He does not, however, account for this 'polarité pre-differentielle.'

Ovaries of Leptinotarsa decemlineata were dissected out in Ringer's solution and placed immediately in the fixing solution. The fluids of Gilson, Altmann, Meves, and Carnoy were most frequently employed. Sections were stained by Benda's method, acid fuchsin, iron hemataxylin, and Mayer's acid hemalum. The best results were obtained with material fixed in Meves' modification of Flemming's solution and stained by the method of Benda.

The general arrangement of the cells in the ovary of an adult beetle is shown in figure 9. The terminal chamber of the ovarian tubule contains three kinds of cells, (1) nurse cells (*n.c.*), (2) young oocytes (*y.o.*) and growing oocytes, and (3) epithelial cells. The nurse cells and oocytes are both derived from the

oogonia; the epithelial cells are of mesodermal origin. As noted above, the investigations of Giardina ('01) and many others upon the genesis of the nurse cells in the ovaries of insects have established the fact that in some species a single oocyte and a definite number of nurse cells arise from a single ultimate oogonium. Wieman ('10b) has followed the history of the oogonia in Leptinotarsa signaticollis through the larval and adult stages, but was unable to find any evidence that the nuclei inaugurate differentiation as in Dytiscus (Giardina, '01; et al.). He concludes that "the process seems to be the result of several distinct cell elements which operate together as a whole" (p. 148) and that the semi-fluid matrix which results from the liquefaction of cells at the base of the terminal chamber may exert a "specific effect on those germ cells coming under its influence, enabling them to develop into ova, while the more distant germ cells become nurse cells" (p. 147). My observations agree with those of Wieman; no definite numerical relations nor nuclear evidence were discovered during the differentiation of the oogonia into oocytes and nurse cells. The data available do not suggest any method of differentiation not already proposed, and still leave the question whether the nurse cells should be regarded as abortive germ cells or true somatic cells, one of personal opinion.

(*2*) *The origin of the pole-disc granules in Leptinotarsa decemlineata.* The pole-disc in Chrysomelid eggs has already been described and figured, and comparisons with similar structures in the eggs of other animals will be made later (p. 461). Previous to the publication of my results ('08, '09) no granules resembling those of the pole-disc had been discovered in the eggs of Chrysomelid beetles, although Wheeler ('89), Lecaillon ('98) and others had studied various species belonging to this family. Wieman ('10a) has attempted to determine the origin of these granules, using the germ cells of Leptinotarsa signaticollis for this purpose. His conclusion, which was arrived at from circumstantial evidence, is "that the granules of the pole-disc consist of particles derived from the food stream of the ovum that form an accumulation in the protoplasm in its posterior part" (p. 187). In a previous paper (Hegner, '09a) I have

described and figured the pole-disc in the egg of Leptinotarsa decemlineata which was preserved shortly before deposition. Since that time a complete series of oocytes have been prepared and examined for the purpose of tracing the history of these granules.

The positions of the stages to be described are indicated in the diagram (fig. 9) and the nuclear and cytoplasmic structures are shown in figures 58 to 67. Two oocytes and a neighboring epithelial cell from position 58 in figure 9 are shown in figure 58. The nuclei of the oocytes are large and contain a distinct spireme; the cytoplasm is small in amount and apparently homogeneous. After a short period of growth, the oocytes form a linear series in the ovarian tubule and become connected with the spaces between the nurse cells by means of eggs strings (fig. 9, *e.s.*) through which the nutritive streams flow into the oocytes. One of the youngest of these oocytes is represented in figure 59 (position 59 in fig. 9). The nucleus is not larger than in those of the earlier stage; its chromatin forms a reticulum, and a distinct nucleolus is present. The cytoplasm, on the other hand, has trebled in amount and within it are imbedded a number of spherical bodies which stain with crystal violet after Benda's method, and appear to be mitochondrial in nature. At a slightly later stage (fig. 60, position 60 in fig. 9) the nucleus is larger and contains several small spherical chromatic bodies besides the nucleolus. The cytoplasm has increased more rapidly in volume and a corresponding increase in the number of mitochondrial granules has also taken place. Further growth results in an increase in the volume of both nucleus and cytoplasm (fig. 61, position 61 in fig. 9), and a slight increase in the number of mitochondria. Whether these bodies developed de novo or by division of the preexisting granules could not be determined.

In succeeding stages growth is very rapid. The cytoplasm (fig. 62, position 62 in fig. 9) still remains homogeneous except for the mitochondria which increase slightly in size and become situated as a rule near the periphery. The nucleus at this time contains a large number of chromatin granules and a diffuse reticulum. Part of an older oocyte is shown in figure 63 (posi-

tion 63 in fig. 9); the cytoplasm has taken a reticular appearance; the mitochondrial granules are present in greater numbers, and the nucleus is larger, oval in shape and contains a distinct reticulum with many chromatin bodies of various sizes. A still older oocyte (fig. 64, position 64 in fig. 9) is interesting, particularly because of the rapid increase in the mitochondria and the localization of these near the periphery. From this stage on the character of the contents changes until, as shown in figure 9, the central part of the oocyte (*ooc*) consists of homogeneous cytoplasm (*cy*), and the outer region of cytoplasm is crowded with granules and spherical bodies of various sizes. Apparently the mitochondria lying near the periphery (figs. 65 and 66) increase in size, gradually losing their affinity for the crystal violet stain and swelling up until they constitute the large yolk globules so numerous in the mature egg. All stages in the evolution of these bodies are illustrated at this time as represented in figure 66. In the meantime material is brought into the egg through the egg string from the nurse cells, thus probably adding several sorts of granules to the contents of the oocyte.

To determine the origin of the pole-disc granules it is necessary to trace the various bodies in the oocytes and the nurse stream up to the time when the pole-disc appears. An egg just before this structure becomes visible the posterior end, as shown in figure 66, consists of cytoplasm, more or less reticular, containing yolk globules of various sizes and a number of small granules of a mitochondrial nature. It is impossible at present to state definitely, however, that these granules increase in number by division to form the pole-disc, or are added to from neighboring regions, since no intermediate stages between the condition here represented and that of the completely formed pole-disc were discovered. Wieman ('10a) claims that the pole-disc granules come from the nutritive stream. I admit that this may be true, but it seems more probable from my preparations that they evolve from granules of mitochondrial nature which, as we have seen, may be traced from the young oocytes.

The origin of the pole-disc granules has not, therefore, been definitely determined and, as in previous communications, it

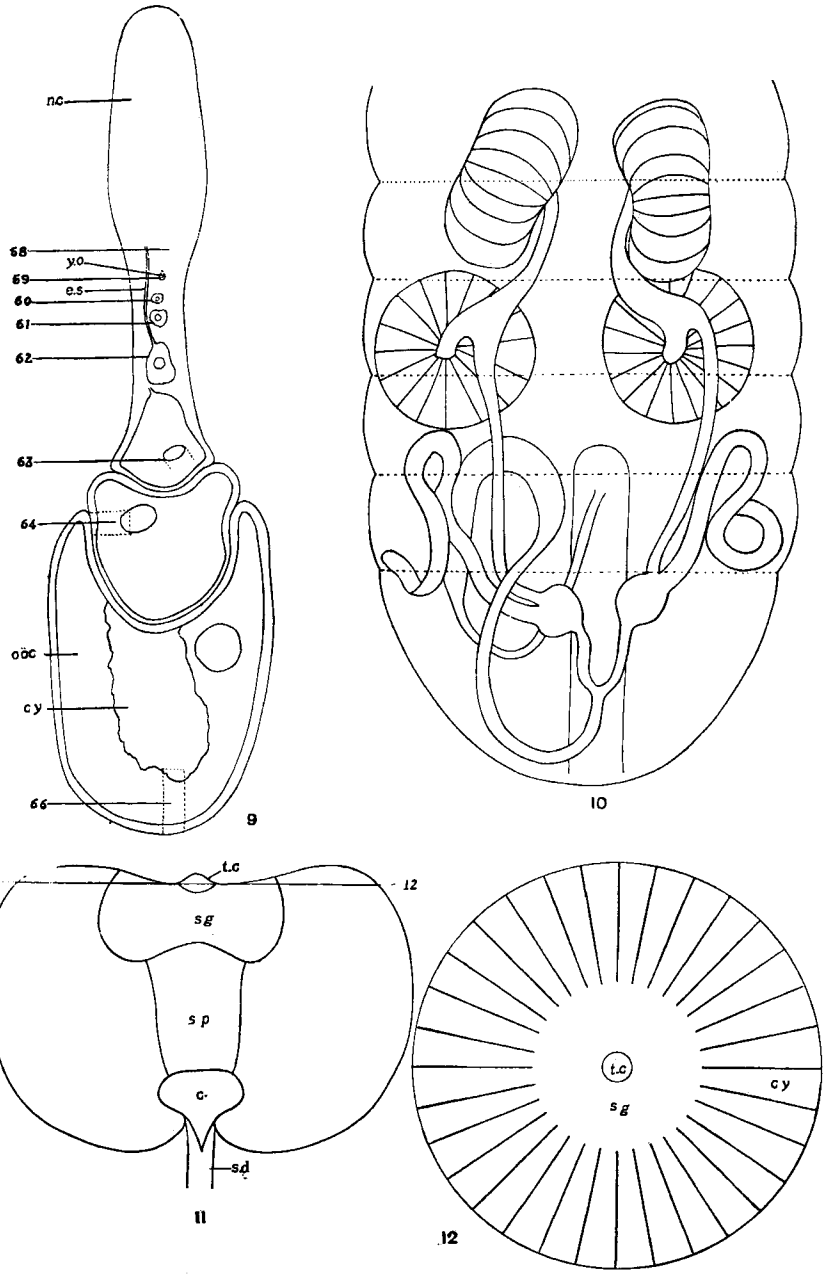
is only possible to suggest that they may be derived from (1) the cytoplasm of the egg, (2) the cytoplasm of the nurse cells, (3) chromatin from the germinal vesicle, (4) chromatin from the nurse cells, (5) nucleolar substance from either germinal vesicle or nurse cells or both, and (6) bodies of mitochondrial nature.

(*3*) *The spermatogonial divisions and formation of cysts in the testes of Leptinotarsa decemlineata.* The material on which this section is based consists of a complete series of stages from the time the male germ gland can be recognized in the half grown embryo to the adult condition. Embryos and young larvae were fixed in toto in hot Tower's fluid and sectioned entire. The germ glands of older larvae, pupae, and adults were dissected in Ringer's solution and transferred at once to the desired fixing fluid. Meves', Gilson's, and Carnoy's solutions were chiefly used for this purpose. Sections were stained with iron hematoxylin, Mayer's acid hemalum, and safranin and light green. All of the figures were drawn from preparations made from material fixed in Carnoy and stained with iron hematoxylin.

The form and position in the abdomen of the adult male reproductive organs are shown in figure 10. There are two pairs of testes, one pair on either side of the body. Each testis consists of a large number of follicles radiating out from near the center. Figure 11 is a diagram of a longitudinal section made from the testis of an old larva. At the lower end is attached the sperm duct (*s.d*) which is connected with a cavity (*c*) within the testis. Just above this cavity is a region containing spermatozoa; above this region is a mass of spermatogonia (*sg*) not yet within cysts; and this mass is capped by a small group of epithelial cells (*t.c.*). The major part of the testis is composed

Fig. 9 Leptinotarsa decemlineata; diagram of an ovarian tubule showing various stages in the development of the oocyte. The figures refer to the positions of cells shown in the figures in plate 9.

Figs. 10 to 12 Leptinotarsa decemlineata. 10, Reproductive organs of male. 11, Longitudinal section through testis of full grown larva. 12, Transverse section through testis of full grown larva in region indicated by line labelled 12 in fig. 11. *c*, cavity of testis; *cy*, region of cysts; *s.d*, sperm duct; *sg*, spermatogonia; *sp*, spermatozoa; *t.c*, terminal cap.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of radiating follicles containing cysts of spermatogonia, spermatocytes, or spermatozoa (*cy*). A transverse section through the distal end of a testis is shown in figure 12 (position 12 in fig. 11). In the center is the terminal cap (*t.c*); surrounding this is a mass of spermatogonia not yet formed into cysts (*sg*); and from this the testicular follicles radiate out to the periphery. Wieman ('10b, '10c) has traced the development of the testis through the larval and pupal stages and I have nothing to add to his account. I wish, however, to describe certain stages in the divisions of the spermatogonia during cyst formation, since Wieman has reported the occurrence of amitotic nuclear division at this time, and I have discovered some cellular connections which makes it possible to compare certain processes in the testis with those known to take place during the differentiation of nurse cells and oocytes in the ovaries of several insects.

In that region of the testis surrounding and underlying the terminal cap (figs. 11, 12, *t.c*) there are a large number of spermatogonia not yet contained in cysts. All stages in cyst formation may be observed here, not only in larval testes but also in those of pupae and adults. The youngest spermatogonia are those lying near the terminal cap. Figure 68 shows a few cells of the terminal cap (*t.c.*) some of the neighboring spermatogonia (*spg*), and several of the epithelial cells (*ep*) which are scattered about among the spermatogonia. Cysts are formed toward the edge of the spermatogonial mass away from the terminal cap and figures 69 to 74 represent certain of the stages observed. The spermatogonia divide apparently exclusively by mitosis. A well developed spindle is formed and this persists after the cell wall has separated the two daughter cells. The spindle fibers which are at first perfectly distinct (fig. 69) unite into a compact strand (fig. 70) which stains dense black in iron hematoxylin after fixation in Carnoy's fluid. In many cases it was impossible to distinguish an intervening cell wall between the daughter nuclei (fig. 71). In either case, however, the spindle remains persist, forming a basic-staining strand with enlarged ends connecting the two nuclei. Since at this time and in all later stages the two or more spermatogonia may be found surrounded

by an envelope of epithelial cells it seems certain that, as Wieman ('10b) maintains, the spermatozoa in a single cyst are derived from a single spermatogonium.

A cyst containing four spermatogonia is represented in figure 72. Here again appear the strongly basic-staining spindle remains connecting the nuclei. These black strands persist until the succeeding mitotic division occurs as figure 73, which was drawn from a section of a cyst containing eight spermatogonia, shows. Spindle remains are still evident in later stages, as in figure 74, which represents a cyst containing thirty-two spermatogonia, but were not observed in cysts containing more than sixty-four cells.

Many investigators have figured spermatogonial divisions which result in rosette-like groups of cells similar to that represented in figure 73. Apparently, however, the remains of the spindle, if present, did not possess such a strong affinity for basic stains. Furthermore, only those of my preparations which were fixed in Carnoy's fluid and stained in iron hematoxylin exhibited these black strands. Similar spindle remains have been observed in Dytiscus (especially by Günthert, '10) and Carabus (Govaerts, '13) during the differentiation of nurse cells and oocytes from oogonia, and there can be little doubt but that the process of cyst formation in the male, as described above, is similar to the differential divisions in the female and may also be compared with the differentiation of spermatocytes and Sertoli cells in mammals (Montgomery, '11; von Winiwarter '12).

In Dytiscus Günthert found that the chromatic mass eliminated from the nucleus always passed to the pole of the oogonium containing the 'Spindelreste.' Govaerts ('13) has pointed out that, although in Carabus and Cicindela no such chromatic mass is demonstrable, still a distinct 'residu fusorial' exists, and that during the differential divisions one cell differs from its sister in the possession of these spindle remains. Polarity is held responsible for the localization of the 'residu fusorial' and the cause of the differential divisions is therefore considered to be a 'polarité pre-differentielle.'

The discovery of these distinct spindle remains in the spermatogonial divisions enables us to homologize one more period in the cycle of the male germ cells with a corresponding period in the cycle of the female germ cells.

Thus the ultimate spermatogonium passes through a certain number of divisions—probably five or six—which correspond to the differential divisions so clearly exhibited by the ultimate oogonia of Dytiscus. Just as in the maturation processes, however, where only one female cell but all of the male cells are functional, so these earlier divisions result in the female in the production of a single oocyte and a number of nurse cells which may be considered abortive eggs, whereas in the male every daughter cell is functional. The limited period of division in the cycle of the male germ cells in man (Montgomery, '11; von Winiwarter, '12) is also similar to those in Dytiscus and Leptinotarsa.

(*4*) *Amitosis in the germ cells of Leptinotarsa.* We have already described what appears to be amitotic nuclear division in the vitellophags of Chrysomelid eggs, and shall now examine certain stages in the germ cell cycle where amitosis has been reported.

Wilson ('00) defines amitosis as "mass-division of the nuclear substance without the formation of chromosomes and amphiaster" (p. 437), and concludes from a review of the literature up to the year 1900, "that in the vast majority of cases amitosis is a secondary process which does not fall in the generative series of cell-divisions" (p. 119). During the past ten years interest in direct nuclear division has been maintained, principally because of the claims of certain investigators that germ cells may multiply in this way and still give rise to functional eggs or spermatozoa.

During amitosis the chromatin remains scattered within the nucleus and does not form a spireme nor chromosomes, and therefore its individual elements, the chromatin granules, do not divide. As a result of this *mass*-division there can be no accurate segregation of chromatin granules in the daughter nuclei as is demanded by the theory that the nucleus, and partic-

ularly the chromatin, contains the determiners of hereditary characteristics. Furthermore, nuclear division without the formation of chromosomes obviously condemns the hypothesis of the genetic continuity of the chromosomes, and hence seriously interferes with current ideas regarding the significance of the accessory chromosomes in the deterimnation of sex. Among the animals in whose germ cells amitosis has been reported are certain amphibia, coelenterates, cestodes, and insects.

Amphibia. Vom Rath ('91, '93), Meves ('91, '95) and McGregor ('99) have recorded amitosis in the germ cells of Amphibia. Meves claims that the spermatogonia of Salamandra divide amitotically in the autumn but return to the mitotic method in the spring, later giving rise to functional spermatogonia. Vom Rath finds amitosis but contends that the cells which divide in this way do not become spermatozoa but are degenerating, being used as nutritive material by the other spermatogonia. The amitotic divisions described by McGregor ('99) in Amphiuma differ in certain respects from those of both Meves and vom Rath. In this species the primary spermatogonia divide by amitosis, their products later divide by mitosis and produce functional spermatozoa. Our knowledge concerning amitosis in the spermatogonia of Amphibia is therefore in an unsatisfactory state, although the observations of Meves and McGregor argue strongly in favor of this method.

Coelenterata. While no direct nuclear divisions were recorded by Hargitt ('06) in the germ cells of Clava leptostyla the absence of mitotic figures in the early cleavage stages of the egg led him to the conclusion that the 'nuclear activity differs greatly from the ordinary forms of mitosis, and appears to involve direct or amitotic division" (p. 229). If this were true the germ cells which are derived from these cleavage cells must be descended from cells which once divided amitotically. This case of supposed amitosis has been cleared up by the subsequent studies of Beckwith ('09) who collected material of Clava very early in the morning and found typical mitotic divisions during the maturation and early cleavage of the egg and no evidence of amitosis.

Cestoda. Child concluded ('04) from a study of the cestode Moniezia expansa that the amitotic method of cell division occurs in the antecedents of both the eggs and the spermatozoa. This writer has published a series of papers upon this subject using Moniezia expansa and M. planissima for his material ('04, '06, '07, '10), and his principal conclusion is that in these species the division of the cells destined to become eggs and spermatozoa is predominantly amitotic. Mitotic division also occurs but comparatively rarely. Cells which have divided amitotically then divide mitotically during maturation and form typical ova.

The nature of the nuclear division in the cestodes was later investigated by Richards ('09, '11) who studied the female sex organs of the same species employed by Child as well as material obtained from Taenia serrata. Richards finds that mitosis unquestionably occurs in the young germ cells but was unable to demonstrate amitosis. Richards claims that amitosis cannot be demonstrated except by the observation of the process in the living material and the subsequent study of this material by cytological methods. Child ('11) agrees with Richards that amitosis cannot be demonstrated in fixed material but nevertheless concludes after an examination of Richard's preparations "that direct division plays an important part in the developmental cycle of Moniezia, in the germ cells as well as in the soma" (Child, '11, p. 295).

Finally Harman ('13) was unable to find any evidence of amitotic divisions in the sex cells of either Taenia teniaeformis or Moniezia and concludes that the conditions which suggest amitosis can just as well or better be explained by mitosis. Experiments with living cells of Taenia were without results, since the cells did not divide when placed in Ringer's solution, although they continued to live outside the body of the host for forty-eight hours. Morse ('11) likewise failed to observe divisions in living cells of Calliobothrium and Crossobothrium which were kept in the plasma of the host.

Insecta. In the Hemiptera amitosis was described by Preusse ('95) in the ovarian cells of Nepa cinerea and similar conditions were reported by Gross ('01) in insects of the same order. Gross,

however, claims that the cells which divide amitotically do not produce ova but are degenerating or secretory.

Foot and Strobell ('11) described in ovaries of Protenor, the amitotic division of certain cells which later produce ova. There is, however, considerable difference of opinion among investigators as to the origin of the ova from the various regions of the insecta ovary and, since Payne ('12) has shown that in Gelastocoris the cells which apparently multiply amitotically do not produce ova, it seems safe to conclude that in Protenor the ova are not descended from cells that divide amitotically.

Amitotic division of germ cells followed by mitotic division has been described by Wieman ('10b, '10c) in the ovaries and testes as well as in the nurse cells of Leptinotarsa signaticollis. Germ cells in both ovary and testis taken from full grown larvae were found in stages of division recognized by Wieman as amitotic. It was difficult to demonstrate actual division of the cytoplasm but that such a division really occurs was inferred because binucleated cells apparently gave rise to spermatocytes with single nuclei. Rapid cell division is assumed by Wieman to account for amitosis. This is brought about by fluctuations in the nutritive supply, or in the case of the testis, by the rapid proliferation of cells during the formation of cysts.

I have studied my preparations of Chrysomelid beetles carefully with the aim of detecting amitotic division and have observed what appears to be direct nuclear division among the nurse cells, but could not demonstrate with certainty this kind of division among the oogonia, or spermatogonia. Three stages in the direct division of nurse cell nuclei in Leptinotarsa decemlineata are shown in figure 67. Oogonia and spermatogonia, however, do not exhibit such clearly defined stages, and after examining my preparations and several slides kindly sent me by Doctor Wieman I am forced to conclude that amitosis has not been demonstrated. It is true that frequently dumb-bell shaped nucleoli occur in certain of the nuclei and frequently two nucleoli are present at opposite ends. Also two nuclei may be surrounded by a single cell wall, but no stages were present which could not be attributed as well or better to mitotic phenomena.

Conclusion. From the evidence at present available we must conclude that amitotic division of the germ cells has not been demonstrated, and that not until such a process is actually observed in living cells will any other conclusion be possible.

