# Contributions toward the Embryology and Anatomy of Polistes pallipes (Hymenopteron).

# I. The Formation of the Blastoderm and the first Arrangement of its Cells.

By

# William S. Marshall and Paul H. Dernehl.

With Plates X and XI.

# Historical.

The earlier students of insect embryology were at a great disadvantage in their work in having to study the egg entirely from an external view; there was hidden from them, either entirely or in part, much that has since been observed. By crushing the egg certain observations were made as to the presence of nuclei or cells within it, a use of transparent eggs led to similar conclusions; this could only lead at first to a supposition that these had anything to do with the cells which later were seen to appear upon the egg's surface. The use of the microtome and modern methods of staining necessarily gave to all later students a means of ascertaining what took place within the egg, also of following the changes and connecting them with each other. It appears, however, in insect embryology, as with so many other branches of zoology, that the earliest workers gained a surprising knowledge of their subject. Before it was conclusively shown that the first segmentation nucleus gave rise by division to the nuclei which later took part in the formation of the blastoderm cells, this was held by some to be their origin, and the later works have given us details but few general conclusions concerning the development of the insect's egg.

Historically considered, the pre-blastodermic development of the Hymenoptera would be short, and it seems to us best to give a general account of what was known concerning the early development

of insects prior to the advent of modern methods of research. Our account is not perfect, a number of references not being at our disposal, and, in some few instances, cited results have been taken from other papers.

HEROLD and KÖLLIKER (29) were two of the first, whose observations on insects eggs could be said to have any embryological bearing. KÖLLIKERS work, which appeared in 1842, was written from observations on *Chironomus*, *Simulia* and *Donacia*. He undoubtedly observed a blastoderm surrounding the Dipterous egg, which covered the entire yolk mass. The nucleated cells of this peripheral layer increased in number, becoming smaller and finally showing more than a single layer. BURMEISTER (8), studying the eggs of *Palingenia*, found a layer of unequal thickness on the surface of the egg, probably the Keimhautblastem. He also saw, although ZADDACH (63) disagrees with this statement, a blastoderm which covered two-thirds of the egg, developing on the third day. LEYDIG (38), figured the ovarian tubules with enclosed eggs of *Aphis*, and figured an early stage containing a number of large nuclei within the egg; in a later stage these are shown nearly surrounding it, occupying a position that would correspond to the blastoderm cells, or rather the nuclei of them.

ZADDACH's (63) paper, published in 1854, on the development of the Phryganids, was much more of an attempt than had yet been made to work out the embryology of any insect. He followed the development through, and than gave considerable space to the discussion of questions having a general and a comparative interest. He first describes the appearance, at the periphery of the egg, of numerous »Punkte und Stellen« from which the yolk withdraws and they then flow together, forming a broad peripheral zone over the egg; this is clearer and more finely granulated than the other contents of the egg. We are unable to make out just what ZADDACH meant; he undoubtedly describes here the Keimhautblastem, but forming in a peculiar way. There next appears a number of round »Flecke«, in this peripheral layer, which are the first blastoderm cells; these are nucleated and surround the egg in a continuous single layer. The cells are at first nearly square, but change by a rounding-off of the corners. ZADDACH describes these blastoderm cells as remaining large over the ventral surface but disappearing dorsally.

A paper by HUXLEY (27) in 1857, gives a figure of an Aphis egg showing a peripheral layer of large nuclei, which, on one side

of the egg, are represented as separated from each other by boundaries thus giving to each separated part the appearance of a cell. Two of the nuclei with surrounding cytoplasm are figured enlarged, each nucleus having in its center a large irregular spot (nucleole). HUXLEY says: "The peripheral clear layer is, on the other hand, in all essential respects, comparable to a blastodermic vesicle; and I see no reason why it should not be called a blastoderm." This blastoderm becomes two layered. HUXLEY thought that the "pseudoval endoplast" (nucleus) divided and gave rise to endoplasts of the germ; or, in other words, the nuclei of the blastoderm cells came from the nucleus of the egg (first segmentation nucleus).