4. HYMENOPTERA

A number of papers have appeared which contain references to the germ glands of Hymenoptera (Hegner, '09a, pp. 245–248). The most important of these from the standpoint of the present discussion are (1) Silvestri ('06, '08) on some parasitic species, and (2) Petrunkewitsch ('01, '03), Dickel ('04), and Nachtsheim ('13) on the honey-bee.

In an endeavor to test the 'Dzierzon theory,' that the eggs which produce drone bees are normally unfertilized, Petrunkewitsch ('01–'03) discovered some unusual maturation divisions. In 'drone eggs' the first polar body passes through an equatorial division, each of its daughter nuclei containing one-half of the somatic number of chromosomes. The inner one of these daughter nuclei fuses with the second polar body, which also contains one-half of the somatic number of chromosomes; the resultant nucleus with sixteen chromosomes, the 'Richtungscopulationskern', passes through three divisions giving rise to eight 'doppelkernige Zellen.' After the blastoderm is completed, the products of these eight cells lie in the middle line, near the dorsal surface of the egg, where the formation of the amnion begins; the nuclei of these cells are small, and lie imbedded in dark staining cytoplasm. Later they are found just beneath the dorsal surface near the point of union of the amnion with the head-fold of the embryonic rudiment. They are next located between the epithelium of the mid-gut and the ectoderm; from here they migrate into the coelomic cavities, and finally, at the time of hatching, form a 'wellenartigen' strand, the germ-gland, extending through the third, fourth, fifth and sixth abdominal segments. The fertilized eggs of the bee were also examined by Petrunkewitsch, but no 'Richtungscopulationskern' was discovered. In these eggs "entstehen die Genitaldrüsen

aus Mesodermzellen, die in die Mesodermröhren von der Bauchseite herindringen." Doubt was immediately cast on these results, although Weismann ('04) vouched for their accuracy. Thus Wheeler ('04) says:

> Even in his first paper there is no satisfactory evidence to show that the cells regarded as derivatives of the polar bodies in the figures on plate 4 are really such, and not dividing cleavage cells or possibly vitellophags . . . . When we take up the second paper we wonder how anybody could regard the figures there presented as even an adumbration of proof that the testes of the drone are developed from the polar bodies.

Dickel ('04) could find no connection between the polar bodies and the cells Petrunkewitsch claims originate from the 'Richtungskopulationskern,' but considers these 'Dotterzellen.' Nachtsheim ('13) agrees with Dickel. "Die im Blastodermstadium am Blastoporus liegenden Syncytien sind Dotterzellen, stehen also zu den Richtungskörpern in keiner Beziehung. Sie finden sich in den befruchteten und unbefruchteten Eiern in gleicher Weise, nicht, wie Petrunkewitsch angegeben hat, nur in den letzteren" (p. 198).

The investigations of Silvestri ('06, '08) on parasitic Hymenoptera are of particular interest, since in both the polyembryonic species and those whose eggs produce a single individual, the keimbahn-determinant is a plasmosome which escapes from the germinal vesicle. Silvestri ('06) first studied Copidosoma (Litomastix) truncatellus, a polyembryonic species which lays its eggs in the eggs of the moths of the genus Plusia. In the germinal vesicle of this species are two nucleoli, one chromatic the other plasmatic (fig. 13, *A*). Just before maturation the plasmosome escapes and becomes situated near the posterior end. Maturation occurs near the anterior pole (fig. 13, *B*). First and second polar bodies are formed, and the first divides, thus making three in all (fig. 13, *C*); these remain near the anterior end, whereas the female nucleus comes to lie near the nucleolus at the opposite pole (fig. 13, *C*). In both fertilized and parthenogenetic eggs the maturation processes, the behavior of the nucleolus, and segmentation are similar. The nucleolus is segregated in

one cleavage cell (fig. 13, *D*) during the first and second divisions, and the cell containing it in the four-cell stage (fig. 13, *E*) is situated dorsally. Then the nucleolus becomes vacuolated and its substance slowly surrounds the nucleus, occupying a large

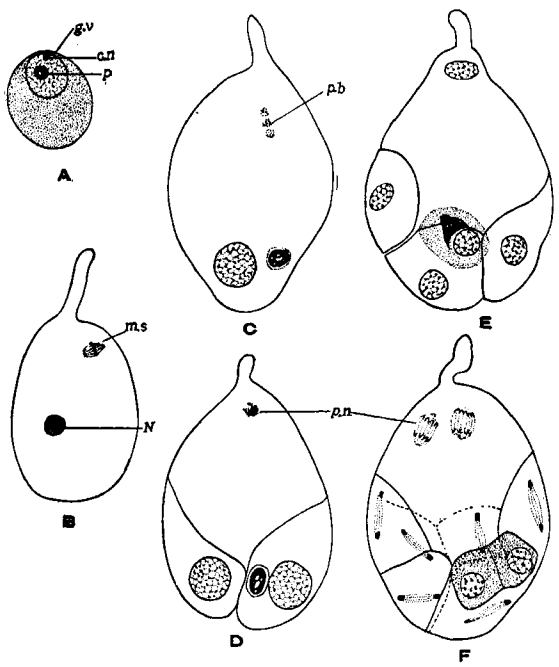


Fig. 13 Copidosoma (Litomastix) truncatellus (redrawn from Silvestri, '06). A, oocyte showing germinal vesicle (*g.v*) containing a chromatin-nucleolus (*c.n*) and a plasmosome (*p*). B, egg, a few minutes after deposition, showing first maturation spindle (*m.s*) and 'nucleolo' (*N*). C, egg about one hour after deposition, showing three polar bodies (*p.b*), the first cleavage nucleus (*c.n*), and the 'nucleolo.' D, egg in two-cell stage, about one and one-half hours old. *p.n*, polar nucleus. E, egg about one and one-half hours old; in four-cell stage. F, egg about four and one-half hours old showing two polar nuclei dividing, two embryonic cells containing nucleolar substance, and six embryonic cells (dividing) without nucleolar substance.

part of the cytoplasm. Division of this cell is not synchronous with that of the other cleavage cells but is slightly slower. When it does divide each daughter cell receives a share of the nucleolar substance (fig. 13, *F*). Silvestri did not trace the cells contain-

ing the substance after the fourth cleavage division when there were four of them present, but he thinks this can be done and expresses his ideas regarding their history and potency. Two embryonic regions are formed in Copidosoma (1) an anterior 'massa germinigena' which produces normal larvae, and (2) a posterior 'massa monembrionale' which produces larvae without genital, respiratory, circulatory, and excretory systems; these he calls sexless larvae. He believes that the cells provided with nucleolar material are germ cells, whereas those lacking this substance are somatic cells, and that the 'massa monembrionale' contains only somatic cells, hence the larvae derived from this region are sexless.

Further studies were made by Silvestri ('08) on other species of parasitic Hymenoptera, and several interesting variations in the behavior of the nucleolus were observed. In Ageniaspis (Encyrtus) fuscicollis, and A. fuscicollis praysincola the structure of the egg is similar to that of Copidosoma and the nucleolus becomes situated in one of the first two blastomeres. The cell with the nucleolus divides more slowly than the other, and, before its cleavage, the nucleolus breaks up into granules which are distributed between the daughter cells. The cleavage stages thus are as follows: (1) A two-cell stage, one cell with the nucleolus; (2) a three-cell stage, one cell with and two without nucleolar material; (3) a four-cell stage, two cells with and two without nucleolar material; (4) a six-cell stage, two with and four without nucleolar material; and (5) a twelve-cell stage, four with and eight without nucleolar material. The further history of the cells containing nucleolar material was not determined.

In Encyrtus aphidivorus, which is not polyembryonic, the nucleolus remains at the posterior end of the egg until a late period of cleavage; then its substance becomes distributed among the primordial germ cells, which, as in Copidosoma and Ageniaspis, divide more slowly than the somatic cells. In this case there seems to be no doubt that the cells containing nucleolar material become germ-cells, whereas all of the rest become somatic cells.

The structure of the egg, formation of polar bodies, segmentation, and distribution of the nucleolar substance was found to be similar in Oophthora semblidis to these processes in Encyrtus aphidivorus (fig. 14).

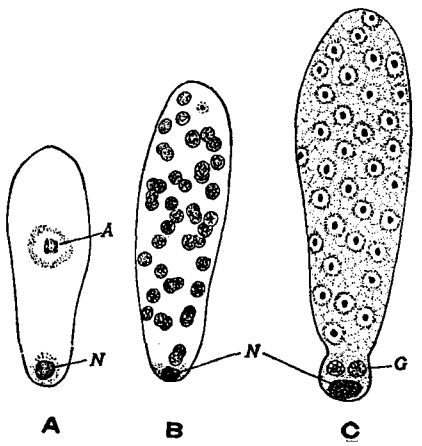


Fig. 14 Oophthora (redrawn from Silvestri, '08). A, egg with germinal vesicle (*A*) and 'nucleolo' (*N*). B, egg containing many cleavage nuclei. C, formation of primordial germ cells (*G*) at posterior end of an egg.

II. THE ORIGIN AND SIGNIFICANCE OF THE KEIMBAHN-DETERMINANTS IN ANIMALS

In the following pages the writer has attempted to describe briefly the history of the Keimbahn-determinants in animals (except the insects), and to determine the genesis, localization, distribution, and fate of these substances. The literature of this subject is rather large and widely scattered, so it was considered advisable to provide selected figures from original sources wherever they would aid in making the discussion more clear. The data regarding the insects have been set forth in the first paper of this series; they will of course be included in the general considerations in this contribution.

1. THE KEIMBAHN IN THE CRUSTACEA

The Keimbahn in the Crustacea is best known in certain Cladocera and Copepoda. Of special interest are the investigations of Grobben ('79), Weismann and Ischikawa ('89), Haecker ('97), Amma ('11), and Kühn ('11, '13).

Grobben ('79) studied the embryology of Moina rectirostris and gives a remarkably fine account of early cleavage stages considering the early date when the work was done. He figures stages showing a foreign body which he considered a polar body, segregated in one of the early blastomeres, the segregation and characteristics of the primordial germ cell and the first entoderm cell, and the division and later history of the germ cells. His results have been, in the main, confirmed by Kühn ('11, '13) and, since the work of the latter has been done with the aid of better methods, an account of Grobben's observations is not necessary here.

The following description quoted from Weismann and Ischikawa ('89) is a brief but adequate account of the discoveries of these authors (fig. 15):

> Die Thatsachen sind, kurz zusammengefasst, die folgenden. In dem befruchtungsbedürftigen Winterei von sechs Arten von Daphniden, welche vier Gattungen angehören bildet sich während der Ovarialentwicklung des Eies eine Zelle in der Eizelle, an Volumen viel kleiner als diese und wie ein fremder Eindringling langsam in ihr sich umherbewegend. Sie entsteht, indem in dem noch jungen und dotterlosen Ei (Moina) ein Theil der Kernsubstanz activ aus dem Keimbläschen in die umgebende Protoplasmamasse austritt, sich zu einem wirklichen Kern (Paranucleus) organisirt und zugleich sich mit einem Zellkörper umbüllt.
>
> Bei der Eiablage gleitet die 'Copulationszelle,' in der Masse des Eikörpers gelegen, mit in den Brutraum und verhält sich zunächst ganz passiv. Nachdem aber die Befruchtung durch eine inzwischen eingedrungene Samenzelle stattgefunden, der Furchungsprocess seinen Anfang genommen und sich mehr oder weniger weit fortgesetzt hat, bewegt sich die Copulationszelle auf eine der im Innern des Dotters versenkten Furchungszellen los, streckt kurze Fortsätze aus und verschmilzt mit ihr in einem förmlichen Copulationsact, indem zuerst die Zellkörper, dann die Kerne der beiden Zellen zusammenschmelzen. Bei zwei Arten geschieht dies schon im Stadium von 2 Furchungszellen, bei den vier andern erst im Stadium von 8 Furchungszellen.....

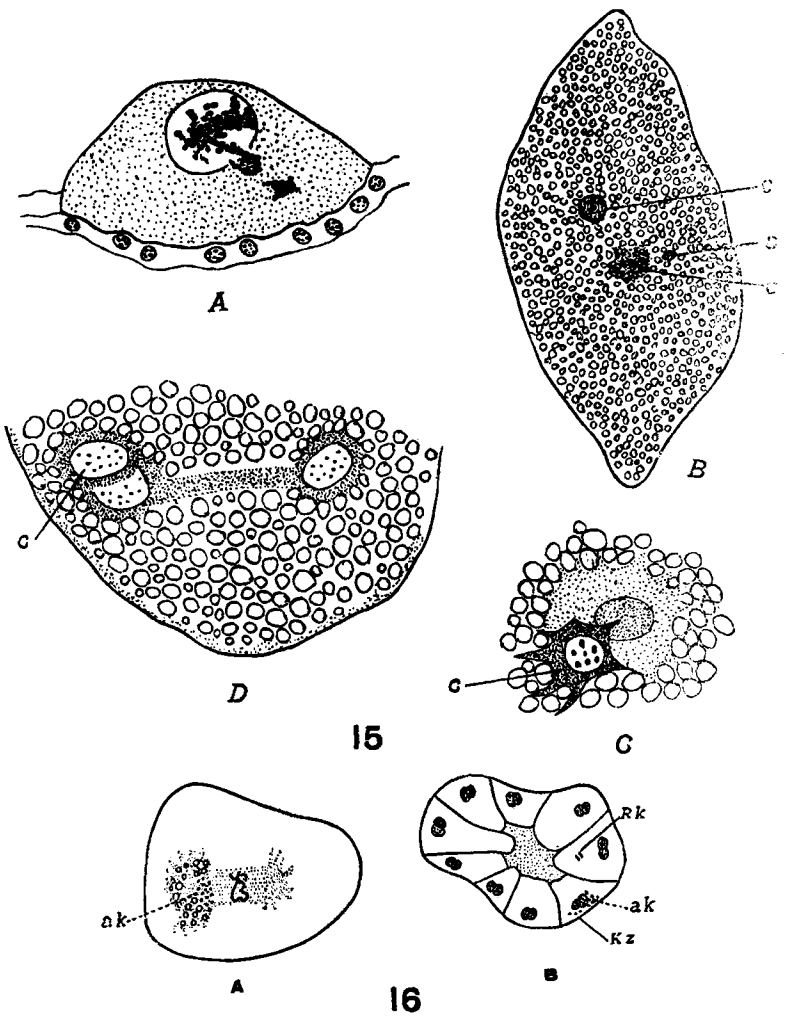


Fig. 15 Moina paradoxa (redrawn from Weismann and Ischikawa, '89). A, egg with substance escaping from germinal vesicle. B, egg containing egg nucleus (*e*), sperm nucleus (*s*) and 'Copulationszelle' (*C*). C, cleavage cell and 'Copulationszelle' (*c*) fusing. D, cleavage cell and 'Copulationszelle' fusing.

Fig. 16 Cyclops (redrawn from Haecker, '97). A, egg showing 'Aussenkörnchen' (*ak*) at one end of first cleavage spindle. B, thirty-two cell stage showing 'Aussenkörnchen' (*ak*) in the primordial germ cell (*Kz*). *Rk*, polar bodies.

Die erstere Thatsache lässt freilich vermuthen, dass es immer dieselbe Furchungszelle sei, mit welcher die Copulationszelle sich verbindet, die letztere deutet darauf hin, dass der Vorgang mit der geschlechtlichen Fortpflanzung etwas zu thun hat (pp. 182–183).

The Keimbahn of Cyclops and some closely allied forms has been very carefully investigated by Haecker ('97) and Amma ('11), with results which are of particular interest so far as germ cell determinants are concerned. In Cyclops, according to Haecker, 'Aussenkörnchen' arise at one pole of the first cleavage spindle (fig. 16, *A*, *ak*); these are derived from disintegrated nucleolar material and are attracted to one pole of the spindle by a dissimilar influence of the centrosomes. During the first four cleavage divisions the granules are segregated always in one cell (fig. 16, *B*, *kz*); at the end of the fourth division these 'Aussenkörnchen' disappear, but the cell which contained them can be traced by its delayed mitotic phase, and is shown to be the primordial germ cell.

The most recent and complete account of the Keimbahn in Copepoda is that of Amma ('11). This author studied the early cleavage stages of eleven species of Cyclops, three species of Diaptomus, one species of Canthocamptus, and one species of Heterocope. Cyclops fuscus var. distinctus is made the basis for the most detailed study, but short descriptions and figures are presented of the others. In all of the sixteen species examined, the stem-cell, which gives rise to the primordial germ-cell, may be recognized, as Haecker ('97) discovered in Cyclops, first by the presence of granules which do not occur in the other cleavage cells, and later by a delayed mitotic division. The process is essentially as described by Haecker.

The following summary of the Keimbahn in Cyclops fuscus var. distinctus is given by Amma:

1. Während der ersten Furchungsteilungen ist eine bestimmte Folge von Zellen, die Keimbahn, durch das Auftreten von Körnchen, die sich bei der Teilung jewels um einen Spindelpol der Teilungsfigur ansammeln, gekennzeichnet (fig. 17, *A*).

2. Die Körnchen oder Ectosomen entstehen immer erstmals während des Stad ums der Diakinese, vermehren sich während der nächst folgenden Phasen noch bedeutend und verschmelzen gegen das Ende

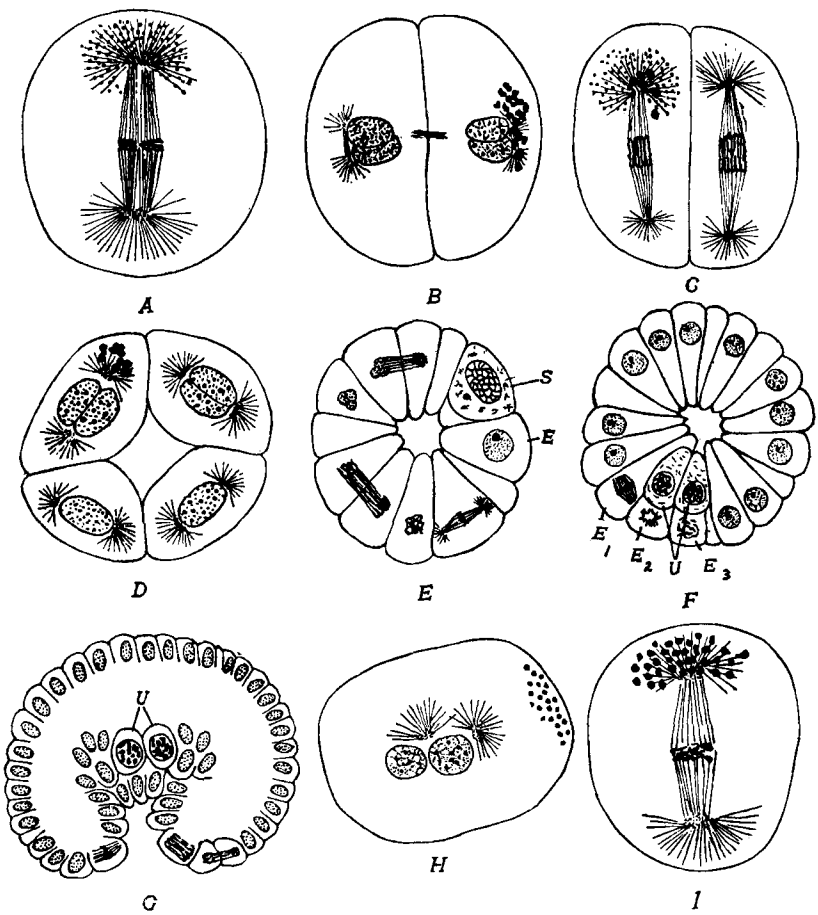


Fig. 17 Cyclops fuscus var. distinctus (*A-G*): Diaptomus coeruleus (*H*), Cyclops vitidis (I); (redrawn from Amma, '11). A, ectosomen at end of first cleavage spindle. B, two-cell stage; ectosomes dissolving. C, old and newly formed ectosomes at end of one of second cleavage spindles. D, eight-cell stage; ectosomes dissolving in stem-cell. E, sixteen- to twenty-eight-cell stage. *S*, cell with, *E*, cell without granules. F, one hundred and twelve-cell stage, with two primordial germ cells (*U*) and three ectoderm cells (*E*). G, two hundred and forty-cell stage. *U*, primordial germ cells. H, appearance of ectosomes before cleavage spindle forms. I, increased production due to carbonic acid gas.

der Teilung zu grösseren, unförmigen Brocken, welche allmählich während des Ruheperiode der Zelle aufgelöst werden. (fig. 17, *B*)

3. Die neue Körnchenzelle geht stets vom körnchenführenden Produkte der alten Körnchenzelle hervor, was direkt dadurch bewiesen werden kann, dass sich in der neuen Körnchenzelle immer noch unaufgelöste Überreste der Ectosomen der alten Körnchenzelle vorfinden; alle Körnchenzellen stammen somit in direkter Linie von einander ab. (fig. 17, *C*).

4. Vom II—Zellenstadium an bleibt die Körnchenzelle immer in der Teilung hinter den andern Furchungszellen zurück; es ergibt sich eine Phasendifferenz, welche in immer stärkeren Masse in den höheren Furchungsteilungen zunimmt (fig. 17, *D.E*).

5. Aus dem körnchenführenden Produkte der Körnchenzelle des vierten Teilungsakts, der Stammzelle *S*, gehen, nachdem diese sich an dem fünften Furchungsschritte nicht beteiligte, gegen Ende des sechsten, im LX—Zellenstadium, die beiden definitiven Urgeschlechtszellen hervor; bei dieser Teilung der *S*-Zelle erscheinen die Ectosomen in ganzen Zellraume. (fig. 17, *F*).

6. In Ausnahmefällen beginnt die *S*-Zelle sich etwas rüher zu teilen, nämlich schon während des Übergangs des XXX—zum LX—Zellenstadium.

7. Die Urgeschlechtszellen verlieren den Verband mit dem Blastoderm, sie werden allmählich in die Tiefe gedrängt (fig. 17, *G*), (pp. 529–530).

An important departure from the usual method of origin of the 'Ectosomen' is recorded from Diaptomus coeruleus. Amma says concerning the process in this species, that whereas "bei andern Formen die Ectosomen bei der ersten Furchungsteilung gewöhnlich erst im Stadium der Diakinese oder der Aquatorialplatte zum Vorschein kommen, treten sie hier schon vor dem Stadium der Copulation der Geschlechtskerne auf" (fig. 17, *H*).

The origin and nature of the Ectosomen are considered by Amma at some length. The hypothesis that these granules arise by the splitting of particles of chromatin from the chromosomes as occurs in Ascaris is rejected (1) because in one species, Dipatomus coeruleus (fig. 17, *H*), the Ectosomen appear before the nuclear membrane breaks down in preparation for the formation of the first cleavage spindle, and (2) because the Ectosomen do not stain as deeply as chromatin but only slightly darker than the cytoplasm. The origin of the Aussenkörnchen (Ectosomen) from the nucleolus, as considered probable by Haecker ('97), could not be confirmed. The condition in Diaptomus coeruleus

is also a serious objection to this theory. The Ectosomen are different from chromidia since chromidia arise from the nucleus and

. . . . man gewinnt im Gegenteil entscheiden den Eindruck, dass die Körnchen ganz unabhängig von den Kernsubstanzen, völlig autogen im Zellplasma entstehen (p. 553).

Wir haben also offenbar in den Chondriosomen und Ectosomen zwei wesentlich voneinander verschiedene Arten von Gebilden vor uns, denen nicht dieselbe Entstehungsursache und dieselbe Bedeutung zukommt (p. 555).

Aus dem ganzen Verlaufe der Körnchenentwicklung geht nun soviel mit Sicherheit hervor, dass man es bei den Ectosomen mit vergänglichen Gebilden zu tun hat, denen keine weiteren Funktionen zukommen, die im Leben der Zelle nicht weiter verwendet werden. In den Prophasen der Kernteilung entstehen die Körnchen zunächst als feine Tröpfchen im Zellplasma; im weiteren Verlauf der Teilung erfahren sie dann noch eine Zunahme, bis sie ungefähr im Stadium des Dyasters ihre höchste Entwicklung erreicht haben. Von hier ab beginnt der regressive Prozess der Körnchen: sie fliessen zu grösseren, unförmigen Klumpen zusammen, welche vom Zellplasma allmählich vollständig resorbiert und aufgelöst werden. Bei der nächsten Teilung der Keimbahnzelle erscheinen dann die Ectosomen wieder von neuem. Um ein einfaches Unsichtbarwerden während der Zellenruhe, wie es, z.B. vom Centrosoma von vielen Forschern angenommen wird, kann es sich bei den Ectosomen nicht handeln, denn vielfach konnten ja neben den neuen, frisch entstandenen Ectosomen noch die Überreste der Ectosomen der letzen Körnchenzelle nachgewiesen werden. Es erfolgt also bei jedem neuen Teilungsschritte tatsächlich eine *Neubildung und Wiederauflösung* der Körnchen.

Gestützt auf diese Tatsachen, möchte ich nun die Ansicht vertreten, dass die Ectosomen als *Abscheidungen, Endprodukte des Kern-Zelle-Stoffwechsels* aufzufassen sind, welche zu bestimmten Zeiten im Plasma der Zelle zur Abscheidung gelangen und wieder aufgelöst werden (p. 557).

According to Amma, if the above hypothesis be correct, a greater amount of Ectosomen would be present if an egg were allowed to develop in carbonic acid gas. The results of a number of experiments with oxygen and carbonic acid gas indicate that a greater amount of Ectosomen occur when the egg is developed in the latter as shown in figure 17, *I*, of an egg of Cyclops viridis placed one hour after deposition in carbonic acid gas for one hour.

When various stains were used it was found that the Ectosomen became colored much like the cytoplasm. For example, when stained in methylen blue followed by eosin, the chromosomes were blue and the Ectosomen and cytoplasm red, and when stained by the methy lgreen-fuchsin-orange G method of Heidenhain the chromosomes were green and the cytoplasm and Ectosomen red.

Amma also attempts to explain the fact that the Ectosomen appear at only one end of the first cleavage spindle and in only one of the cleavage cells until the two primordial germ cells are formed. He rejects Haecker's hypothesis that the centrosomes possess an unequal influence upon the Ectosomen and that one centrosome attracts all of them because it is stronger than the other, and is inclined to favor the idea that the Ectosomen are the visible evidence of an organ-forming substance which is thus distinguished from the rest of the cytoplasm as 'Körnchenplasma.' Amma's statement is, *"dass im Zellplasma des noch ungefurchten Copepodeneies ein vom übrigen Eiplasma qualitativ verschiedenes Körnchenplasma existiert, welches die organbildende Substanz, die Anlagesubstanz für die Geschlechtsorgane darstallt"* (p. 564).

Kühn ('13) has studied the Keimbahn in the summer egg of a Cladoceran, Polyphemus pediculus, and has confirmed certain parts and corrected other portions of the work done by earlier investigators—Grobben ('79), Samassa ('93), and Weismann and Ischikawa ('89). In this species usually one (but sometimes two or three) of the nurse cells (fig. 18, *A*) passes into the egg before cleavage. This cell (or cells) becomes imbedded near the periphery at the vegetative pole (fig. 18, *B*, *n*). During each of the early cleavage divisions this nurse cell is confined to one cell (fig. 18, *C–E*) which gives rise during the third cleavage (8 to 16-cell stage) to the primordial germ cell, containing the remains of the nurse cell (fig. 18 *E*, *K*) and to the primordial entoderm cell which does not receive any part of the nurse cell (fig. 18, *E*, *e*). The primordial germ cell and primordial entoderm cell do not divide as quickly as the other blastomeres during the succeeding cleavage stages, a fact that aids in their identification. While the egg is undergoing cleavage, the nurse cell is gradually chang-

ing so that when the sixteen-cell stage is reached it has become disintegrated into dark staining granules and fragments of various forms and sizes (fig. 18, *E*). During the division of the 'Keimbahnzelle' (From 16-32 cell stage) these granules and fragments are about equally distributed between the daughter cells (fig. 18, *F*). A similar distribution takes place in succeeding divisions of the primordial germ cells and this is accompanied by a further decrease in the size of the dark staining granules.

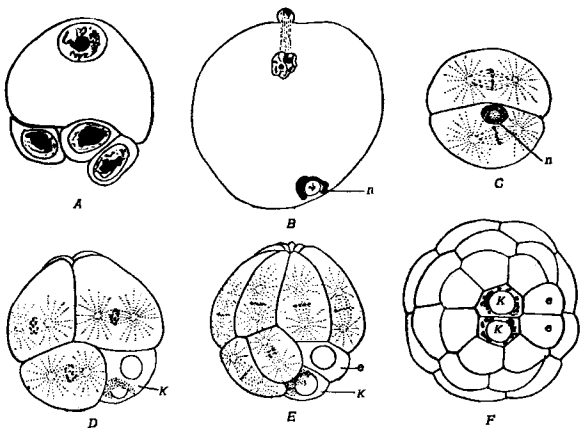


Fig. 18 Polyphemus pediculus (redrawn from Kühn, '11, '13). A, egg with three nurse cells. B, egg at close of maturation. *n*, 'Nahrzellenkern.' C, two-cell stage; view of vegetative pole. D, eight to sixteen-cell stage. *K*, 'Keimbahnzelle.' E, sixteen- to thirty-cell stage; *e*, entoderm cell. F, thirty-two-cell stage from vegetative pole. *K*, primordial germ cells; *e*, entoderm cells.

A blastula of 236 cells is figured by Kühn which shows at the vegetative pole four primordial germ cells lying next to eight entoderm cells and bordered by twelve mesoderm cells. During gastrulation this group of twenty-four cells becomes surrounded by the ectoderm cells, and the primordial germ cells may then be recognized as the anlage of the reproductive organs.

Kühn discusses the origin and significance of the 'Nahrzellenkern,' and compares this structure with similar bodies which have been found in the primordial germ cells of other animals, but is unable to arrive at any final conclusion.

In certain Cladocera and Copepoda, as we have seen, there are visible substances within the cytoplasm of the egg which becomes segregated in, and render distinguishable, the primordial germ cells. Some species belonging to these and other groups of Crustacea have been studied in which such a visible substance peculiar to the primordial germ cells is absent.

Samassa ('93) not only failed to find the primordial germ cell during the cleavage stages of Moina rectirostris, but claims that the germ cells arise from four mesoderm cells. Kühn ('08), from a study of the parthenogenetic generation of Daphina pulex and Polyphemus pediculus, also derives the germ cells from the mesoderm. Vollmer ('12) could not distinguish the germ cells of Daphnia magna and D. pulex in the developing winter eggs until the blastoderm was almost completed and Muller-Calé ('13) could not find these cells in Cypris incongruens until the germ layers were fully formed. McClendon ('06) has shown that in two parasitic copepods, Pandarus sinuatus and an unnamed species, the primordial germ cell is established at the end of the fifth cleavage (32-cell stage) instead of at the end of the fourth as Haecker ('97) found in Cyclops. It is suggested that this delay may be due to the large amount of yolk present. The stem cell from which it arises is, however, not made visibly different from the rest of the blastoderm by peculiar granules as is the case in Cyclops.

Bigelow ('02) has described in Lepas anatifera and L. fascicularis certain stages which may bring the forms in which no early segregation of the germ cells has been discovered into line with the apparently more determinate species. In Lepas the yolk, which at first is evenly distributed within the egg, passes to the vegetative pole and becomes segregated in one of the first two cleavage cells (cd^2). At the 16-cell stage the yolk lies within the single entoblast cell ($d^{5\cdot1}$), which occupies a position corresponding to that of the primordial germ cell in Moina. In this connection may be mentioned the fact that in many animals the germ cells are supposed to come from the entoderm and are characterized by the possession of much yolk.

2. THE KEIMBAHN IN THE NEMATODA

The classical example of the Keimbahn in animals is that of Ascaris megalocephala as described by Boveri ('87, '92). The first cleavage division of the egg of Ascaris results in two daughter cells, each containing two long chromosomes (fig. 19, *A*). In the second division the chromosomes of one cell divide normally

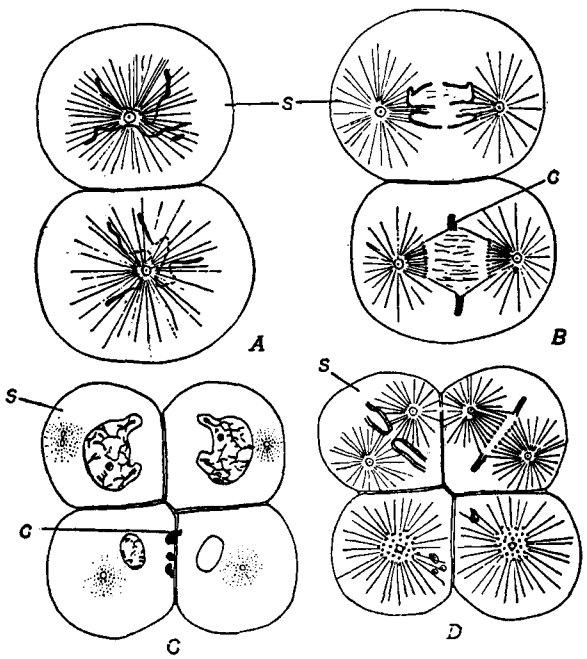


Fig. 19 Ascaris (redrawn from Boveri, '92). A-D, stages showing chromatin-diminution in all cells except the stem-cell (*s*).

and each daughter cell receives one half of each (fig. 19, $\bar{*}B, \bar{*}s$). The chromosomes of the other cell behave differently; the thin middle portion of each breaks up into granules (fig. 19, *A*) which split, half going to each daughter cell, but the swollen ends (fig. 19, *B*, *C*) are cast off into the cytoplasm. In the four-cell stage there are consequently two cells with the full amount of chromatin and two with a reduced amount. This inequality in the amount

of chromatin results in different sized nuclei (fig. 19, *C*); those with entire chromosomes (*s*) are larger than those that have lost the swollen ends (*c*). In the third division one of the two cells with the two entire chromosomes loses the swollen ends of each; the other (fig. 19, *D*, *S*) retains its chromosomes intact. A similar reduction in the amount of chromatin takes place in the fourth and fifth divisions and then ceases. The single cell in the 32-cell stage which contains the full amount of chromatin has a larger nucleus than the other thirty-one cells and gives rise to all of the germ cells, whereas the other cells are for the production of somatic cells only. The cell lineage of Ascaris is shown in the accompanying diagram (fig. 21).

Meyer ('95) extended the study of chromatin diminution to other species of Ascaris. In A. lumbricoides no diminution takes place until the four-cell stage; then three of the nuclei become deprived of part of their chromatin. A diminution of this sort had been described by Boveri as a variation in the process observed in A. megalocephala. In A. rubicunda the differentiation of the cleavage cells seems to resemble A. megalocephala more than it does A. lumbricoides. Only late cleavage stages of A. labiata were obtained by Meyer, but there is no doubt that a similar process occurs here. The general conclusion is reached that the cleavage cells of all Ascaridae undergo a chromatin diminution.

Bonnevie ('01), however, while able to confirm Meyer's results so far as A. lumbricoides is concerned, could discover no process of diminution in Strongylus paradóxus, and Rhabdonema nigrovenosa.

The elimination of chromatin from all of the somatic cells of Ascaris and not from the germ cells led to the conclusion that the germ plasm must reside in the chromatin of the nucleus. The more recent experimental investigations of Boveri ('10a,'10b), indicate, however, that it is not the chromatin alone that determines the initiation of the diminution process, but that the cytoplasm plays a very important rôle. Dispermic eggs were found to segment so as to produce three types as follows (fig. 20, *A*, *B*):

Type I, with one stem-cell (*P*) and three primordial somatic cells (*AB*);

Type II, with two stem cells and two primordial somatic cells; and

Type III with three stem cells and one primordial somatic cell.

Figure 20, *B* shows a cleavage stage of Type II. Here are represented two stem cells (*P*) with the complete amount of chromatin, both of which are preparing to divide to form the

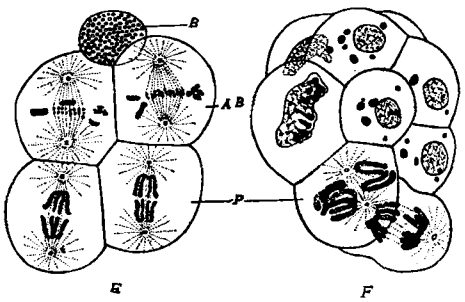


Fig. 20 Ascaris (redrawn from Boveri '10b, F, chromatin-diminution in a centrifuged egg. E, chromatin-diminution in a dispermic egg. AB, somatic cells; *p*, stem-cells.

stem cells (P_2) of the next generation. From the study of these dispermic eggs Boveri ('10b) draws the following conclusions:

Durch die simultane Vierteilung eines dispermen Ascaris-Eies entstehen (vielleicht mit ganz seltenen Ausnahmen) Zellen, welche die gleiche Wertigkeit besitzen, wie diejenigen, die durch Zweiteilung eines normal-befruchteten Eies gibildet werden, nämlich die Wertigkeit AB oder P_1. Es können drei Zellen die Qualität AB besitzen oder zwei oder eine; dem jeweiligen Rest kommt die Qualität P_1 zu. Schon beim Uebergang vom vierzelligen zum achtzelligen Stadium lässt sich aus der Teilungsrichtung mit Sicherheit diagnostizieren, welche der vier primären Blastomeren als AB, welche als P_1 aufzufassen sind; und diese Wertbestimmung wird durch die weiteren Schicksale der vier Zellfamilien in jeder Hinsicht bestätigt (p. 157).

The opinion is expressed that it is "die einrichtigen plasmatischen Qualitäten des sich entwickelnden Zellenkomplexes" which cause the injurious results of dispermy, and that if, of the

three types of dispermic eggs described, the cells could be isolated in pairs, one AB-cell paired with one P_1-cell, an embryo, normal except in size, would result from each pair.

Eggs which were strongly centrifuged cut off at the beginning of the first cleavage a granular ball at the heavy pole. This phenomenon was previously reported by Hogue ('10) and such

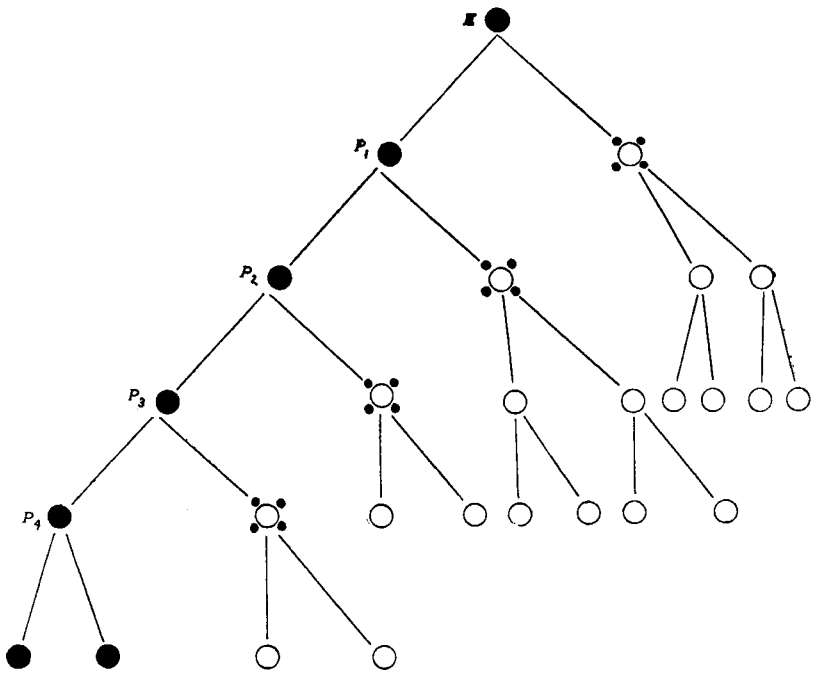


Fig. 21 Ascaris (from Boveri, '10b); diagram showing segregation of primordial germ cells. *E*, egg; P_1, P_2, P_3, stem-cells; P_4, primordial germ cell; circles represent somatic cells.

eggs were termed 'Balleier.' In these eggs the two cells of the four-cell stage which are adjacent to the 'Ball' undergo the diminution process; the remaining two are stem-cells which give rise to the germ cells. Thus there are two 'Keimbahnen' proceeding side by side in a single egg and four primordial germ cells are produced instead of two as in normal eggs (see fig. 21).

Miss Hogue's experiments with centrifuged force led her to conclude that there must be an 'unsichtbare Polarität' or 'Protoplasmaachse' in the egg of the Ascaris. Boveri agrees with this and considers further that the initiation of the diminution process is not determined by the chromatin but by the cytoplasm of the egg. He states that

> Was aber auch hier durch weitere Untersuchungen noch erreicht werden mag, Eines halte ich für sicher, dass sich alles, was über die Wertigkeit der primären Blastomeren bei abnormer Furchung ermittelt worden ist, durch die Annahme sehr einfacher Plasmadifferenzen erklären lässt, wogegen die Hypothese einen differenz erenden Wirkung des Kerns in jeder Form auf unüberwindliche Schwierigkeiten stösst (p. 206).

3. THE KEIMBAHN IN SAGITTA

Sagitta has proved to be of considerable importance to those interested in the Keimbahn of animals. Hertwig ('80) figures the four primitive germ cells in the gastrula and later stages, proving that these cells are early set aside in embryonic development. Recently the work of Elpatiewsky ('09, '10) has given Sagitta a new importance, since this writer has found within the fertilized egg a cytoplasmic inclusion which is intimately associated with the segregation of the germ cells. The presence of this inclusion has been confirmed by Buchner ('10a, '10b) and Stevens ('10) and several ideas have been expressed regarding its origin, fate and significance.

Elpatiewsky ('09) found in Sagitta, at the time when the male and female nuclei were lying side by side in the middle of the egg, a body situated near the periphery at the vegetative pole (fig. 22, *B*, *x*). This body, which he called the 'besondere Körper,' consists at first of 'grobkörnigen' plasma which stains like chromatin but not so intensely; later it condenses into a round homogeneous body with a sharp contour. During the first five cleavage divisions the 'besondere Körper' is always confined to a single cell. At the completion of the fifth cleavage (32-cell stage), the blastomere containing this cytoplasmic inclusion is recognizable as the first 'Urgeschlechtszelle' (fig. 22, *C*, *G*), and

its larger sister cell as the first 'Urentodermzelle' (fig. 22, *C*, *E*). The primordial germ cell is the last to divide during the sixth cleavage and the 'besondere Körper' does not, as before, pass entire into one of the daughter cells, but breaks up into a number of pieces, part of which are included in each of the two daughter cells (fig. 22, *D*, *E*, *X*). One of these daughter cells apparently

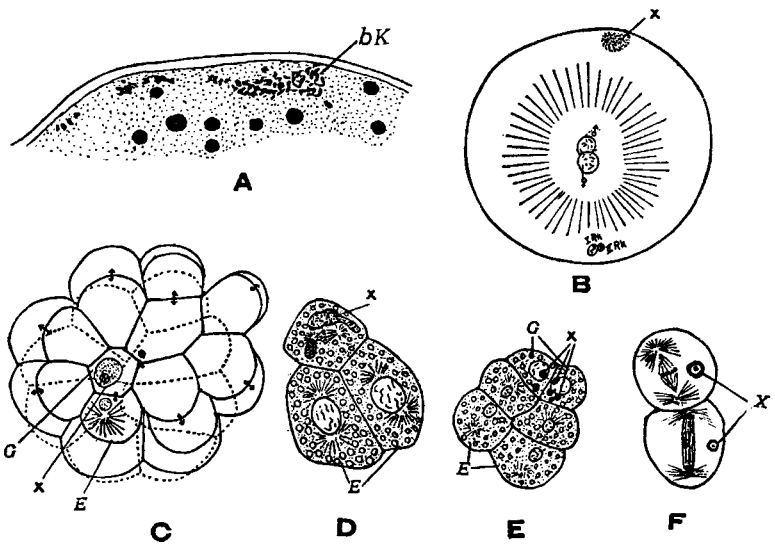


Fig. 22 Sagitta (redrawn from Elpatiewsky, '09, '10). A, first appearance of the 'besondere Körper' (*bK*) in the egg. B, egg with germ nuclei fusing. *X*, 'besondere Körper.' C, thirty-two cell stage; the primordial germ cell (*G*) contains the 'besondere Körper' (*X*). D, division of 'besondere Körper' (*X*) during division of primordial germ cell. E, two primordial germ cells showing unequal distribution of 'besondere Körper' (*X*). F, division of first two primordial germ cells; one dividing more rapidly than the other.

acquires more of the 'besondere Körper' than the other. This division appears to Elpatiewsky to be differential, separating the primordial oogonium from the primordial spermatogonium, the latter being the cell which receives the larger portion of the 'besondere Körper' and which during the next (seventh) division is slightly delayed (fig. 22, *F*). Subsequent to the seventh cleavage the remains of the 'besondere Körper' become pale and

gradually disappear, apparently dissolving, and, in the four germ cells resulting from the next division, only occasionally can stained granules from this body be distinguished.