The following year LEUCKART (37), in giving the development of Melophagus, describes the yolk as withdrawing from the cell membranes leaving a clear space filled with fluid, which was largest at the poles. The »egg-nucleus« he lost sight of but saw in the egg a number of clear bladder-like spots. He disagrees with ZAD-DACH (63) who called these spots nucleated cells, and held that they later formed the blastoderm. These »cells« were at first few in number and far apart, but occurring in some eggs in greater numbers than in others. LEUCKART observed some of these dividing, and then goes on to declare that they are not cells, »sondern vielmehr Körper, die den Zellenbildungsprozeß erst einleiten«. He did not observe the origin of the blastoderm cells, but thought they came from the »egg-nucleus« by a process of budding. According to LEUCKART the blastoderm was but one layer in thickness. He compares the segmentation of the insects egg with that of other animals, and describes it as a sort of superficial segmentation.

A number of brief observations on insect's eggs made by RATHKE (47) were gathered by HAGEN and published in 1861. RATHKE, in *Naucoris*, observed the blastoderm cells, but was unable to tell if they surrounded the egg or were present in groups upon its surface. Each cell contained a spherical nucleus and one to two nucleoles. He also described the egg of a beetle as having a clearer layer on the periphery in which were a number of blastoderm cells. The egg of *Liparis* was described as having this same peripheral mass and containing more than a single layer of cells. In Phryganid eggs he noticed the same layer, "Embryonal substanz«, but not so evenly distributed over the egg; he here also noticed that the ventral surface developed first. The next year ROBIN (48, 49) observed a number of half round bodies in the egg of *Tipulaires*, which became

rounded and then flattened against each other; they finally were cut off from the yolk and formed a continous layer over the egg (Blastoderm). The cells which formed this layer originate by a process of budding. He describes a second and a third layer, both also formed by budding.

WEISMANN (59), working in 1865 on the development of the Diptera, noticed the clear peripheral layer covering the egg, which had been seen by others, and called it the Keimhautblastem. In this the blastoderm cells appeared by a process of free cell formation and spread out upon the eggs surface. The nuclei appear first, they are cut off in little masses of protoplasm to form the cells, which, rounded at first, later become prismatic. An inner Keimhautblastem appears just inside of the blastoderm which it supplies with nourishment, gradually decreasing in amount and finally disappearing. In *Musca* WEISMANN describes the Keimhautblastem as appearing first at the anterior pole of the egg and from here spreading over the entire surface. He also notices that cells of the blastoderm migrate into the yolk and noted the division of the blastoderm cells. KUPFFER (34), working in 1866 on the development of *Chironomus*, agrees with WEISMANN regarding the early development of the egg.

agrees with WEISMANN regarding the early development of contractional, agrees with WEISMANN regarding the early development of the egg. METSCHNIKOW (41), in 1866, found that in Simulia the blastoderm covers the entire egg; it is of the same thickness excepting at the posterior pole where it is thicker. The cells of the blastoderm are cylindrical, each containing a nucleus with a nucleole; they lengthen becoming of a different shape at the poles than those covering the rest of the egg. In Cecidomyia, a single nucleus was first noticed within the egg; this divides and by repeated division and a wandering of the nuclei, a zone of them is formed which finally passes to the periphery where cells are formed. These are at first spherical, but later become cylindrical. By viviparous aphids METSCHNIKOW found an early stage in the egg's development which contained but a single nucleus. This divides and the two resultant nuclei, at first near each other in the center of the egg, separate; they divide, the division continues and results in a layer of nuclei at the periphery. The blastoderm cells form and lengthen, becoming largest at the broad end of the egg. METSCHNIKOW was against WEISMANN (59) in his free cell formation theory, holding that the cleavage nuclei were nuclei only, and that they gave rise to the nuclei of the blastoderm cells.

Three years later MELNIKOW (40) observed in the eggs of

Donacia a number of clear spots near the periphery; they appeared first on the ventral surface: he followed WEISMANN (59) in believing their origin to be a free cell formation. These nuclei later become the nuclei of the blastoderm cells which surround the egg in a single layer. The blastoderm of the ventral surface becomes thicker than the rest, this thickening beginning at the middle of the ventral surface and extending towards both poles but more marked at the anterior. By *Pediculus* a similar blastoderm formation was observed.