Buchner ('10a, '10b) had no difficulty in finding the 'besondere Körper' of Elpatiewsky and in tracing it during the cleavage stages. He claims that it originates from the 'accessory fertilization cell' described by Stevens ('04) as degenerating after the egg breaks away from the oviducal wall, and that it is chromidial in nature and should therefore be called 'Keimbahnchromidien.' Stevens ('10), however, has carefully examined abundant material from Sagitta elegans and S. bipunctata and could trace no connection between the 'accessory fertilization cell' and the 'besondere Körper,' the latter appearing for the first time at the stage when the egg and sperm nuclei lie side by side in the middle of the egg, thus confirming Elpatiewsky's conclusions. She admits the possibility of the origin of the 'besondere Körper' from granules of the accessory fertilization cell, provided this material loses its staining capacity for a period, and suggests also that the granules of chromatin-like material extruded from the nucleus of the egg during maturation may take part in its formation. Miss Stevens also believes with Elpatiewsky that the 'besondere Körper' divides unequally between the two daughter cells of the primordial germ cell and that this is a differential division. She was unable, however, to detect any constant difference between either the cytoplasm or the nuclei of oogonia and spermatogonia. It is worthy of mention that Elpatiewsky ('10) believes that the 'besondere Körper' may originate "aus dem achromatischen Kernkörper."

The differentiation of oogonia and spermatogonia from indifferent germ cells during the development of hermaphrodites is a subject of great interest and importance. Attempts have been made, especially with molluscs (Ancel '02, '03; Buresch, '11; Boveri, '11; Schleip, '11), but without as definite results as are desirable. The early differentiation of germ cells into oogonia and spermatogonia in Sagitta, which was pointed out by Hertwig ('80), has been employed by Pedaschenko ('99) as a basis for a theory of sex determination in a copepod, Lernaea branchialis.

In this crustacean there are at one cleavage stage four primordial germ cells which resemble the quartette of cells described by Hertwig in Sagitta, two of which were proved by him to be oogonia and the other two spermatogonia. Pedaschenko supposes that the four primordial germ cells form two pairs, a pair lying on either side of the median line. Each pair becomes a single cell by fusion and the probable disintegration of one nucleus. One cell of each pair is believed by Pedaschenko to be male, the other female; and the sex of the cell whose nucleus does not degenerate determines the sex of the resulting individual, since a persisting female cell would form an ovary and a persisting male cell a testis. Thus is a potentially hermaphroditic organism changed to a dioecious organism.

4. THE KEIMBAHN IN VERTEBRATES

Animals from all classes of vertebrates have been employed for determining the origin of the germ cells, but in no case have these cells been traced back to cleavage stages. Early authors, and even many writers at the present time, believed in the germinal epithelium theory of Waldeyer ('70). This investigator first distinguished germ cells in the epithelium covering the genital ridge and thought that they evolved from these epithelial cells. The gonotome theory of Rückert ('88) and Van Wijhe ('89) holds that germ cells arise in the embryo from a certain part of the mesoblastic segments called by the latter the 'gonotome;' from here they are carried into the peritoneum. There can be no doubt from the most recent investigations that the germinal epithelium and gonotome theories are incorrect, and that the germ cells of vertebrates are formed at a much earlier period, giving good basis for the idea that these cells arise from cleavage cells as has been abundantly proved for many invertebrates. Some of those who have advocated such an early origin of germ cells are Nussbaum ('80) in the trout and frog, Eigenmann ('92, '96a, '96b) in Cymatogaster, Wheeler ('99) in the lamprey, Beard ('00, '02) in Raja and Pristiurus, Nussbaum ('01) in the chick, Woods ('02) in Squalus, Allen ('06, '07, '09) in Chry-

semys, Rana, Amia and Lepidosteus, Rubaschkin ('07, '09, '10, '12) in the chick, cat, rabbit and guinea-pig, Kuschakewitsch ('08) in Rana, Jarvis ('08) in Phrynosoma, Tschaschin ('10) in the chick, von Berenberg-Gossler ('12) in the chick, Schapitz ('12) in Amblystoma, and Fuss ('12) in the pig and man. This is by no means a complete list, but indicates the range of forms studied and the current interest in this subject. No attempt will be made here to review this mass of literature, but interesting facts will be selected from several papers in the list.

In the first place, the vertebrates do not furnish as favorable material for germ cell studies as do many of the invertebrates, and a large number of the contributions have not added anything particularly important to our knowledge of the subject, but have simply demonstrated that similar conditions prevail among members of different classes, orders, etc. One author (Eigenmann, '91, '96) believes that the germ cells in Cymatogaster are differentiated as such in the thirty-two cell stage. This, however, was not proved and no confirmatory data have since been furnished. Within the past three years, however, several communications have been published which give us hope of really tracing the Keimbahn back into the cleavage stages. Before this time some of the characteristics by means of which germ cells could be distinguished in vertebrate embryos were as follows: (1) the presence of yolk, (2) an amoeboid shape, (3) large size, and (4) slight staining capacity. Of the more recent investigations, I shall mention those carried on by Dodds ('10), Rubaschkin ('10, '12), Tschaschin ('10), and von Berenberg-Gossler ('12).

One fact discovered by Dodds ('10) in the teleost, Lophius, is of special interest, namely that the germ cells in the embryos of this fish cannot be definitely distinguished previous to the appearance in their cytoplasm of a body which stains like a plasmosome (fig. 23, *C*). Germ cells are undoubtedly segregated before this period, but they exhibited no characteristics with the methods employed which rendered them distinguishable. Dodds believes that this cytoplasmic body is extruded plasmosome material, probably part of one of the two plasmosomes possessed by many of the cells at this period. Thus far Lophius

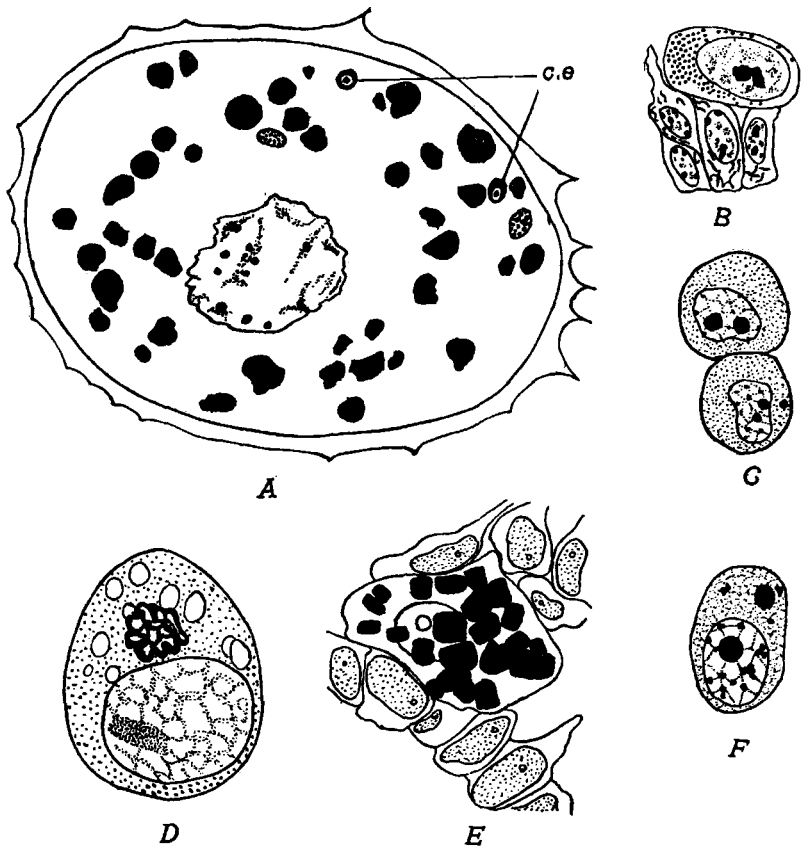


Fig. 23 A, oocyte of the cat, showing the 'corps enigmatique' (*c.e*); (redrawn from van der Stricht, '11). B, one germ cell and several somatic cells from a guinea-pig embryo; (redrawn from Rubaschkin, '12). C, two germ cells from the embryo of Lophius with plasmosome extruded into cytoplasm; (redrawn from Dodds, '10). D, germ cell of chick, showing 'Netzapparat'; (redrawn from von Berenberg-Gossler, '12). E, a germ cell of Raja batis, filled with yolk material and surrounded by somatic cells; (redrawn from Beard, '02). F, oogonium of a sponge, (redrawn from Jörgensen, '09).

is the only vertebrate in whose primordial germ cells an extra-nuclear body has been found.

Rubaschkin, in 1910, announced the results obtained with the eggs of the guinea-pig by certain methods designed to bring

into view the chondriosomes. He shows that the chondriosomes of the undifferentiated cells are granular, and that as differentiation proceeds, these granules unite to form chains and threads (fig. 23, *B*). "Zwischen den somatischen und Urgeschlechtszellen existiert ein Unterschied in der Structur, welcher sich dadurch offenbart, dass die Urgeschlechtszellen primitive körnige Chordriosomen besitzen, während die somatischen Zellen mit veranderten, d.h. fadenförmigen Chondriosomen ausgestattet sind" (p. 428). The germ cells are those which remain in an undifferentiated condition situated in the posterior part of the embryo among the entoderm cells. Tschaschkin ('10) in the same year, came to a similar conclusion from studies made with chick embryos. Rubaschkin ('12) has also extended his investigations on guinea-pig embryos. The accompanying diagram (fig. 24) shows the fertilized egg and the early cleavage cells all alike (in black); some of their descendants become differentiated into the somatic cells of the germ layers (circles), but others (in black) remain in a primitive condition and are recognizable as the primordial germ cells (*p.g.c.*); these remain at rest for a considerable period, but finally multiply and become part of the germinal epithelium (*g.ep.*).

Von Berenberg-Gossler ('12) considers the 'Netzapparat' in the primitive germ cells of the chick of particular importance (fig. 23, *D*) comparing it with the 'wurstförmige Körper' described by Hasper ('11) in Chironomus (p. 385, fig. 1). The appearance of this structure in the 'Keimbahnzellen' is thought to be due to the long period during which these cells do not divide.

Certain events take place during the spermatogenesis of mammals which are concerned with the differentiation of germ cells. I refer to the formation of the Sertoli cells of man, as reported by Montgomery ('11), and in part confirmed by von Winiwarter ('12). The Sertoli cells are intimately connected with the germ cells in the mammalian testis and probably perform three functions: (1) they nourish the spermatocytes; (2) they provide the spermatic fluid; and (3) they exert some chemico-tactic stimulus which serves to orient the spermatozoa into bundles. The origin of the Sertoli cells has been for many

years in doubt. Many investigators claim that they arise from cells other than germ cells; these writers have been called by Waldeyer ('06) 'dualists.' An equal number of authorities believe that both Sertoli cells and spermatogonia originate from primordial germ cells; these are the 'monists.'

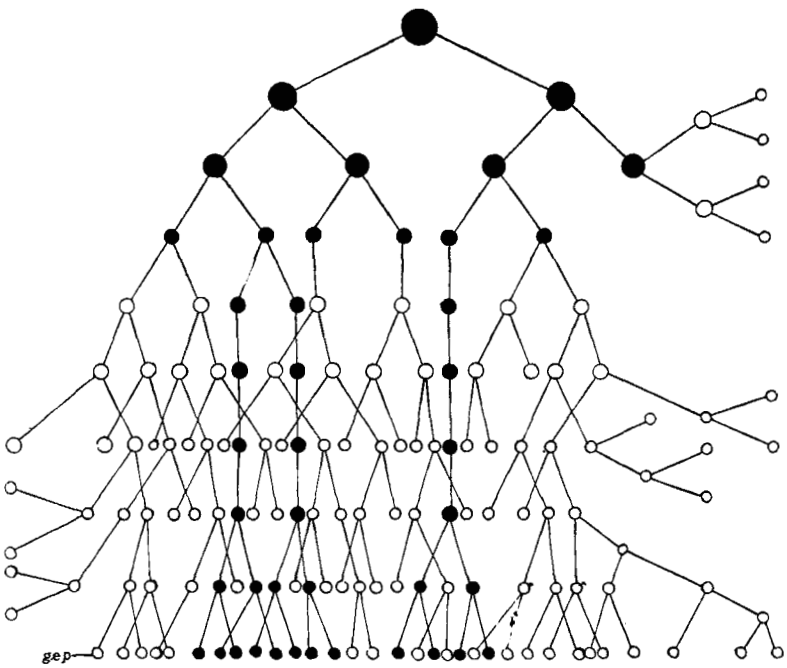
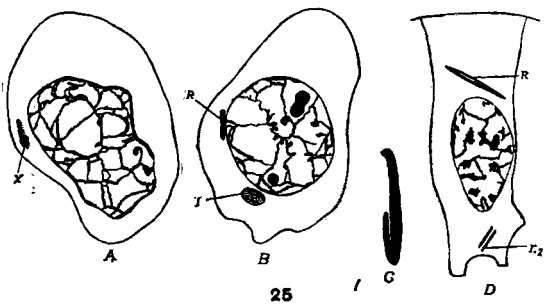


Fig. 24 Diagram to show the history of the germ cells in the embryo of the guinea-pig. *g.ep*, germinal epithelium; (from Rubaschkin, '12).

The researches of Montgomery and von Winiwarter have decided the question, at least so far as man is concerned, in favor of the monists. Montgomery's results are as follows: Of thirty antepenultimate spermatogonia examined, twenty-three contained each a rod-shaped structure (fig. 25, *B*, *R*) and it seems probable that this peculiar body, which is identified by von Winiwarter with the 'cristalloide de Lubarsch' (Lubarsch, '96), is present in every cell of this generation. This rod is considered

by Montgomery to be of cytoplasmic origin and is termed by him a 'Sertoli cell determinant.' During the division of the antepenultimate spermatogonia the rod passes undivided into one of the daughter cells; thus one-half of the penultimate spermatogonia possess a rod, the other half do not. Of the forty-nine



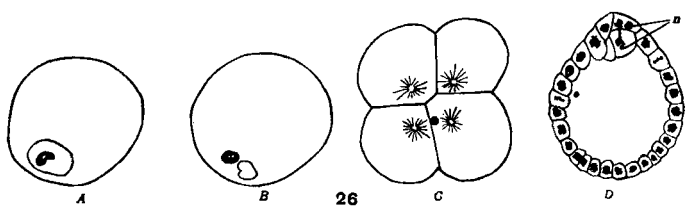


Fig. 25 Stages in the formation of the Sertoli cell in man; (redrawn from Montgomery, '11). A, spermatogonia containing granular inclusion (*X*) from which 'Sertoli cell determinant' may arise. B, antepenultimate spermatogonium showing rod (*R*) and idiozome (*I*). C, division of rod. D, a Sertoli cell containing a divided rod (*R*) and two rodlets (r_2).

Fig. 26 Aequorea forskalea (redrawn from Haecker, '92). A, freshly laid egg with germinal vesicle containing nucleolus. B, egg one-half hour after laying; nucleolus has escaped from nucleus. C, four-cell stage; nucleolus in one blastomere. D, blastula; certain cells contain nucleolar-like inclusions (n).

penultimate spermatogonia examined, twenty-four exhibited a rod and twenty-five did not. This result has been confirmed by von Winiwarter. When the rod-containing penultimate spermatogonia divide there is a similar segregation of the rod in one of the daughter cells, hence only one-fourth of the cells resulting from the divisions of the antepenultimate spermatogonia possess a rod. Of one hundred and forty-two cells of this genera-

tion studied by Montgomery, twenty-five were found with a rod and one hundred and seventeen without. That this ratio is less than one to three (1:3) is explained by the fact that some of the spermatogonia with rods may already have become Sertoli cells. The further history of the rod in the Sertoli cell is as follows: A primary rodlet is produced by a splitting of the rod (fig. 25, *C*) after which the rod either disappears at once or else persists for a time, in which case it may split longitudinally as shown in figure 25, *D*, *R*. However, in four-fifths of the cells examined (one hundred in number) the large rod disappeared before the growth of the Sertoli cell had begun. Each primary rodlet splits longitudinally into two approximately equal parts, called secondary rodlets (fig. 25, *D*, r_2), which persist until the end of the cycle of the Sertoli cell.

Neither Montgomery nor von Winiwarter was able to determine the origin of the rod. They do not consider it mitochondrial in nature, although it may arise from granules lying in the cytoplasm. Montgomery found in one cell a mass of granules from which the rod may have developed (fig. 25, *A*, *X*), and von Winiwarter noted that the rod had a granular appearance in the earliest stages he examined. It is also perfectly distinct from the idiozome (see fig. 25, *B*, *I*) and is apparently not directly derived from the nucleus. Von Winiwarter is not as certain as Montgomery regarding the history of the spermatogonia, the 'cristalloide de Lubarsch,' and the 'gâstonnets accessoires,' as he calls the rodlets. He was unable to decide regarding the number of spermatogonial divisions and believes it to be indeterminate. He finds, contrary to Montgomery, the rod persisting in fully developed Sertoli cells, and considers the fragmentation or fission of the rod to form the primary rodlets as doubtful. Further investigations with more favorable material are very desirable, but notwithstanding certain differences of opinion between the two writers whose results have been briefly stated above, it seems certain that Sertoli cells and germ cells are both derived from primordial germ cells, and that the Sertoli cells differ from the ultimate spermatogonia in the possession of a peculiar rod probably of cytoplasmic origin. Montgomery considers this a sort

of secondary somatic differentiation (the Sertoli cells representing the soma of the testis); the first somatic differentiation occurring when the tissue cells become differentiated from the germ cells in the embryo.

It may be worth mentioning at this place that among the invertebrates, instances of changes which take place in the germ cells of the male are known which may be brought into line with the facts in the history of the Sertoli cells of man. For example, in the spermatogenesis of the parasitic copepod, Laemargus muricatus, McClendon ('06) found that the cells in some of the groups of four spermatids became filled with an achromatic substance which resembled yolk—a substance called 'Austreibestoff' by Heider ('79). The origin of this substance could not be determined, but it probably came from the nucleus. The cells thus affected serve as nurse cells for the spermatozoa.

5. THE KEIMBAHN IN OTHER ANIMALS

It is not possible in this place to give either a general account or detailed account of the Keimbahn as it has been described in groups such as the Porifera, Coelenterata, etc., since we are here interested especially in the peculiar substances which apparently determine the Keimbahn, and thus far no results of importance have been obtained from studies of these animals. Under the above heading, however, I wish to mention a few stages in the development of certain forms, widely separated in the animal kingdom, which have either been compared or can be compared with conditions such as we have described in the preceding portions of this paper.

Metanucleoli. Haecker's ('97) belief that the 'Aussenkörnchen' in Cyclops are of nucleolar origin and Silvestri's ('06, '08) discoveries in parasitc Hymenoptera indicate that in certain instances the nucleoli may play some rôle in the differentiation of the primordial germ cells.

The large nucleolus in the germinal vesicle of the medusa, Aequorea forskalea (fig. 26, *A*), according to Haecker ('92), disappears from the germinal vesicle about half an hour after the egg is laid, and a similar body becomes evident near the egg

nucleus which has in the meantime become smaller (fig. 26, *B*). These two bodies are considered by Haecker to be identical, and the term 'Metanucleolus' has been applied to them. The metanucleolus is, in each division up to the sixty-four cell stage, segregated intact in one cell. Its further history was not traced, but in the blastula, when the cells at the posterior pole begin to differentiate, nucleolar-like bodies appear in some of them which are absent from the undifferentiated blastula elements (fig. 26, *D*, *n*). These may be the descendants of the metanucleolus.

A body similar to the metanucleolus was also discovered by Haecker near the copulating germ nuclei in the egg of Aurelia aurita, but its history could not be determined because of the large amount of yolk present. Haecker identifies the metanucleolus of Aequorea with the spherical body described by Metschnikoff ('86) near the egg nucleus of the Mitrocoma annae, and considered by him as a sperm nucleus. A similar interpretation is given by Haecker for the cytoplasmic inclusion ('Spermakern') found by Boveri ('90) in Tiara. Similarly the 'Kleinkern' which Chun ('91) discovered in the egg cells of the Stephanophyes superba, and the bodies described by Hertwig ('78) near the maturation spindles of Mytilus and Sagitta, resemble very closely the metanucleolus of Aequorea.

Furthermore, the metanucleolus is considered by Haecker homologous to the 'Paracopulationzelle' described by Weismann and Ischikawa in the winter eggs of certain Daphindae (p. 434, fig. 15) and in both cases it is considered probable that these peculiar bodies are restricted to the 'Keimbahnzellen' of the embryo.

In the eggs of Myzostoma, Wheeler ('97) found that the nucleolus of the germinal vesicle does not dissolve soon after it is cast out into the cytoplasm during the formation of the first maturation spindle, but remains visible at least until the eight-cell stage, at which time it lies in the large posterior macromere, a cell which "very probably gives rise to the entoderm of the embryo." Later embryonic stages were not studied. According to Wheeler "the nucleoli are relegated to the entoderm cells as the place where they would be least liable to interfere in the

further course of development and where they may perhaps be utilized as food material after their disintegration" (p. 49).

McClendon ('06) has likewise described a body embedded in the cytoplasm of the egg of Myzostoma clarki which he derives from the 'accessory cells,' which, as Wheeler ('96) has shown, attach themselves to either pole of the oocytes. These 'accessory cells' are really the 'Nahrzellen' of other authors. The cleavage of the egg was not studied. Buchner ('10b) suggests that this body described by McClendon and the 'nucleolus' of Wheeler are identical and that through them the Keimbahn may be determined.

A metanucleolus has also been described by Hartmann ('02) in Asterias glacialis. The germinal vesicle of the ovarian egg possesses a 'Keimfleck' which contains all of the plastin and the chromatin of the nucleolus. During the maturation the germinal vesicle breaks down, the chromosomes escape from the 'Keimfleck' and the rest of this body becomes imbedded in the cytoplasm near the maturation spindle.

Granules. Granules of various sorts which are segregated in particular blastomeres have been noted in the eggs of various animals and may have some relation to the Keimbahn. For example, among the molluscs, Blochmann ('81) has described the appearance of a group of granules in the early cleavage cells of Neritina which finally reach the velar cells. It is also probable that Fol ('80) observed similar granules in the 16-cell stage of Planorbis. In the same category, no doubt, belong the bodies figured by Fujitas ('04) in the 4-cell to the 16-cell stages of Siphonaria lying at the vegetative pole, and the 'Ectosomen' described and figured by Wierzejski ('06) in Physa. These granules appear at the vegetal pole in the blastomeres of Physa during the second cleavage; are at first imbedded in the entoderm mother cells, but finally become localized in the ectoderm cells. They periodically appear and disappear, and may, as suggested by Wierzejski, represent only "eine besondere Erscheinung des Stoffwechsels" (p. 536).

Similarly in the rotifer, Asplanchna, Jennings ('96) has traced a cloud of 'granules' from the eight-cell stage until the seventh

cleavage, when this mass forms part of the smaller entodermal cell. In Lepas there has also been recorded (Bigelow, '02) a segregation of granules in one blastomere. Many other substances, granular in form, have been described in the eggs of animals, some of them at least having migrated there from the somatic tissue. Blockmann ('87) discovered a number of bacteria-like rods in the undeveloped eggs of Blatta germanica; these rods multiplied by division and were considered symbiotic bacteria. 'Bacterienartige Stäbchen' were also noted by Heymons ('95) in the eggs of Periplanata orientalis and Ectobia livida; these sink into the yolk and disappear. More recently a report of Buchner ('12) indicates that these bodies are really organisms which seem to be symbiotic and not parasitic, although it remains to be proved what advantage the host receives from their presence. Of a similar sort are the Zooxanthellae which Mangan ('09) has shown enter the developing ovum from the parental tissues. All of these organisms become in some way imbedded in the germ cells, but, so far as we know, never serve to distinguish the Keimbahn, although a more selective distribution within the developing animal would obviously be greatly to their advantage.

Vander Stricht ('11) has compared the 'besonderer Körper' found by Elpatiewsky ('09, '10) in the egg of Sagitta with several bodies, the 'corps enigmatique,' which he discovered in the oocyte of the cat (fig. 23, *A*). One or two of these 'corps enigmatique' are present in the young oocyte originating from a few (one to five) cytoplasmic safraninophile granules which are visible at the beginning of the growth period. At first they lie near the nucleus, but as the size of the oocyte increases, they become situated near the periphery. Usually three parts can be recognized in the 'corps enigmatique:' "granulation centrale, couche intermédiaire et couche corticale foncée." As the term applied to them indicates, the functions of these bodies were not determined. The following suggestion is, however, made: "il est possible que cet élément nous montre, des l'origine, la 'Keimbahn' ainsi que les premières cellules génitales constituées" (p. 425).

A body stained deeply by nuclear dyes which was found by O. Van der Stricht ('09) in the bat at the time of the first cleavage mitosis, may be similar to the 'corps enigmatique' of the cat. It is also worth recording that Jörgensen ('10) finds that the oogonium of the sponge at the earliest stage when it can be recognized, contains a granular body not present in the somatic cells (fig. 23, *F*).

6. THE GENESIS, LOCALIZATION, DISTRIBUTION AND FATE OF THE KEIMBAHN-DETERMINANTS

It is customary to be suspicious of any peculiar bodies revealed to us in fixed and stained material under high magnification. There can be no doubt, however, that most, if not all, of the cytoplasmic inclusions mentioned in this paper are realities and not artifacts. Some of them have been seen in the living eggs; most of them have been described by several investigators; they occur after being fixed and stained in many different solutions; and their presence is perfectly constant. The genesis, localization, and fate of these bodies are difficult to determine, and their significance is problematical; but the writer has attempted in the following pages to draw at least tentative conclusions from the evidence available and to indicate what still needs to be done.

A. The genesis of the Keimbahn-determinants

The writers who have discussed the origin of the Keimbahn-determinants have derived them from many different sources. In a few cases they are known to be nuclear in origin, consisting of nucleolar or chromatic materials; they are considered differentiated parts of the cytoplasm by some investigators; in some species they are extra-cellular bodies, such as nurse cells.

Table 1 indicates the number and diversity of the animals in which Keimbahn-determinants have been described, and shows the increasing interest given to this subject within recent years, over half of the papers listed having been published since 1908. Several cases have been referred to in the text, but omitted from the table because of insufficient evidence regarding

their connection with the primordial germ cells. The list as given includes representatives of the Coelenterata, Chaetognatha, Nematoda, Arthropoda, and Vertebrata. The terms applied to the various substances have been chosen evidently because of their genesis, position in the egg, or supposed function.

a. From nuclear substances: Nucleoli. It is certain that bodies of a nucleolar nature behave as Keimbahn-determinants. Three

TABLE 1

Principal cases of visible substances concerned in differentiation of germ cells (in chronological order)

NAME OF SPECIES, GENUS, OR GROUP	NAME APPLIED TO SUBSTANCE	AUTHORITY	DATE
Chironomus nigroviridis	Dotterkörnchen	Weismann	1863
Miastor	Dottermasse	Metschnikoff	1866
Moina rectirostris	Richtungskörper	Grobben	1879
Chironomus	Keimwulst	Ritter	1890
Daphnidae	Paracopulationszelle	Weismann and Ischikawa	1889
Aequorea	Metanucleolus	Haecker	1892
Ascaris megalocephala	Chromatin	Boveri	1892
A. lumbricoides, A. rubicunda, A. labiata	Chromatin	O. Meyer	1895
Cyclops	Aussenkörnchen, Ektosomen	Haecker	1897, 1903
Calliphora	Dotterplatte	Noack	1901
Dytiscus	Anello cromatico	Giardina	1901
Apis mellifica	Richtungskörper	Petrunkewitsch	1901
Parasitic Hymenoptera	Nucleolo	Silvestri	1906, 1908
Chrysomelidae	Pole-disc	Hegner	1908
Miastor metraloas	polares Plasma	Kahle	1908
Sagitta	besonderer Körper	Elpatiewsky	1909
Guinea-pig	Chondriosomes	Rubaschkin	1910
Chick	Chondriosomes	Tschaschkin	1910
Lophius	Extruded plasmosome	Dodds	1910
Ascaris	Plasmadifferenzen	Boveri	1910
Chironomus	Keimbahnplasma	Hasper	1911
Copepoda	Ectosomen	Amma	1911
Polyphemus	Nahrzellenkern	Kühn	1911, 1913
Sagitta	Keimbahn-chromidien	Buchner	1910
Man	Sertoli cell determinant	Montgomery	1911

or more kinds of bodies are spoken of as nucleoli. Of these may be mentioned (1) the true nucleoli or plasmosomes, (2) karyosomes or chromatin-nucleoli, and (3) double-nucleoli consisting of usually a single principal nucleolus (Hauptnucleolus of Flemming) and one or more accessory nucleoli (Nebennucleoli of Flemming). Many nucleoli have been described which may perhaps represent intermediate stages in the evolution of one of the types mentioned above into another.

The young ovarian egg of most animals contains a single spherical nucleolus ('Keimfleck,' or germinal spot) but the number may increase greatly during the growth period. Usually during the formation of the first maturation spindle the nucleolus escapes from the nucleus into the cytoplasm where it disappears, often after breaking up into fragments. Many theories have been advanced regarding the origin, function and fate of the nucleoli of the germinal vesicle. They are considered by some of chromatic origin, arising as an accumulation of the chromatin (Retzius, '81; Mertens, '93; Foot and Strobell, '11; Payne, '12, and many others), or from the chromatin by chemical transformation (Schneider, '91; Obst, '99). Others consider them extranuclear in origin (Montgomery, '99).

Many functions have been attributed to the nucleoli; of these the following may be mentioned: (1) They function as excretory organs (Balbiani, '64; Böhm, '88; Hodge, '94; Bambeke, '97). (2) Nucleoli play an active rôle in the cell, since they serve as store-houses of material which is contributed to the formation of the chromosomes (Flemming, '82; Korschelt, '95; Lubosch, '02; G. T. Hargitt, '09; Jordan, '10; Foot and Strobell, '11) and may give rise to kinoplasm (Strasburger, '95) or 'Kinetochromidien' (Schaxel, '10). (3) Nucleoli are passive by-products of chromatic activity; they become absorbed by active substances (Haecker, '95, '99). (4) Nucleoli represent nutritive material used by the nucleus into which it is taken from the cytoplasm (Montgomery, '99).

Undoubtedly the various bodies known as nucleoli originate in different ways, have different histories and execute different functions.

In the particular cases to be discussed here the nucleoli are not temporary structures, as is usually true, but persist for a comparatively long interval after the germinal vesicle breaks down. The most important and convincing evidence of the functioning of a nucleolus as a Keimbahn-determinant is that furnished by Silvestri ('06, '08) in parasitic Hymenoptera. Here, as shown in figures 13 and 14 and described on page 429, the nucleolus escapes from the germinal vesicle, comes to lie a considerable distance away at the opposite (posterior) pole of the egg, and later is segregated in the cytoplasm of the germ cells, apparently playing some rôle in the determination of the latter. Several events in the history of this nucleolus are unusual. (1) The nucleolus leaves the germinal vesicle before the nuclear membrane dissolves, whereas usually this body is not cast out into the cytoplasm until after the spindle has begun to form; (2) The nucleolus does not become granular and disappear but persists intact for a considerable period; (3) It comes to occupy a definite position in the egg, i.e., at the posterior pole; (4) During cleavage the nucleolar material becomes distributed apparently equally among all of the primordial germ cells, and is absent from all of the somatic cells. Silvestri has given us no data regarding the escape of the nucleolus from the germinal vesicle and the writer is at present unable to account for this peculiar behavior, although he is now at work on the growing eggs of a polyembryonic, hymenopterous parasite which he hopes will enable him to determine this point, as well as to trace the history of the nucleolus back into early stages.[2]

As we have already noted, in a few instances the nucleolus does not disappear during the maturation divisions but persists for a time as a 'metanucleolus' (p. 457). The nucleoli of these parasitic Hymenoptera are of this sort. They are evidently of a different nature from the usual type and are hence saved from immediate disintegration in the cytoplasm. The localization of this nucleolus at the posterior end of the egg is the result,

[2] This has since been completed and published in the Anat. Anz., Bd. 46, pp. 51-69, 1914.

either of its own activity, or of that of the surrounding cytoplasm, or a combination of these. Gravity can have no decided effect upon it (Herrick, '95) since its position is constant, whereas the posterior end of the egg with respect to gravity is not. It also seems hardly possible that oxygenotactic stimuli are the cause of its change of position as has been suggested by Herbst ('94, '95), for the migration of the blastoderm-forming cells from the center to the surface of the eggs of certain arthropods. Concerning the fate of this nucleolus, one of the most difficult phenomena to explain is its fragmentation at a definite developmental stage and the apparently equal distribution of its substance to the primordial germ cells during their multiplication.

Haecker ('97) has suggested that the 'Aussenkörnchen' which appear in the egg of Cyclops during the formation of the first cleavage spindle may be nucleolar in nature. Later ('03) this idea was withdrawn and more recently Amma ('11) has likewise been unable to sustain this hypothesis. The most convincing data furnished by Amma are that in an allied form, Diaptomus coeruleus (fig. 17, *H*), these granules appear before the cleavage spindle is formed and before the nucleoli of the pronuclei have disappeared.

The remaining forms in which nucleoli have been considered as Keimbahn-determinants are merely suggestive. In Aequorea, Haecker ('92) traced the metanucleolus, which arises from the germinal vesicle, into certain cells of the blastula. Similar bodies appear in Mitrocoma (Metschnikoff, '86), Tiara (Boveri, '90), Stephanophyes (Chun, '91), Myzostoma (Wheeler, '97), and Asterias (Hartmann, '02), but their fate has not been determined.

It seems probable that in all these cases the same influences may be at work regulating the time, the place, and the method of localization of the nucleoli. Silvestri has made no attempt to explain this behavior. The writer can only conclude (1) that the 'nucleolo' of Silvestri and the 'metanucleoli' of other authors differ in nature from ordinary plasmosomes, chromatin-nucleoli, and double-nucleoli; (2) that these bodies are definitely segregated in a certain part of the egg or in a certain blastomere, probably by protoplasmic movements; and (3) that their disinte-

gration and the distribution of the resulting fragments or granules are controlled by reactions between them and the substances in which they are imbedded.

Chromatin. In two genera of animals the differentiation of the primordial germ cells is accompanied by a diminution of the chromatin in the nuclei of the somatic cells, so that eventually the nucleus of every germ cell is provided with the full complement of chromatin, whereas the nucleus of every somatic cell lacks a considerable portion of this substance, which remains behind in the cytoplasm when the daughter nuclei are reconstituted. These two genera are Ascaris and Miastor. This diminution process was described by Boveri ('92) in the former and confirmed by O. Meyer ('95) and Bonnevie ('01), and by Kahle ('08) in Miastor and confirmed by Hegner ('12). For details of these processes reference should be made to figures 3, 4, and 32 and pp. 390 and 442. It may be pointed out here that, although the final results are similar, the process differs in the two genera. In Ascaris both ends of each chromosome are split off, whereas in Miastor approximately one-half of each daughter chromosome is left behind to form the 'Chromosomenmittelplatte' (fig. 32, *cMp*) and later the 'Chromatinreste' (fig. 34, *cR*).

The elimination of chromatin during the maturation and early cleavage divisions of the egg, as well as during the mitotic divisions of other kinds of cells, has often been recorded. For example, Wilson ('95, p. 458) estimates that only about one-tenth of the chromatin in the germinal vesicle of the starfish is retained to form the chromosomes during the first maturation division, and Conklin ('02) finds that "in Crepidula the outflow of nuclear material occurs at each and every mitosis" (p. 51). Furthermore, Rhode ('11) argues that chromatin diminution is a normal histological process, and describes such phenomena in blood cells, nerve cells, and cleavage cells of several Amphibia, comparing conditions with the chromatin-diminution in Ascaris and Dytiscus. His conclusion is as follows:

In der Histogenese der allerverschiedensten Gewebe tritt uns also die Erscheinung entgegen, dass die sich entwicklenden Zellen, bzw.

Kerne einen Teil ihres Chromatins abstossen, d.h. also eine Chromatindiminution erfolgt, wenn auch die Befunde selbst im speziellen von den bisher beobachteten in der Einleitung beschriebenen Fällen der Chromatindiminution etwas abweichen.

Eine Chromatindiminution tritt also nicht nur am Anfang und Ende der Keimbahn, wie es bisher angegeben worden ist, sondern in den verschiedensten Entwicklungsstadien und bei den verschiedensten Geweben und Tieren ein, sie hat also offenbar eine allgemeine Bedeutung (pp. 24–25).

Diminution processes similar to those in Ascaris and Miastor have not been discovered in other animals, although investigators have been on the watch for such phenomena and have studied allied species, e.g., the work of Hasper ('11) on Chironomus and my own work on the Chrysomelid beetles (pp. 410 to 411). If, therefore, there be a similar difference in chromatin content between the germ cells and somatic cells in all animals, the elimination of chromatin from the latter must take place by the transformation of the basichromatin of the chromosomes into oxychromatin which passes into the cytoplasm during mitosis, or else by the more direct method advocated by the believers in the chromidia hypothesis.

The causes of the diminution of chromatin in Ascaris and Miastor are unknown. Recently Boveri ('10) has concluded from certain experiments on the eggs of Ascaris (p. 444) that in this form it is the cytoplasm in which the nuclei are imbedded which determines whether or not the latter shall undergo this process. Kahle ('08) does not explain the cause of the diminution in Miastor. To the writer it seems more important to discover why the nuclei of the Keimbahn cells *do not* lose part of their chromatin, since the elimination of chromatin during mitosis is apparently such a universal phenomenon. I would attribute this failure of certain cells to undergo the diminution process, not to the contents of the nucleus alone, but to the reaction between the nucleus and the surrounding cytoplasm. As stated in a former paper (Hegner, '09):

In Calligrapha all the nuclei of the egg are apparently alike, potentially, until in their migration toward the surface they reach the 'Keimhautblastem;' then those which chance to encounter the granules of

the pole-disc are differentiated by their environment, i.e., the granules, into germ-cells. In other words, whether or not a cell will become a germ-cell depends on its position in the egg just previous to the formation of the blastoderm (pp. 287–288).

Similarly, in Ascaris, the cleavage nuclei are conceived as similar so far as their 'prospective potency' is concerned, their future depending upon the character of their environment, i.e , the cytoplasm. In the egg of Miastor, the cleavage nucleus IV (fig. 3) does not lose part of its chromatin because of the character of the reaction between it and the substance of the 'polares Plasma.' In Chrysomelid beetles (Hegner, '08, '09a) and Chironomus (Hasper, '11) however, although no diminution process has been discovered in the nuclei which encounter the pole-disc or 'Keimbahnplasma,' the other nuclei in the egg, so far as known, are similar in this respect. The nuclei of the primordial germ cells, however, may be distinguished easily from those of the blastoderm cells in Chrysomelid beetles (figs. 53, 54), proving conclusively that a differentiation has taken place, either in one or the other. This differentiation probably occurs in the nuclei which take part in the formation of the blastoderm since the nuclei of the germ cells (fig. 53) retain more nearly the characteristic features of the preblastodermic nuclei (fig. 55), whereas those of the blastoderm cells (fig. 54) change considerably.

In some cases the eliminated chromatin may have some influence upon the histological differentiation of the cell, since it is differentially distributed to the daughter cells, but in Ascaris and Miastor no mechanism exists for regulating the distribution of the cast out chromatin and there is consequently no ground for the hypothesis that "in Ascaris those cells which become body cells are the ones that include the cast-off chromosome ends in their cytoplasm, and it will probably be found that these ejected chromosome parts engender such cytoplasmic differentiations as characterize the body cells" (Montgomery, '11, p. 192).

Chromidia. To several of the bodies listed in table 1 on page 461 as Keimbahn-determinants has been ascribed an origin from the chromatin of the germinal vesicle. Many cases of the elimination of chromatin from the nuclei of growing oocytes are

to be found in the literature. Blochmann ('86) discovered a process of 'budding' in the oocytes of Camponotus ligniperda resulting in the formation of 'Nebenkerne.' These appear first as small vacuoles lying near the nucleus; later they contain small staining granules and acquire a membrane. The 'Nebenkerne' grow in size and increase in number, while the nucleus of the oocyte becomes smaller. Stuhlmann ('86) described a similar phenomenon in about a dozen different species of Hymenoptera. The oocyte nucleus in all species examined becomes localized near the anterior end; then the small nuclear-like bodies form around it at its expense. The time of their production varies in the different species; in some they appear in the very young eggs; in others not until a much later stage has been reached. Sometimes they fuse to form a large 'Dotterkern' lying at the posterior pole of the egg; or they may remain separate and later become scattered. Paulcke ('00) also noted nuclear-like bodies near the oocyte nucleus of the queen bee, and Marshall ('07) has likewise found them in Polistes pallipes. In this species the nuclear-like bodies form a single layer around the nucleus; later they come to lie near the periphery of the oocyte and finally disappear. Loewenthal ('88) has described what appears to be chromatin in the cytoplasm of the egg of the cat, and an elimination of chromatin was noted by van Bambeke ('93) in the ovarian egg of Scorpaena scrofa. In none of these species, however, have Keimbahn-determinants been discovered.

According to Buchner ('10b) the 'besonderer Körper' in the egg of Sagitta and, in fact, Keimbahn-determinants in most other animals are of a chromidial nature, representing the trophochromatin demanded by the binuclearity hypothesis. The term chromidia was introduced by R. Hertwig in 1902 and applied to certain chromatin strands and granules of nuclear origin in the cytoplasm of Actinosphaerium. Goldschmidt ('04) transferred the chromidia hypothesis to the tissue cells of Ascaris. Since then chromidia have been described in the cells of many animals, including both somatic and germ cells. Thus far the group of zoologists which favor the chromidia idea has not received very extensive backing, but the fact remains

that chromatin particles are in some cases cast out of the nuclei in the oocytes of certain animals and continue to exist as such in the cytoplasm for a considerable period. It is also possible that, as Buchner ('10b) maintains, the Keimbahn-determinants may be in reality 'Keimbahnchromidien.'

This view was suggested by the writer in 1909 (p. 274) to account for the origin of the pole-disc granules in the eggs of Chrysomelid beetles. It was thought that here, as in the Hymenoptera (Blochmann, '86; et al.), chromatin granules might be cast out of the nuclei of the oocytes, and that these granules might gather at the posterior end to form the pole-disc. It was also suggested that chromatin granules from the nurse-cell nuclei might make their way into the oocyte and later become the granules of the pole-disc. It should not be forgotten, moreover, that these granules stain like chromatin. Finally, mention should be made of the 'anello cromatico' of Giardina ('01) which is associated with the differentiation of the oocytes in Dytiscus (p. 414).

Conclusion. Certain Keimbahn-determinants consist of nucleolar material which is derived from the germinal vesicle and which persists until the primordial germ cells are established. In some cases the Keimbahn cells are characterized by the possession of the complete amount of chromatin, in contrast to the somatic cells which lose a part of this substance. Since, however, the chromatin-diminution process does not occur in many species, it is not a universal phenomenon, and consequently cannot be of fundamental importance. Most of the evidence, on the other hand, points toward the conclusion that all of the cleavage nuclei are qualitatively alike, and that the cytoplasm is the controlling factor.

b. From cytoplasmic or extracellular nutritive substances: Yolk and nurse cells. It was pointed out on page 450 that one of the characteristics used to distinguish primordial germ cells from other embryonic cells is the presence of yolk material within them. In many vertebrates the yolk globules persist in the primordial germ cells until a comparatively late stage, and indeed are often so numerous as practically to conceal the nuclei of these cells. A large

number of the Keimbahn-determinants which have been described are supposed to consist of nutritive substances. Some of the earliest investigators were aware of the yolk content of the primordial germ cells. For example, in Chironomus Weismann ('63) found four oval nuclei lying in the 'Keimhautblastem' at the posterior end of the egg; each of these, he says, "besassen einen Kreisrunden, klaren, etwas röthlich schimmernden Kern, und in einigen Lagen ausserdem noch ein oder zwei Dotterkörnchen." These are the 'Polzellen.' In another dipteron (Simula *sp.*) Metschnikoff (1866) records four or five pole-cells which "bestehen ausser einem Kerne noch aus einer die feinsten Dotterkörnchen enthaltenden Zellsubstanz." The same author ('66) also states that when the pseudovum in the paedogenetic larva of Miastor contains twelve to fifteen nuclei, "Man bemerkt zunächst, dass der am spitzen Pole des Pseudovums liegende Keimkern von einer dicken dunkeln Dottermasse schärger umgeben wird und mit dieser zusammen bald in eine besondere, 0.017 mm. grosse, membranlose Zelle sich abschnürt." This gives rise to the pole-cells.

In certain Daphnidae, Weismann and Ischikawa ('89) describe a 'Paracopulationszelle' which is derived from the contents of the germinal vesicle (p. 433); but the recent work of Kühn ('11, '13) renders it probable that this body is nothing but the remains of a nurse cell. The 'Dotterplatte' discovered by Noack ('01) at the posterior end of the egg of Calliphora (fig. 2) is considered by this investigator to consist of yolk elements. In previous communications (Hegner, '08, '09a, '11b) the writer has discussed the probability that the pole-disc in Chrysomelid eggs consists of nutritive material, and Wieman ('10a) also has offered arguments for this view.