In 1869 GANIN (16) found that the blastoderm cells in Hymenoptera arise first at the posterior pole and then spread, the cells at the poles being the largest, dorsally the smallest. In another paper published the same year on the development of *Platygaster* (GANIN, 17), he endeavoured to compare the entire yolk to the Keimhautblastem of other insect eggs. In a third paper he studied the development of the Diptera (GANIN 15); he showed the appearance of nuclei in a peripheral layer and distinguished a convex ventral surface. The same year BRANDT (4) found that in the formation of the blastoderm by Odonata the cells appear at the periphery in groups and that later the blastoderm becomes thickened on one part of the surface.

In 1870 GRIMM (20) studied the development of *Chironomus*, finding a layer, the Keimhautblastem, covering the egg; this was in some parts thicker than in others. A single nucleus gave rise, by division, to others, and these moved to the periphery of the egg where a blastoderm was formed. The blastoderm cells later divide transversely, resulting in the formation of two layers. GRIMM (21) also described the same process of blastoderm formation in *Docophorus*.

BOBRETZKY (3), in 1870, studied the development of two Lepidoptera by sectioning the egg, and he showed conclusively that the nuclei of the blastoderm cells come from those nuclei which are earlier within the egg. These «cells« within the egg are amoeboid and connected by fine processes. The blastoderm cells are at first large and far apart, becoming smaller and packing closely together. Some «cells« remain in the yolk. He held that the amoeboid cells came from the first segmentation nucleus. Previous to this paper by BOBRETZKY, appeared two important works on insect embryology, the one by KOWALEVSKY (31), the other by BÜTSCHLI (9).

# The Formation of the Blastoderm.

The general form of the egg of *Polistes* is ovoid with one pole wider than the other. The widest part of the egg is towards the

anterior end, the egg tapering towards the posterior pole at the extremity of which is the narrowest part. From this general shape there are some deviations either in a relative lengthening or shortening of the long axis, also in a suppression of the pointed end, some few eggs shortening so much as to become nearly spherical. The narrower posterior end is used for attachment in a cell of the nest towards the opening of which the anterior end points. One surface of the egg is concave or flattened, opposed to which is a convex surface. There are often found irregularities in which either the concavity or the convexity, or both, may be suppressed. The convex surface becomes the ventral, the concave the dorsal aspect, of the egg. This same orientation has been observed in Hymenoptera by KOWALEVSKY (31) and BÜTSCHLI (9) for Apis, by CARRIÈRE and BÜRGER (11) for *Chalicodoma* and by GANIN (16) for *Formica*.

The eggs are attached, one in each cell, to that wall which is nearest the center of the nest. In a few cells two eggs were found which must have led, later, to the death of one or both of the larvae which developed from these eggs. The point of attachment of the egg is usually about two-thirds the depth of the cell. In the young nests an egg is found in each cell, but as soon as these develop to larvae the eggs are then found only in the youngest, outermost, cells which have been added to the nest. The nest increases in size, the eggs soon becoming larvae, the larvae, pupae, and we then find that even the marginal row of cells is in part filled with pupae and eggs can then be obtained from a few only of these outermost cells. The development of the wasps in the central cells is finally completed and when the mature wasps leave them they are used again, eggs being found both in these central cells as well as some of those comprising the marginal row. Both from what has been said, and the fact that early development proceeds rapidly, the exceeding difficulty of procuring many eggs in their earliest stages of development is appreciated. The occurrence of the wasps also varies greatly in different years, and with this, the abundance or scarcity of nests.

The many eggs of *Polistes* sectioned by us have failed to show any stages in the formation of the polar cells, the union of the pronuclei or the earliest divisions of the cleavage nuclei. We begin our account of the formation of the blastoderm after the earliest divisions of the cleavage nuclei have already occurred.

The earliest stage which we have found (Fig. 1), shows a small

number, six, of nuclei within the egg; these are not central in their position, but all lie nearer the anterior than the posterior pole, confined to the anterior half of the egg. A similar occurrence, as to position, of the few nuclei at first found within the egg, has been observed for a number of other insects by WEISMANN (59) in *Chironomus*, KOWALEVSKY (31), GRASSI (19) and DICKEL (14) in *Apis*, BOBRETZKY (3), in *Pieris*, HEIDER (22) in *Hydrophilus*, and CARRIERE and BÜRGER (11) in *Chalicodoma*. What relation this localization of the first few nuclei has to the position of the first segmentation nucleus within the egg we are unable to say; whether the latter originally occupied the same relative position or wandered there from some other part of the egg. Other than their being in a certain part of the egg there is no arrangement of these first nuclei relative to each other or any axis of the egg itself. Whenever, during these early stages, any nuclear divisions were noticed, they were always mitotic.

The nuclei divide, increasing in number, all remaining for some time within the anterior half of the egg, showing the same irregular arrangement as was at first observed. A little later a change begins to take place, it being noticeable that, while predominately irregular in position, some definite arrangement of the nuclei within the egg is suggested (Fig. 3). As the nuclei increase in number most of them move away from their original position wandering towards the surface of the egg (Fig. 4); a few, however, remain near their original positions and are thus nearer the median part of the egg than the others. The nuclei can now be divided, although such a division is not as yet well defined, into two groups; one group, the larger, comprising those nuclei, the cleavage nuclei, which have moved furtherest from their original position; the other, the smaller group, containing those nuclei, the yolk nuclei, which remain nearest the original position.

At a somewhat later stage it becomes apparent that the nuclei are moving more rapidly towards the posterior pole than in any other direction. This is clearly seen by comparing Figs. 2 and 4. If in each of these two figures we would draw a line connecting all of the nuclei which are outermost, the outlines thus formed would be quite different, more circular in the first figure, the younger egg, while in the second figure, the outline would more closely follow that of the egg itself. Passing on to a still somewhat older egg (Fig. 6) we find that the arrangement already suggested becomes very pronounced, one group of nuclei, the outermost, arranging

themselves in a zone or belt with an outline very similar to that of the egg, a second group, the nuclei of which remain near the center of the egg and show a very irregular arrangement. Those of the first group, the cleavage nuclei, and those of the second group, the yolk nuclei, both add to their number by mitotic division.

As just mentioned all the nuclei present possess the power of dividing mitotically and are as yet different from each other only in their position within the egg. There now appears a difference in the secondary origin of the various nuclei. The cleavage nuclei, when clearly defined as such, increase only by mitosis of nuclei belonging to this group; the yolk nuclei increase by mitotic divisions of their own number and also in the following manner. As the cleavage nuclei wander towards the periphery of the egg a few drop behind and add themselves to the second, inner group, the yolk nuclei. Sections of eggs showing the zone of cleavage nuclei well out towards the periphery would show nuclei just within the zone and at some distance from most of the yolk nuclei. We could see no indication that these nuclei ever rejoined the zone, and, as yolk nuclei are in later stages present in this same part of the egg, it appeared most probable that these nuclei, whose origin was from the nuclei of the zone, remained in the yolk and became yolk nuclei.

The division of nuclei within the egg and the fact that they all came originally from the first segmentation nucleus has been known for some time, METSCHNIKOW (41); the question concerning the kind of division being, however, a subject of dispute. WITLACZIL (62) found for the eggs of *Aphis* that the nuclei within the egg divided amitotically his figure showing a nucleus of a »biskuitförmige Gestalt«. According to WILL (61), the division of the cleavage nuclei occurs mitotically. In 1889 HEIDER (23) for *Hydrophilus* and WHEELER (60) for *Blatta* and *Doryphora* described the division as mitotic. CHOLODKOWSKY (13) described amitosis as occurring in the yolk nuclei of *Phyllodromia*, SCHWARTZE (51) holding that in *Lepidoptera* division is not always the same, occurring mitotically in the cleavage nuclei but not discovering any mitotic figures in those nuclei within the center of the egg. HEYMONS (26) found mitosis in the cleavage nuclei in some *Orthoptera*, but in *Forficula* a direct division of the yolk nuclei. TSCHUPROFF (35) distinguishes two kinds of nuclei in the eggs of *Odonata*, the one kind dividing by mitosis, the other amitotically. CARRIÈRE and BÜRGER (11) found that the yolk nuclei of *Sialis* divided amitotically. These latter for *Chalicodoma*, and Zeitschrift f. vissensch. Zeologie, LXXX, Ed.