Kühn ('11, '13) has presented what appears to be certain evidence that the Keimbahn-determinants in the egg of the Cladoceron, Polyphemus pediculus, arise from one or more nurse cells. The granules segregated in certain cleavage cells of Neritina (Blochmann, '82) Asplanchna (Jennings, '96), Lepas (Bigelow, '02), Siphonaria (Fujitas, '04), and Physa (Wierzejski, '05) may be of a nutritive nature and these cells may be the stem cells

from which the germ cells of these animals eventually arise. The hypothesis that the nucleoli consist of food substance also argues in favor of the idea that the Keimbahn-determinants are nutritive.

The importance of these nutritive substances to the primordial germ cells can be stated with some degree of certainty. According to some authorities the primordial germ cells remain in the primitive condition and do not undergo differentiation at the same time, or at least at the same rate, as do the other embryonic cells. On this account their yolk contents are not at first utilized, since their metabolic activities are so slight. This is more especially true of the vertebrates, in which it has been suggested (Hegner, '09a, p. 276), that the yolk contents of the germ cells are transformed into the energy of motion during the characteristic migration of these cells into the germinal epithelium. Why these nutritive substances are segregated in the primordial germ cells is more difficult to answer.

Finally, it is interesting to note that the differentiation of the indifferent germ cells of Helix arbustorum into spermatogonia or oogonia has been found to depend upon nutrition (Buresch, '11). "Ob aber eine indifferente Geschlechtszelle sich in männlicher oder weiblicher Richtung weiter entwickeln wird, das können wir schon sehr früh sagen, nämlich nach der Lage diesen Zelle näher oder weiter von einen Nährzelle" (p. 327).

Yolk nucleus. There are many bodies in the cytoplasm of growing oocytes which have been called yolk nuclei and which may be responsible for the origin of the Keimbahn-determinants. Some of these bodies have already been considered, but the term 'yolk nucleus' has been applied to so many different cytoplasmic inclusions (Munson, '12) that no attempt will be made here to describe them nor to trace their history.

Mitochondria. The condition of the chondriosomes in the primordial germ cells of certain vertebrates (Rubaschkin, '10, '12; Tschaschkin, '10) and the theories proposed regarding the rôle of these bodies in heredity make it necessary to refer to them briefly here. A review of the literature on mitochondria, chondriosomes, plastosomes, etc., would be superfluous since

this has been done by Benda ('03) Prenant ('10), Faure-Fremiet ('10), and especially Duesburg ('11). At the present time it is difficult to make any definite statement regarding the origin, nature, and significance of the various cytoplasmic inclusions that have been grouped under the general title of mitochondia. It seems probable that we are concerned with a number of different sorts of inclusions, and with various stages in their evolution.

Mitochondria appear to be present in practically all cells, at least at some period in their existence. They have been observed in plants as well as in animals, and in living as well as in fixed and stained cells. Many terms have been applied to them of which the most frequently employed are mitochondria (Benda, '03), chondriosome (Meves, '08) plastochondria (Meves, '10) and plastosomes (Meves, '10). The advocates of the chromidia believe that these granules include mitochondrial formations (Goldschmidt, '04) and that the latter are therefore of nuclear origin, i.e., chromatic (Popoff, '07; Wassilieff, '07; Buchner, '10; Jörgensen, '10; et al.). On the other hand, the majority of investigators consider the mitochondria as cytoplasmic bodies (Vejdovsky, '07; Meves, '08; Duesberg, '11; Wilke, '12; et al.). Various functions have been ascribed to the mitochondria. Benda considered them to be motile; Regaud ('09) thinks that they fix and concentrate various substances in the cell ('fonction électique' of Renaut); and Meves ('08) maintains that they represent an important heredity substance, with the same relation to the cytoplasm that the chromosomes have to the nucleus.

Of particular interest to us are the results of Rubaschkin ('10, '12) and Tschaschin ('10) on the germ cells of vertebrates. In the guinea-pig and chick the chondriosomes of the cleavage cells are spherical and all similar, but, as development proceeds, those of the cells which become differentiated to produce the germ layers unite to form chains and threads, whereas those of the primordial germ cells remain in a spherical and therefore primitive condition (figs. 23, *B*; 24). This distinction between the mitochondrial nature of the primordial germ cells and the surrounding somatic cells may enable us to trace the Keimbahn

in vertebrates back into cleavage stages—something that has not been accomplished as yet.

It seems too early to speculate as to the influence of the mitochondria upon the primordial germ cells. That they are bearers of hereditary qualities and that those brought into the egg by the spermatozoon fuse with those of the egg as described by Meves ('08, '11) is doubted by many observers. Montgomery ('12) has shown that in Peripatus the mitochondria are entirely cast out of the spermatozoon during its metamorphosis, and that, at least in this species, the male cell does not contribute any of these bodies to the egg during fertilization. The same writer (Montgomery, '11) proposes an hypothesis to account for the segregation of germ cells as follows: "Any cleavage cell which failed to receive mitochondria, or failed to receive particular ones or a particular amount of them, would be incapacitated from engendering such somatic specializations (fibrillar structures), it would thereby become a germ cell" (p. 791). This substitution hypothesis is also offered by Montgomery, that the mitochondria of the prospective germ cells remain unaltered or latent, while those of the other cells undergo developmental changes. As we have already noted, evidence of such a condition had already been supplied and a similar hypothesis proposed by Rubaschkin ('10) and Tschaschin ('10); but so far as I know there are no data which enable us to sustain the first hypothesis. The data given in a previous part of this paper show that the pole-disc in Chrysomelid eggs may arise from mitochondria, but this does not seem very probable.

An examination of the various Keimbahn-determinants listed in table 1 (p. 461) has led the writer to conclude that none of them is of a mitochondrial nature, but the results obtained by the special methods employed by students of mitochondria give us good reason to hope that other substances may be made visible which will help to clear up the problem of primary cellular differentiation.

Metabolic products. Among the most difficult cases to explain are those of Sagitta and certain copepods, since here the Keimbahn-determinants apparently arise de novo in the cytoplasm.

Buchner's ('10b) contention that the 'besonderer Körper' of Sagitta is the remains of the 'accessory fertilization cell' of Stevens ('04) is not sustained by either Stevens ('10) or Elpatiewsky ('10). The nucleolar nature of the 'Aussenkörnchen' in Cyclops ('97) was later discarded ('03) and the conclusion was reached "dass ich die Aussenkörnchen ähnlich wie die Nukleolen, für temporare, nicht-strukturierte Abscheidingen oder Zwischenprodukte des Kern-Zelle-Stoffwechses halte, welche in ganz bestimmten Zustanden der Zelle zur Abscheidung gelangen bezw. wieder aufgelöst werden" (p. 308–309). Amma ('11) has considered this subject at some length, and after rejecting the possibilities of these being of (1) chromatic, (2) nucleolar, (3) chromidial, or (4) mitochondrial origin, concludes that they are transitory structures and "dass die Ectosomen als Abscheidungen Endprodukte des Kern-Zelle–Stoffwechsels aufzufassen sind," (p. 557). In this way the Keimbahn-determinants in copepods are satisfactorily explained, and a similar explanation may be applied to Sagitta, although with less certainty.

c. From a differentiated part of the cytoplasm. A review of the literature on the Keimbahn-determinants and the investigation of these substances in the eggs of insects force me to conclude that the fundamental organization of the egg is responsible for the segregation of the primordial germ cells, whereas the visible substances simply furnished evidence of this underlying organization. As I have stated elsewhere (Hegner, '08, p. 21), regarding the Keimbahn-determinants in beetles' eggs, "the granules of the pole-disc are therefore either the germ cell determinants or the visible sign of the germ cell determinants." The writer's experiments have thus far failed to determine the exact function of these granules. When the posterior end of a freshly laid beetle's egg is pricked with a needle, not only the pole-disc granules flow out, but also the cytoplasm in which they are imbedded (Hegner, '08). If a small region at the posterior end be killed with a hot needle the pole-disc is prevented from taking part in the development of the egg, but so also is the surrounding cytoplasm. Eggs thus treated continue to develop and produce embryos without germ cells, but, as a rule, a part of

the posterior end of the abdomen is also absent (Hegner, '11a). The pole-disc granules and the cytoplasm containing them are moved by centrifugal force toward the heavy end of the egg and the latter is proved to be quite rigid, but eggs thus treated do not develop sufficiently normally to enable one to decide whether the pole-disc produces germ cells in its new environment or not.

That the germ cells of Chironomus arise from a prelocalized substance was stated by Balbiani ('85) in these words, "les glandes genitals des deux sexes ont une origine absolutment identifique, naissant de la même substance et au même point de l'oeuf." Later Ritter ('90) expressed the opinion that the 'Keimwulst' of Chironomus consists "aus feinkörnigem Protoplasma," an opinion concurred in by Hasper ('11) who terms it 'Keimbahnplasma.' The similar material in Miastor metraloas—the 'polares Plasma'—is considered a special sort of protoplasm by Kahle ('08) and I can confirm this for Miastor americana. Further evidence of the protoplasmic nature of the substances which become segregated in the primordial germ cells is furnished by Boveri's experiments on Ascaris. In 1904 this investigator concluded from a study of dispermic eggs that the diminution process is controlled by the cytoplasm and not by an intrinsic property of the chromosomes, and that the chromosomes of nuclei lying in the vegetative cytoplasm remain intact, whereas those of nuclei imbedded in the animal cytoplasm undergo diminution. This conclusion has been strengthened by more recent experimental evidence (Boveri, '10) both from observation on the development of dispermic eggs and from a study of centrifuged eggs (fig. 19, p. 378). Boveri's results furnish a remarkable confirmation of the conclusions reached by the writer from a morphological study of the germ cells of Chrysomelid beetles and expressed in the following words: "All the cleavage nuclei in the eggs of the above named beetles are potentially alike until in their migration toward the periphery they reach the 'Keimhautblastem.' Then those which chance to encounter the granules of the pole-disc are differentiated by their environment, i.e., the granules, into germ-cells; all the other cleavage products become somatic cells."

Here, however, the pole-disc granules were considered the essential substance.

The appearance of the Keimbahn-determinants at a certain time and in a certain place, and their determinate segregation point unmistakably to an underlying regulating mechanism. These phenomena have some definite relation to the fundamental organization of the egg and require an investigation of our present knowledge of this subject.

The isotropism of the egg, as postulated by Pflüger, and the 'cell interaction' idea, especially developed by O. Hertwig and Driesch, have given way before the beautiful researches tending to uphold the hypothesis of 'germinal localization' proposed by His and championed by so many investigators within the past two decades. The starting point for embryological studies has shifted from the germ layers to the cleavage cells and from these to the undivided egg. Organization, which Whitman ('93) maintains precedes cell-formation and regulates it, is now traced back to very early stages in the germ cell cycle and is held responsible for the cytoplasmic localization in the egg.

One of the fundamental characteristics of the egg is its polarity. It has been known for about thirty years that the eggs of insects are definitely oriented within the ovaries of the adults. Hallez in 1886, finding this to be true of the ova of *Hydrophilus* and *Locusta*, expressed the fact in his "Loi de l'orientation de l'embryon chez les insectes" as follows: "La cellule-oeuf possede la meme orientation que l'organisme maternal qui l'a produit: elle a un pole cephalique et un pole caudal, un cote droit et un cote gauche, une face dorsale et une face ventrale; et ces differentes faces de la cellule-oeuf coincident aux faces correspondentes de l'embryon." Moreover, gravity and the action of centrifugal force have no effect upon polarity of insect eggs (Hegner, '09b). Giardina ('01) has found that during the divisions of the oogonia in Dytiscus, a rosette of sixteen cells is produced, one of which is the oocyte and the other fifteen nurse cells. The rosette thus formed possesses a definite polarity coincident with the axis of the oocyte which is identical with that which was present in the last generation of oogonia. Similarly in Miastor (fig. 27) the

polarity of the oocyte is recognizable as soon as the mesodermal cells, which serve in this species as nurse cells, become associated with it.

The germ cells of other animals also possess a precocious polarity, as evidenced by their implantation in the germinal epithelium (e.g., Wilson, '03; Zeleny, '04, in Cerebratulus), the position of the nucleus, the formation of the micropyle (Jenkinson, '11), etc. This is true not only for the invertebrates, but, as Bartelmez ('12) claims, "the polar axis persists unmodified from generation to generation in the vertebrates and is one of the fundamental features of the organization of the protoplasm" (p. 310). Furthermore, experiments with centrifuged force seem to prove that the chief axis of the egg is not altered when substances are shifted about, but is fixed at all stages (Lillie, '09; Morgan, '09; Conklin, '10). Bilaterality also is demonstrable in the early stages of the germ cells of many animals, and like polarity, seems to be a fundamental characteristic of the protoplasm.

It is somewhat difficult to harmonize the various results obtained, especially by experimental methods, from the study of egg organization. As the oocytes grow, the apparently homogeneous contents become visibly different in some animals, and when the mature eggs develop normally these 'organ-forming substances' are segregated in definite cleavage cells and finally become associated with definite organs of the larva.

Conklin ('05) has shown "that at least five of the substances which are present in the egg [of Cynthia] at the close of the first cleavage, viz., ectoplasm, endoplasm, myoplasm, chymoplasm, and chordaneuroplasm, are organ-forming substances." Under experimental conditions

> . . . . they develop, if they develop at all, into the organs which they would normally produce; and conversely, embryos which lack these substances, lack also the organs which would form from them. . . . . Three of these substances are clearly distinguishable in the ovarian egg and I do not doubt that even at this stage they are differentiated for particular ends (p. 220). . . . . The development of ascidians is a mosaic work because there are definitely localized organ-forming substances in the egg; in fact the mosaic is one of organ-form-

ing substances rather than of cleavage cells. The study of ctenophores, nemertines, annelids, mollusks, ascidians and amphibians (the frog) shows that the same is probably true of all these forms and it suggests that the mosaic principle may apply to all animals (p. 221).

The same writer has also proved from his study on Phallusia ('11) that these various substances exist even when they are not visible in the living egg. It is interesting also to note that Duesberg ('13) finds the 'myoplasm' of Cynthia to be crowded with plasmosomes, differing in this respect from other egg regions.

Experiments, especially those of Lillie ('06), Morgan and Spooner ('09), Morgan ('10) and Conklin ('10) have shown that in many eggs the shifting of the supposed organ-forming substances has no influence upon development, and leads to the conclusion that these visible substances play no fundamental rôle in differentiation, but that the invisible ground substance is responsible for determinate development. The eggs of different animals, however, differ both in time and degree of organization, and the conflicting results may be accounted for by the fact that specification is more precocious in some than in others.

The most plausible conclusions from a consideration of these observations and experiments are that every one of the eggs in which Keimbahn-determinants have been described, consists essentially of a fundamental ground substance which determines the orientation; that the time of appearance of Keimbahn-determinants depends upon the precociousness of the egg; that the Keimbahn-determinants are the visible evidences of differentiation in the cytoplasm; and that these differentiated portions of the cytoplasm are definitely localized by cytoplasmic movements, especially at about the time of maturation.

B. The localization of the Keimbahn-determinants

One of the characteristics of the Keimbahn-determinants is their regular appearance at a certain stage in the germ cell cycle, according to the species in which they occur, and their constant localization in a definite part of the egg, or in one or more definite cleavage cells. Keimbahn-determinants are recognizable in

many insect eggs before fertilization is accomplished, and even before the oocyte has reached its maximum size. We know that in Chironomus the 'Keimwulst' (Ritter, '90) or 'Keimbahnplasma' (Hasper, '11) is present when the egg is laid, at which time the pronuclei as a rule have not yet fused. This is true also of the 'Dotterplatte' in Calliphora (Noack, '01). There can be little doubt, however, that these substances are present as such in the eggs before fertilization, judging from our knowledge of the history of similar materials in the eggs of other insects. The 'pole-disc' in the eggs of Chrysomelid beetles (Hegner, '08; Wieman, '10a) and the 'polares Plasma' in Miastor (Kahle, '08; Hegner, '12) are recognizable some time before fertilization and cannot therefore arise because of any influence exerted by the spermatozoon. Moreover, in Miastor the eggs thus far examined have all been parthenogenetic. In parasitic Hymenoptera the 'nucleolo' leaves the germinal vesicle in both fertilized and parthenogenetic eggs before the egg is laid. In only one animal, not an insect, has a similar occurrence been noted, namely, in Polyphemus, where, according to Kühn ('11, '13) the Keimbahn-determinants consist of the remains of one or more nurse cells (fig. 18). In the Daphnidae (Weismann and Ischikawa, '89) the 'Paracopulationszelle' arises from material cast out by the germinal vesicle; in Aequorea (Haecker, '92) the 'Metanucleolus' is likewise derived from the germinal vesicle; in Ascaris (Boveri, '92) chromatin diminution occurs during the two to four-cell stage; in Cyclops (Haecker, '97, '03) and other copepods (Amma, '11) the 'Aussenkörnchen' or 'Ectosomen' become visible soon after fertilization (Diaptomus), but usually not until the pronuclei fuse (other species); in Sagitta the 'besonderer Körper' (Elpatiewsky, '09, '10) or 'Keimbahnchromidien' (Buchner, '10b) appear to arise de novo after fertilization, although, if Buchner's contention that they are the remains of the accessory fertilization cells be correct, they should be classed with the 'Nahrzellenkern' described by Kühn ('11, '13) in Polyphemus.

It is thus evident that the Keimbahn-determinants become visible, wherever they have been described, either just before

or just after the eggs are fertilized, or, in parthenogenetic forms, shortly before maturation and cleavage are inaugurated.

The localization of the Keimbahn-determinants at the time of their appearance seems to be predetermined. In insects the posterior end of the egg is invariably the place where these bodies occur. In species whose eggs undergo total cleavage they are, as a rule, under normal conditions, segregated in one definite blastomere from the two-cell stage up to the thirty-two cell stage, and are then distributed among the descendants of the single primordial germ cell. In Ascaris it is normally the cell at the posterior (vegetative) pole that fails to undergo the diminution process. It seems therefore that there must be some mechanism in the egg which definitely localizes the Keimbahn-determinants.

The segregation of these substances in one blastomere at the first cleavage division is a result of their previous localization, but in later cleavage stages events are more difficult to interpret. Both Haecker ('97) and Amma ('11) have attempted to explain the distribution of the 'Ectosomen' in copepods by postulating a dissimilar influence of the centrosomes resulting in the segregation of these granules at one end of the mitotic spindle in the dividing stem-cell. According to Zeigler's hypothesis, the centrosomes during unequal cell division are heterodynamic, and Schönfeld ('01) believes that the synizesis is due to the attraction of the chromosomes by the centrosomes. It is well known that in many cases where unequal cell division occurs, one aster is larger than the other, and this may be the true interpretation of the phenomena, but to the writer it seems more probable that the entire cell contents undergo rearrangement after each cell division, possibly under the influence of the material elaborated within the nucleus and set free during mitosis. Elpatiewsky ('09) also believes in the unequal attractive force of the centrosomes in Sagitta, as indicated in the following quotation:

> Nach der vierten Teilung kommt der besondere Körper in dem Wirkungskreis eines Zentrosomas, nämlich desjenigen, welcher näher der Polarfurche liegt. Fast die ganze 'Energie' dieses Zentrosomas wird für die Ueberwindung der Vis inertiae des besonderen Körpers

verbraucht; dieser wird dem Zentrosoma genähert und umschliesst es wie mit einer Kappe, so dass er im optischen Durchschnitt stets Hufeisen oder Sichelform aufweist. Infolge davon wird die wirkung dieses Zentrosomas auf das Zellplasma nur sehr schwach, dieses Zentrosoma kann nur einen kleinen Plasmateil beherrschen, und die resultierende Zelle wird viel kleiner, als die Schwesterzelle. Diese kleine Zelle, die den besonderen Körper bekommen hat, liegt näher zum vegetativen Poles, als die grössere Schwesterzelle, und stellt die erste Urgeschlechtszelle G (d^{111}), die grössere Schwesterzelle die erste Urentodermzelle E (d^{112}) vor (p. 231).

In Ascaris, certain copepods, Sagitta, Polyphemus and certain Daphnidae, the Keimbahn-determinants are not segregated in one cleavage cell after about the thirty-two cell stage, but their substance is distributed at the next division between the daughter cells. In the insects, such as Chironomus, Miastor and Chrysomelid beetles, where, on account of the superficial cleavage, the Keimbahn-determinants are not segregated in blastomeres, the primordial germ cells, from the beginning, consist almost entirely of the Keimbahn material or this material plus the matrix in which it is imbedded. Hence in these cases the Keimbahn-determinants are localized at a determined point during each cleavage stage, instead of being carried about by the movements of the egg contents or of the blastomeres, but, as in the eggs which undergo total cleavage, the determinants are distributed between the daughter cells as soon as the primordial germ cells are established. The reason for this appears to be that localizations occur in holoblastic eggs at each cleavage and that not until the thirty-two cell stage or thereabouts does the Keimbahn material become entirely separated from other organ-forming substances and segregated in a single cell. When this point is finally reached this Keimbahn material must necessarily become divided between the daughter cells.

In practically all known cases the daughter cells of the primordial germ cells are equal in size and each receives an equal portion of the Keimbahn-determinants. This is certainly to be expected from their constitution and future history. Sagitta, however, differs in this respect for the remains of the 'besonderer Körper' appear to be unequally distributed between the two

daughter cells of the primordial germ cells (fig. 22) and both Elpatiewsky ('09, '10) and Stevens ('10), therefore, consider this as probably a differential division whereby in this hermaphroditic animal the substance of the male primordial germ cell is separated from that of the female. More work is necessary to make certain of this point.

Conclusion. Keimbahn-determinants are definitely localized in the egg and in definite cleavage cells. This localization is first observable just before or just after the eggs are fertilized, or, in parthenogenetic forms, shortly before maturation and cleavage are inaugurated. Some mechanism in the egg must be responsible for this localization. Heterodynamic centrosomes may have some influence so far as the segregation of the Keimbahn-determinants in cleavage cells is concerned, but the movement of the egg contents seems to be a more probable cause of localization.

C. The fate of the Keimbahn-determinants

It is unfortunately impossible to trace the Keimbahn-determinants throughout the entire germ cell cycle. The question of their fate, however, is an important one. As we have seen, they become apparent shortly before or just after the inauguration of the maturation divisions, and remain intact for a brief period during the early cleavage stages. They persist in insects as definitely recognizable granules (fig. 8, *F*) for some time after the primordial germ cells are segregated; then they gradually break up into finer particles, leaving no trace of their existence behind except in so far as they give the cytoplasm of the germ cells a greater affinity for certain dyes. In Chironomus they may still form distinct masses after the definitive germ glands have been formed (fig. 1). The ectosomes in the copepods are temporary bodies which appear to rise de novo during the formation of each mitotic figure in the early cleavage stages; then break down and disappear. Practically all of the other Keimbahn-determinants persist during early cleavage and then disappear as distinct visible bodies as soon as the primordial germ cells

are definitely segregated. What becomes of them during the comparatively long period between their disappearance in the primordial germ cells and their reappearance in the oocytes or mature eggs can only be conjectured. They seem to disintegrate into very fine particles which become thoroughly scattered within the cell body and mixed with the cytoplasm. It has been suggested (p. 397) that they may retain their physiological characteristics and become concentrated again in the growing oocytes into morphologically similar bodies, increasing in the meantime, by multiplication or in some other way, until they equal in mass those of the preceding generation of germ cells. On the other hand, they may all, like the ectosomes of copepods, be temporary structures, produced at a certain time and place under similar metabolic conditions, and becoming associated with particular parts of the cell contents, may thus be constant in their distribution.

Several ideas have been advanced regarding the fate of the eliminated chromatin in Ascaris. The ends of the chromosomes which are cast out into the cytoplasm are not equally distributed among the daughter cells nor does there appear to be any mechanism for their definite unequal division. These facts argue against the theory that these cast out chromatin bodies serve as determinants and also make improbable the hypothesis that they enable the somatic cells to differentiate, whereas the germ cells which do not undergo the diminution process remain in an indifferent condition since their cytoplasm lacks this material (Montgomery, '11, p. 792). However, the fact that during the early cleavage divisions in some animals (p. 465) large amounts of chromatin escape from the nucleus and are differentially distributed to the daughter cells, is evidence that nuclear material may play some important rôle in the progressive changes of cleavage cells.

It has been shown that in many animals the germ cells do not multiply for a considerable period during the early developmental stages. This period also coincides with that during which the Keimbahn-determinants, as a rule, disappear. For example, the germ cells of Chrysomelid beetles multiply until there are about sixty-four present, at which time they constitute a group

at the posterior end of the egg and the embryo has just started to form; no further increase in number occurs until the larval stage is reached and the definitive germ glands are established. As soon, however, as the embryo has reached a certain developmental stage, the germ cells migrate into it, and it looks very much as though they remain quiescent until the somatic cells are "able to protect, nourish, and transport" them.

The number of primordial germ cells during the 'period of rest' is perhaps most definitely known in Miastor, where, as one group of eight and later as two groups of four each, they are present throughout a large part of embryonic development.

In vertebrates also, a long period exists, during which division of the primordial germ cells does not take place (fig. 24) and, at least in several species, certain cell contents (the mitochondria) remain in an indifferent condition (Rubaschkin, '10; Tschaschkin '10; fig. 23, *B*). These facts all indicate that these cells remain in a primitive condition and do not undergo the histological differentiations characteristic of somatic cells, a view which, however, has been objected to (Eigemann, '96). The disappearance of the Keimbahn-determinants and the yolk globules of vertebrates during this period have suggested that these substances are nutritive in function, furnishing energy to the migrating germ cells.

The fact of this long rest period, followed by rapid multiplication of the oogonia and spermatogonia, during which no important specializations occur, and later succeeded by the remarkable changes which occur in both the oocytes and spermatocytes has led to the suggestion (Montgomery, '11, pp. 790–792) that in the germ cell cycle there is a series of changes parallel with that of the somatic cycle. In the development of both cycles preformation and epigenesis proceed at the same time. The chromosomes seem to be preformed elements of the germ cells, since they are apparently the most stable constituents. The cytoplasm, on the other hand, undergoes a series of epigenetic changes such as the formation of an idiozome, the development of mitochondria, the appearance of a sphere and the metamorphosis of the spermatozoon.

Finally, we must inquire into the fate of the Keimbahn-determinants in the male germ cells. Does the Keimbahn material in these cells increase in amount, as has been suggested for the oocytes, and is it localized in the spermatogonia, spermatocytes, or spermatozoa as a definite, visible substance? We know from the investigations of Meves ('11) that the plastosomes in the spermatozoon are carried into the egg, in the case of Ascaris, and there fuse with the plastosomes of the ovum. Whether Keimbahn-determinants act in a similar manner is unknown. There are, however, certain cytoplasmic inclusions in the male germ cells which have been compared with similar structures in the oocytes; for example, the chromatic body described by Buchner ('09) in the spermatogenesis of Gryllus (see p. 415), and the plasmosome which is cast out of the nucleus of the second spermatogonia in Periplaneta and disintegrates in the cytoplasm (Morse, '09). That Keimbahn-determinants from the spermatozoon are not necessary for the normal production of germ cells is of course evident, since some of the species with which we are best acquainted (for example, Miastor) are parthenogenetic.

7. CONCLUSIONS AND SUMMARY

1. The most interesting period in the germ-cell cycle is that extending from the formation of the ultimate oogonia and spermatogonia to the complete segregation of the germ cells in the developing egg. A little known and important part of this period is that during which, in some animals, visible substances (Keimbahn-determinants) peculiar to the germ cells, appear, become localized in a definite part of the egg or in certain blastomeres, and are equally distributed among the primordial germ cells (pp. 376–379).

2. The Keimbahn in animals was first traced in dipterous insects. Keimbahn-determinants appear in the eggs of all Diptera that have been carefully studied. The most detailed reports have been upon Miastor, Chironomus, and Calliphora (figs. 1–2, pp. 380–387).

3. In Miastor there are a definite number of cell divisions during the multiplication of the oogonia, namely, six. The somatic cells lose part of their chromatin by diminution processes, whereas the germ cells possess a complete amount of chromatin. The nurse cells are of mesodermal origin. A peculiar mass of cytoplasm becomes situated at the posterior end of the oocyte; within this one of the first eight cleavage nuclei (with a complete amount of chromatin) becomes imbedded; it is then cut off from the rest of the egg as the primordial germ cell. The origin of this peculiar mass of cytoplasm could not be determined, but several hypotheses are offered to account for its genesis (figs. 27–40, 3–5, pp. 387–398).

4. The eggs of the ovoviviparous dipteron, Compsilura and of the willow-cone gall fly, Cecidomyia, contain Keimbahn-determinants which have a history like that of similar bodies in other insects (figs. 41–43, 6, pp. 398–399).

5. An early segregation of germ cells has been reported for certain Chrysomelid beetles and Keimbahn-determinants have been found in the eggs of those carefuly examined. A résumé of the writer's previously published results is given (figs. 7–8, pp. 400–408).

6. An examination of all stages in the early cleavage of Chrysomelid eggs failed to reveal a chromatin-diminution process such as occurs in Ascaris and Miastor. The conclusion is reached that the cleavage nuclei are all potentially alike and that the cytoplasm controls their differentiation into the nuclei of blastoderm cells, primordial germ cells, and vitellophags. What appears to be amitotic nuclear division among the vitellophags is described (figs. 44–57, 7–8, pp. 408–413).

7. No nuclear changes were observed in the germ-cell cycle of Leptinotarsa resembling those recorded by Giardina ('01) and others in Dytiscus resulting in the formation of nurse cells and ultimate oogonia (pp. 413–417).

8. The pole-disc granules in Chrysomelid eggs form a recognizable mass, just before the oocyte reaches its full size. This genesis could not be definitely determined, but several methods of origin are suggested. The growth of the oocyte is described and figured (figs. 58–66, 9, pp. 417–420).

9. In the testis of Leptinotarsa the germ cells in each cyst arise from a single spermatogonium. Spindle remains connect the daughter spermatogonia up to the time when sixty-four cells are present in each cyst. This process is homologous to the differential divisions in Dytiscus and other beetles, and certain Hymenoptera, during which an ultimate oogonium and a definite number of nurse cells arise from a single oogonium (figs. 68–74, 10–12, pp. 420–424).

10. What appears to be amitotic muclear division was found among the nurse cells of Leptinotarsa, but no nuclear phenomena were observed among the oogonia or spermatogonia which could be interpreted as amitosis and which could not be regarded as phases of mitosis (fig. 67, p. 427.)

11. A brief statement is given of certain phenomena that have been recorded during the segregation of the germ cells in the Hymenoptera (figs. 13–14, pp. 428–432).

12. The Keimbahn in the Crustacea is best known in certain Cladocera and Copepoda. In some species the Keimbahn-determinants seem to be temporary bodies which represent the "Endprodukte des Kern-Zelle-Stoffweschsels." In others they appear to originate from nurse cells which enter the oocyte (figs. 15–18, pp. 432–441).

13. In several species of Ascaris a determinate segregation of germ cells has been recorded and a chromatin-diminution process discovered. The most recent work indicates that this diminution process is controlled by the cytoplasm and is not initiated by the nuclei (figs. 19–21, pp. 442–446).

14. The origin of the 'besondere Körper,' which serves as a Keimbahn-determinant in Sagitta, has not been determined. It apparently is unequally distributed when the primordial germ cell divides, and the daughter cell which receives the larger portion is considered the primordial spermatogonium; the other is the primordial oogonium (figs. 22, pp. 446–449).

15. The vertebrates do not furnish as favorable material for germ cell studies as do the invertebrates. No definite Keimbahn-determinants have been discovered in them but bodies have been described in the cell body of some of them which give

us hopes of tracing the germ-cell cycle back to cleavage stages. A peculiar rod has been found in the spermatogonia of man which is regarded as a Sertoli-cell-determinant (figs. 23–25, pp. 449–456).

16. Metanucleoli and granules of various kinds have been described in the mature eggs and early cleavage stages of a number of animals, which may, upon closer study, be found to play some rôle in the segregation of the germ cells (fig. 26, pp. 456–460).

17. A table is given (p. 461) of the principal described cases of the occurrence of the Keimbahn-determinants in animals. These bodies in certain species arise from nuclear substances, such as metanucleoli, chromidia, or the 'achromatishen Kernkörper' (pp. 462–469).

18. Yolk globules are characteristic of the primordial germ cells and may play some rôle in their genesis; nurse cells are known to become Keimbahn-determinants in some Crustacea; and in certain other Crustacea the Keimbahn-determinants are considered metabolic products; the mitochondria of germ cells differ from those of the surrounding somatic cells (pp. 469–474).

19. Observations and experiments indicate that the egg is definitely organized and that this organization is continuous throughout the germ cell cycle. The Keimbahn-determinants are bodies which enable us to determine the position of that part of the egg substance which controls the production of the primordial germ cells, and to identify the stem cell and ultimately the primordial germ cells when they are definitely established (pp. 474–478).

20. Keimbahn-determinants become visible at a definite time and place, usually just before or just after maturation takes place and cleavage begins. The localization of these bodies is determined by the organization of the cytoplasm and takes place during cleavage, either under the influence of the centrosomes, or, more probably, by rearrangements of the egg contents (pp. 478–482).

21. The Keimbahn-determinants become distributed apparently equally (except in Sagitta) between the daughter cells of the primordial germ cell. As a rule they then gradually disintegrate and disappear, and hence cannot be traced throughout

the entire germ cell cycle. It seems probable, however, that the particular kind of cytoplasm (germ-plasm) marked by the presence of these bodies is continuous throughout the germ cell cycle. In some animals (e.g., Miastor, fig. 32) it constitutes the entire substance (with the exception of the nucleus) of the primordial germ cell; this is also true in other animals, but, as shown in figure 17, a succession of cleavage divisions occurs before the germ-plasm is entirely separated from the somatic-plasm. The nature of the Keimbahn-determinants is uncertain, but their origin from metanucleoli, nurse cells, and possibly the nutritive stream suggests that they may play a rôle in the nutrition of the germ cells during the period extending from their segregation until the formation of the definitive germ glands (pp. 482–485).

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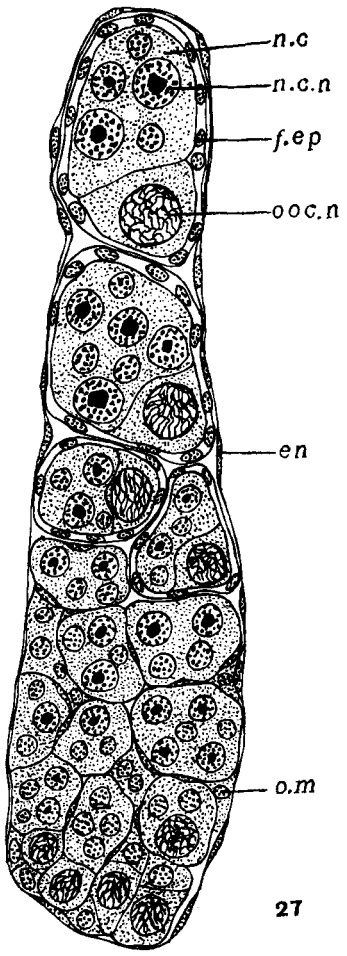
ABBREVIATIONS

b.c, blastoderm cell
c, cytoplasm
ch, chorion
cMp, 'Chromosomenmittleplatte'
c.n, cleavage nucleus
cR, 'Chromatinreste'
en, ovarian envelope
ep, epithelial cell
f.ep, follicular epithelium
f.n, female nucleus
g.v, germinal vesicle
m, mesoderm
m.s, maturation spindle
n.c, nurse chamber
n.c.n, nurse cell nucleus
o.m, ovarian mesoderm
ooc, oocyte
ooc.n, oocyte nucleus
oog, oogonium
p.b, polar body
pd, pole-disc
p.g.c, primordial germ cell
p.Pl, 'polares Plasma'
s, spermatozoon
spg, spermatogonium
t.c., terminal cap



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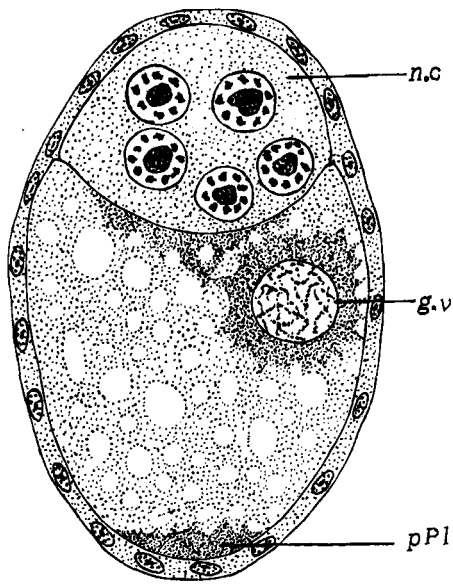

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EXPLANATION OF FIGURES

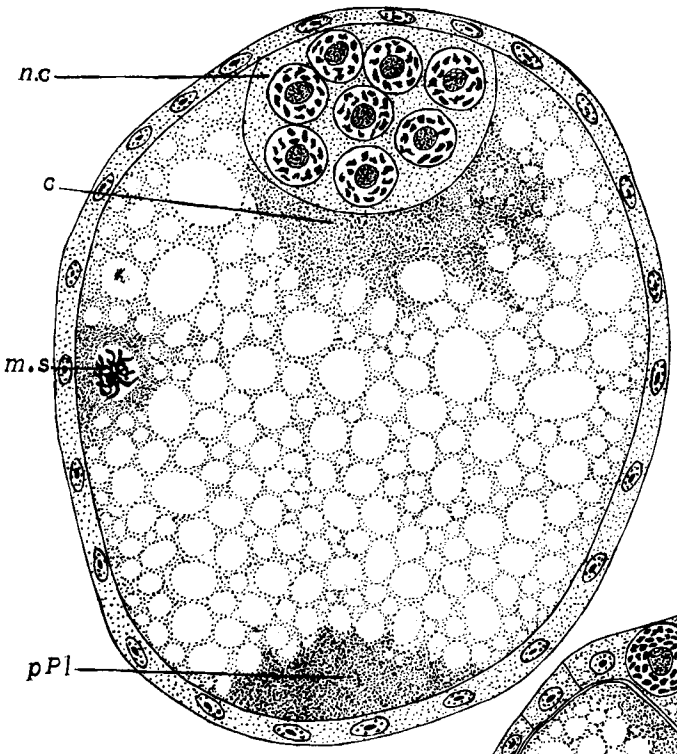
Miastor americana Felt

27 A section through the ovary of Miastor showing the accumulation of mesoderm cells to form the nurse chamber (*n.c*), and the follicular epithelium (*f.ep*).

28 Longitudinal section through a nearly grown oocyte.

29 Longitudinal section through an oocyte at the time of polar body formation.

30 Longitudinal section through a mature egg showing the female nucleus (*f.n*), and the polar body (*p.b*) already divided.



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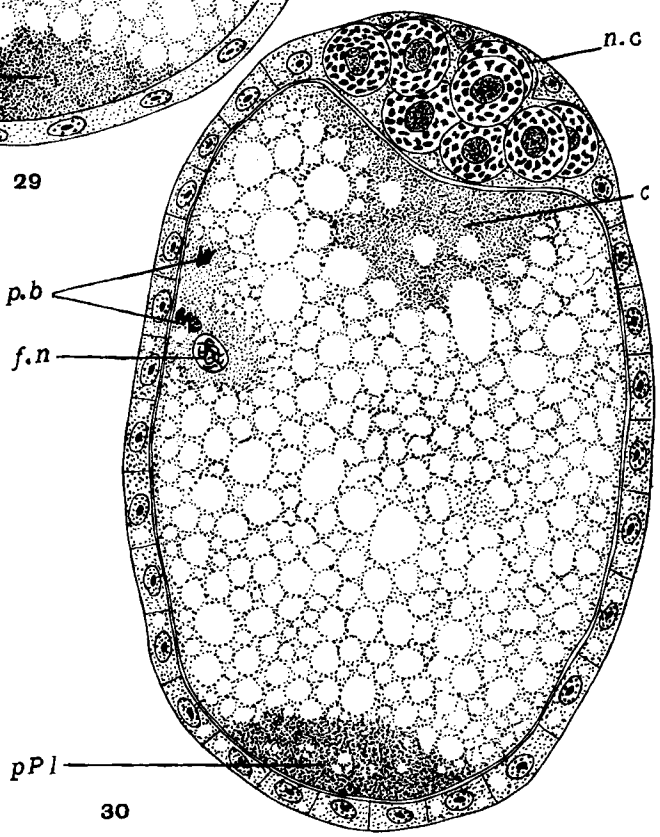

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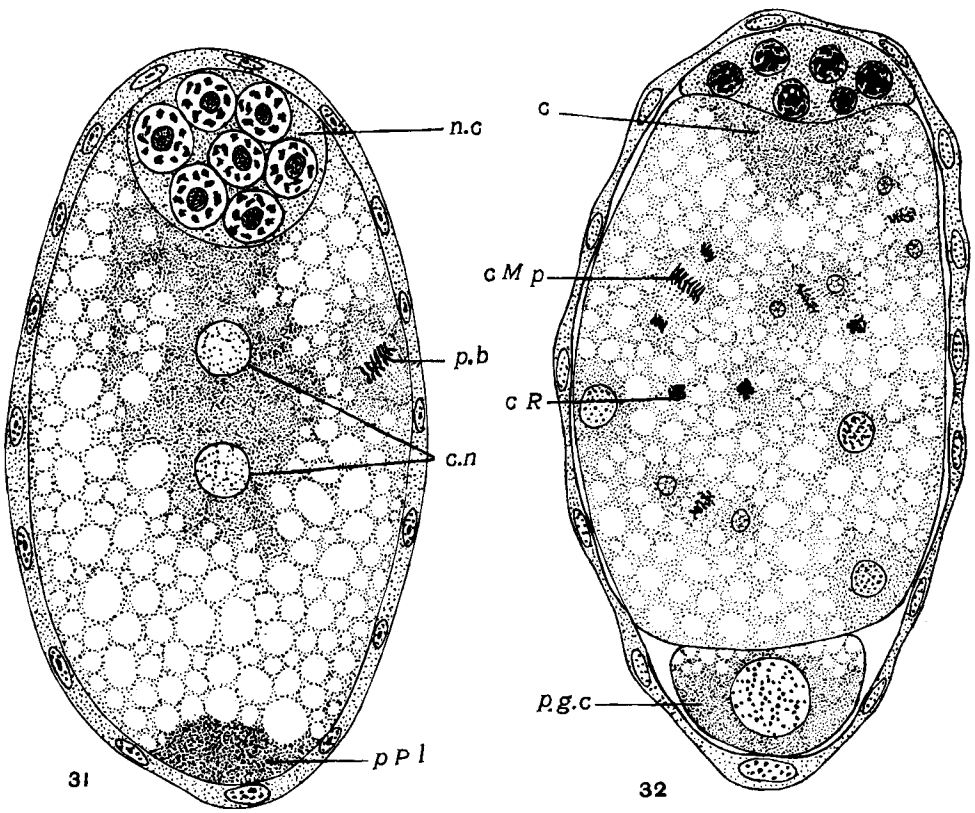
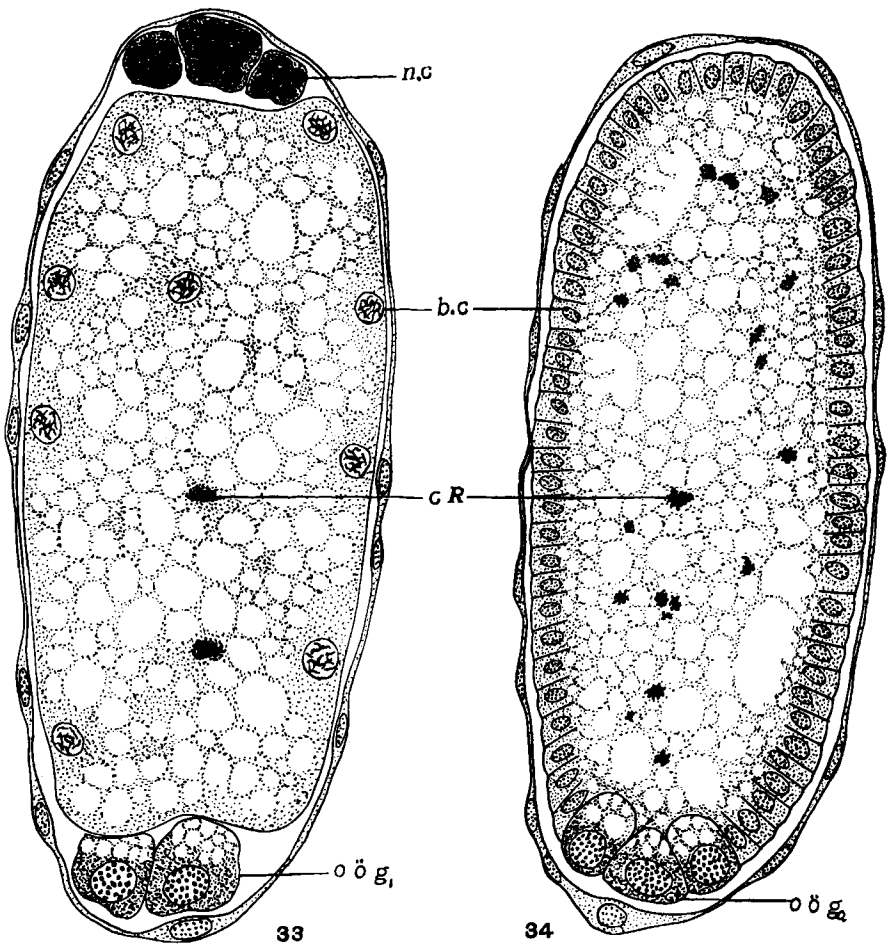


EXPLANATION OF FIGURES

Miastor americana Felt

31 Longitudinal section through an egg containing two cleavage nuclei (*c.n*) and a dividing polar body (*p.b*).

32 Longitudinal section through an egg with a single primordial germ cell (*p.g.c*), several nuclei undergoing the chromatin-diminution process, and several clumps of cast-out chromatin (*cR*).

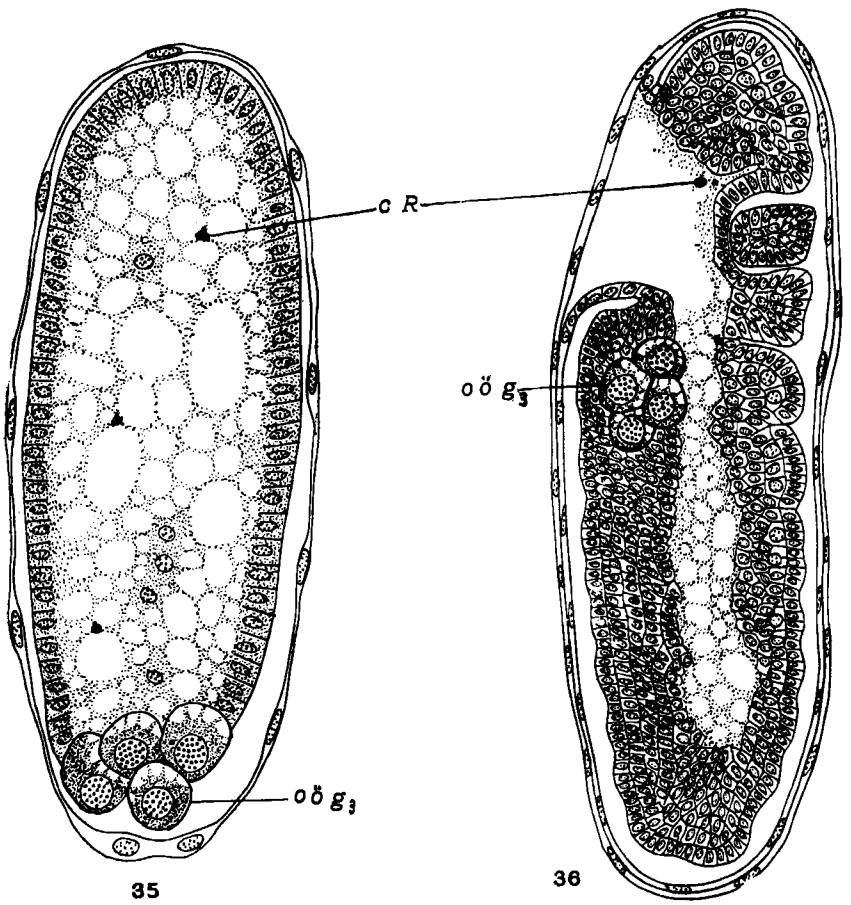


EXPLANATION OF FIGURES

Miastor americana Felt

33 Longitudinal section through an egg with two primordial germ cells (*oog*). The somatic cells (*b.c*) are preparing to form the blastoderm.

34 Longitudinal section through an egg with four germ cells (oog_2).

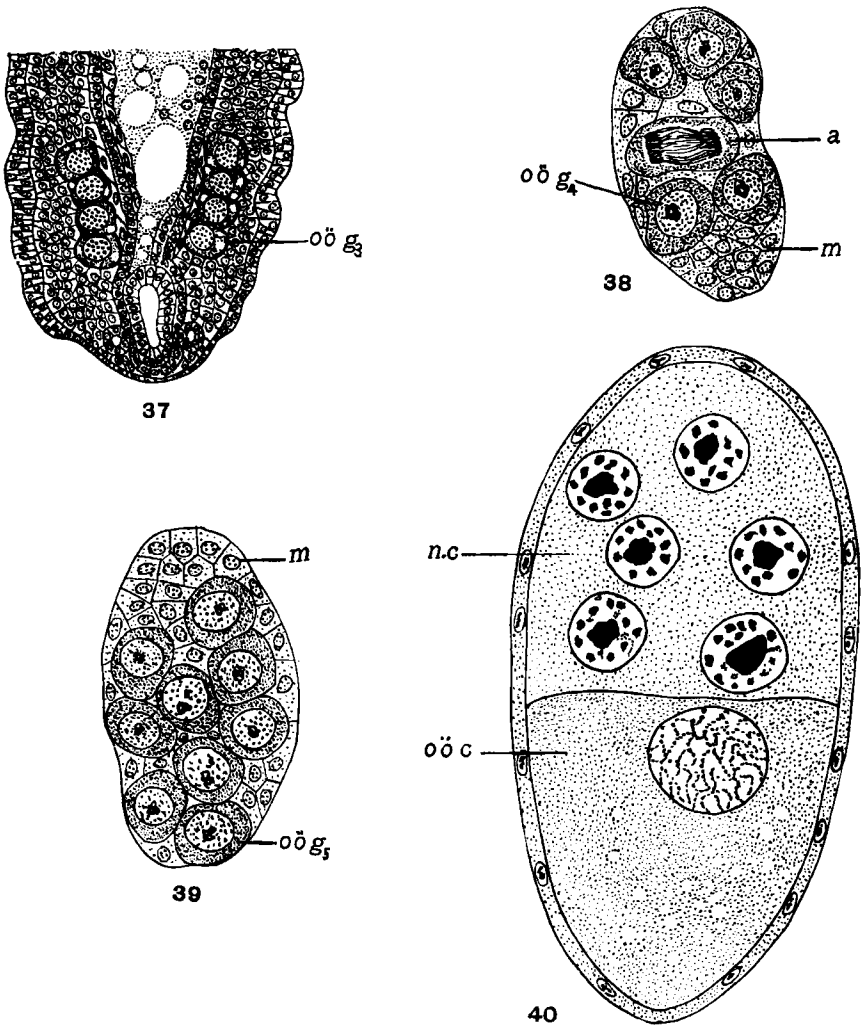


EXPLANATION OF FIGURES

Miastor americana Felt

35 Longitudinal section through an egg with eight germ cells (oog_3).

36 Sagittal section through an embryo with eight germ cells (oog_3) near the end of the tail-fold.



EXPLANATION OF FIGURES

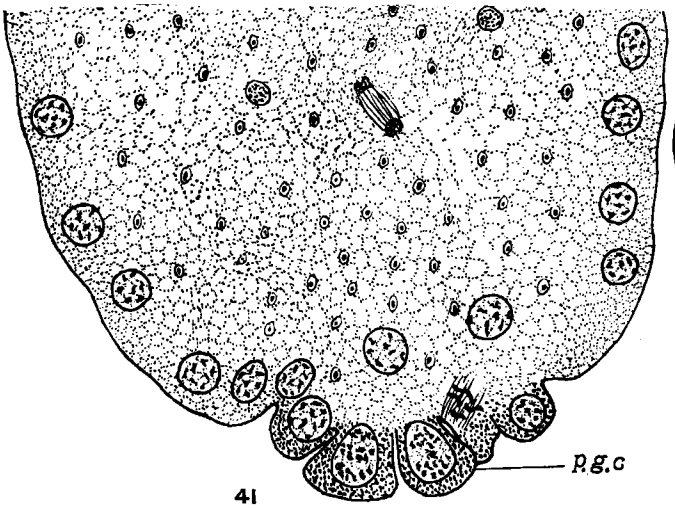
Miastor americana Felt

37 Frontal section through the posterior end of an older embryo showing two rows of four germ cells each (oog_3).

38 A section through an ovary containing eight oogonia (oog_4), one of which is dividing by mitosis (*a*).

39 A section through an ovary containing sixteen oogonia (oog_5).

40 A section through a young oocyte (*ooc*) and the accompanying nurse cells (*n.c*).



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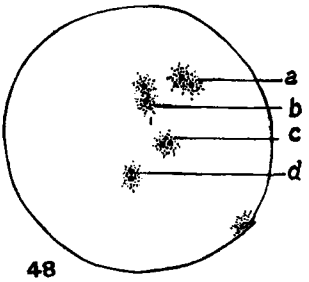

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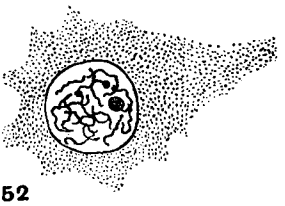

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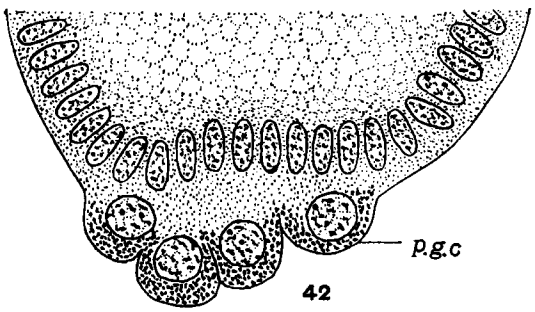

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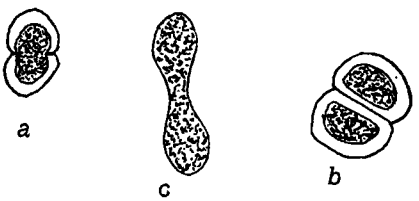

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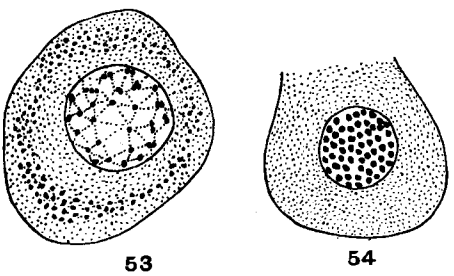

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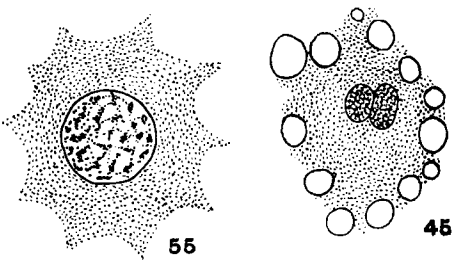

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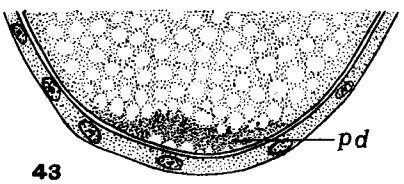

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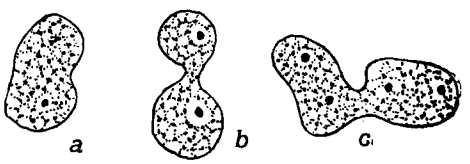

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41 Compsilura concinnata; section through posterior end of egg showing formation of primordial germ cells (*p.g.c*).

42 A slightly later stage than in figure 41.

43 Cecidomyia strobiloides; section through posterior end of egg just before deposition.

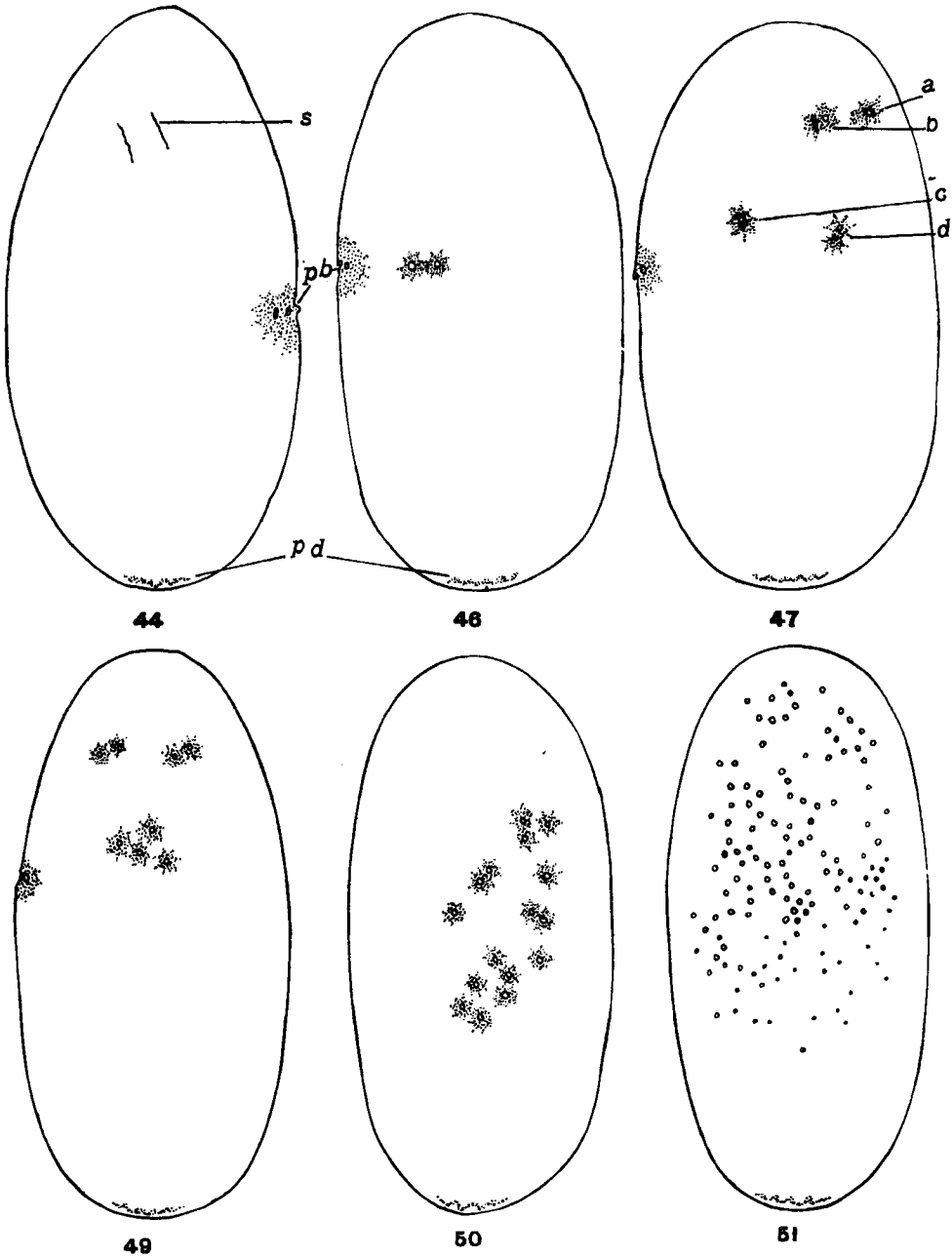
44–55 Calligrapha.

44 Section through an egg containing one polar body (*p.b*), a female nucleus, and two spermatozoa (*s*).

45 The fusion of male and female nuclei.

46 Egg containing two polar bodies and two cleavage nuclei.

47 Egg containing four cleavage nuclei.



48 Transverse section of egg containing four cleavage nuclei.

49 Egg containing e ght cleavage nuclei.

50 Egg containing sixteen cleavage nuclei.

51 Egg containing 133 cleavage nuclei.

52 One cleavage nucleus from egg shown in figure 50, enlarged.

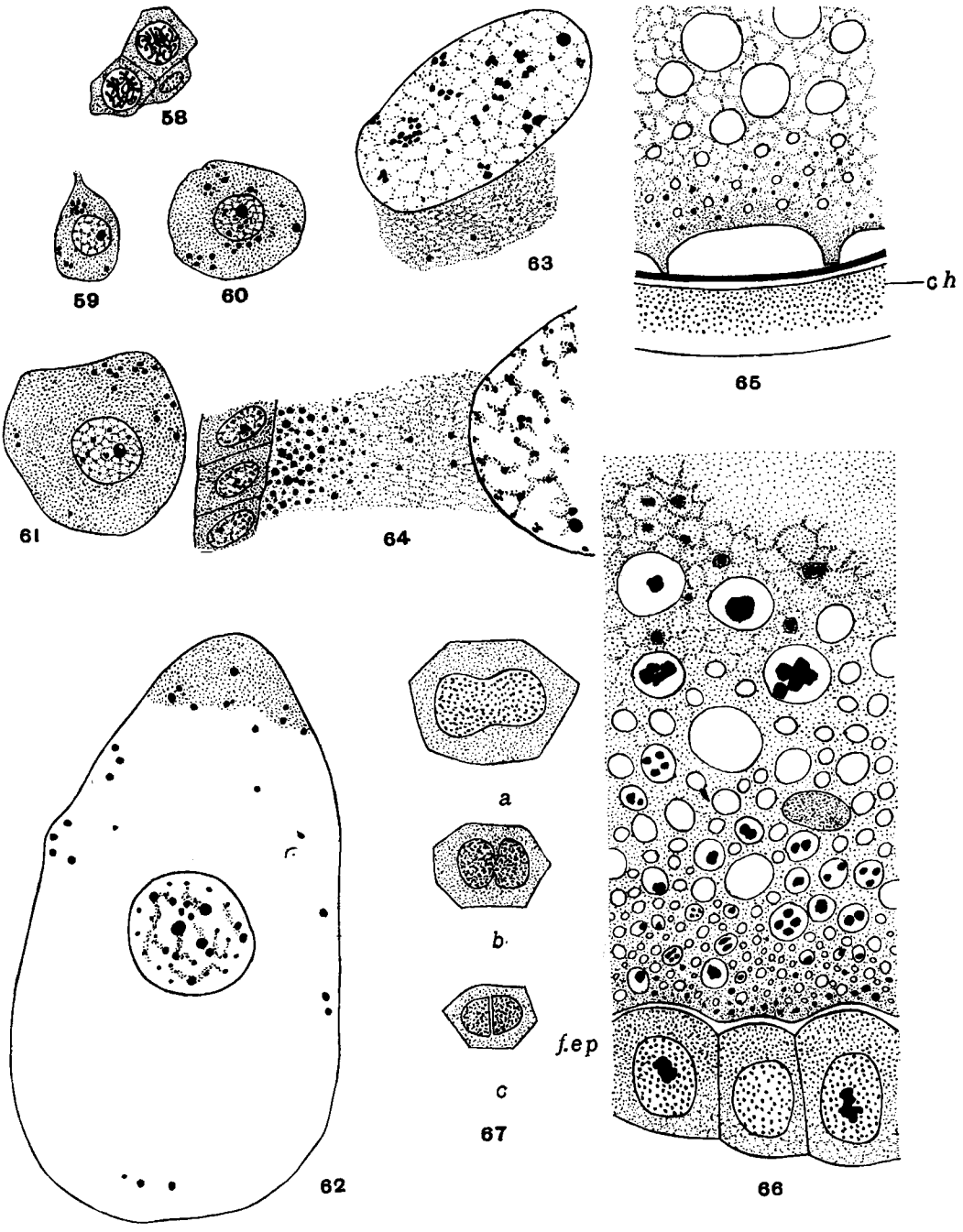
53 Primordial germ cell just after separation from the egg.

54 Blastoderm cell.

55 Vitellophag.

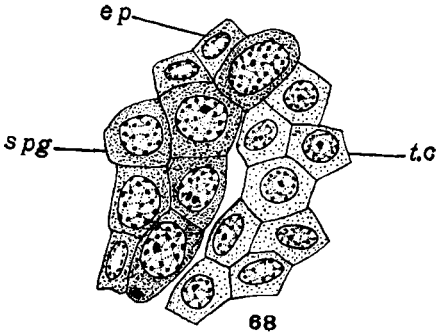
56 Leptinotarsa decemlineata; nuclear division of vitellophags.

57 Nuclear division of vitellophags in a centrifuged egg.



Leptinotarsa decemlineata

58 Two oocytes and one epithelial cell from position 58 in figure 9.
59 Oocyte from position 59 in figure 9.
60 Oocyte from position 60 in figure 9.
61 Oocyte from position 61 in figure 9.
62 Oocyte from position 62 in figure 9
63 Part of oocyte from position 63 in figure 9.
64 Part of oocyte from position 64 in figure 9.



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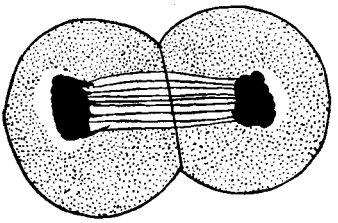
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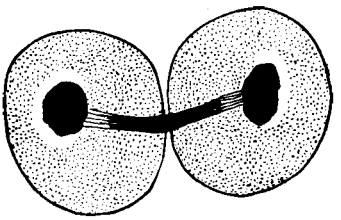

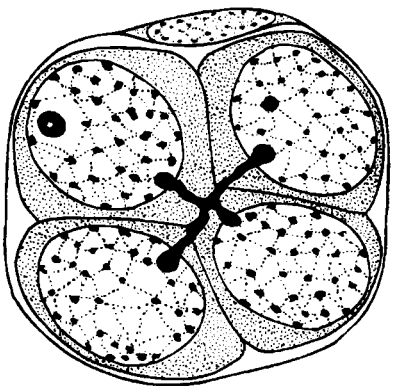

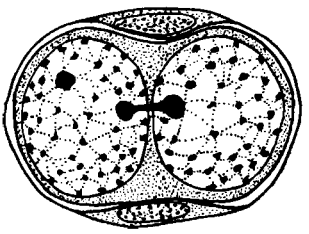

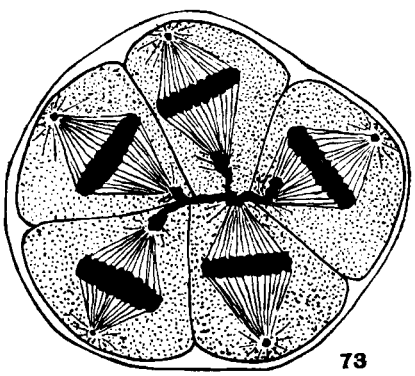
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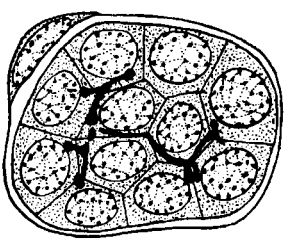

65 Part of posterior end of oocyte from position 65 in figure 9.

66 Part of posterior end of egg shortly before ready to lay.

67 Nuclear division among nurse cells.

68 Spermatogonia (*spg*), cells from terminal cap (*t.c*) and epithelial cells (*ep*).

69 Mitotic division of spermatogonium.

70 Later stage of same process.

71 Binucleated spermatogonial cell within epithelial envelope.

72 Four spermatogonia connected by spindle remains.

73 Spermatogonia from a cyst containing eight cells.

74 Section through a cyst containing 32 spermatogonia.