DICKEL (14) for Apis, have described the zone of cleavage nuclei, at about a similar stage, as appearing in a pyriform arrangement in longitudinal section; a line connecting all the cleavage nuclei in the *Polistes* egg never had this outline, but was in general much more the shape of the egg. KOWALEVSKY (31) also noted the same pyriform arrangement.

We have already noted that in the egg of Polistes all nuclei divide mitotically and that the only difference between any of them is in their position within the egg. Repeated mitosis adds largely to the number of nuclei in each group, the zone of cleavage nuclei becoming more and more clearly defined. In the earliest stages we were unable to distinguish the cleavage - from the yolk - nuclei, and could not with certainty determine to which group any of the nuclei then present would belong. A similarity of the nuclei in the egg was early noted by CHOLODKOWSKY (13), and DICKEL (14), has lately called attention to this fact. In our study of Polistes we have failed to find at any stage a definite arrangement of the dividing nuclei either relative to each other or to the surface or the axes of the egg. Such a definite arrangement has, however, been noticed in the eggs of other insects. BLOCHMANN (2) found that the nuclei forming the zone divided tangentially and radially; in this way some of the newly formed nuclei remained with those of the zone, others passing inside it to become yolk nuclei. HEYMONS (26) has described the division of the cleavage nuclei, when near the periphery, as parallel to the egg's surface. SCHWARTZE (51) found that the nuclei of the zone, which was concentric to the periphery of the egg, divided paratangentially to the surface. WILL (61) figures nuclei at the periphery, and before reaching it as dividing, but not all in any one definite direction, and HENKING (25) found that the nuclei in the eggs of Pyrrhocoris do not divide in a tangential or any other definite direction. GRABER (18) and WHEELER (60) both found the axes of the dividing nuclei all parallel to the longitudinal axis of the egg. HEYMONS (26) noted in Forficula a definite arrangement of the spindle in the dividing cleavage nuclei.

In the earlier stages it was noticed that all or the majority of the nuclei were within the anterior half of the egg. This arrangement is different in older stages and one showing the zone of cleavage nuclei clearly defined would give a great similarity between the anterior and the posterior halves of the egg, the former half containing a slightly greater number of yolk nuclei than the latter.

It would also show the cleavage nuclei a little nearer the anterior than the posterior end. Later stages in development than have yet been described (Fig. 6) show a much greater number of nuclei in each group, a nearer approach of the zone of cleavage nuclei to the egg's periphery, and a greater regularity in its arrangement. A different arrangement of the zone of cleavage nuclei has been noticed in other Hymenoptera. CARRIÈRE and BÜRGER (11) for Chalicodoma and both KOWALEVSKY (31) and DICKEL (14) for Apis have seen that the zone assumes a pyriform outline the widest part nearest the anterior pole of the egg. By Polistes, as soon as approximately half of the cleavage nuclei have wandered into the posterior half of the egg, they assume an outline very similar to that of the egg. The cleavage nuclei nearest the posterior end travel a much greater distance than do the others. When eggs at a similar stage are cut transversely the zone of cleavage nuclei is seen to lie near the surface being evidently at all parts equidistant from it (Fig. 5). There is as yet no intimation as to which surface of the egg will later become ventral or which dorsal. The yolk nuclei are scattered irregularly within the zone, but, as in the longitudinal section, there is no regularity in their distribution. Nothing bearing any resemblance to the rosette arrangement noticed in transverse sections of Dipterous eggs by KOWALEVSKY (32) and GRABER (18) was seen. The entire segmentation in *Polistes* is very similar to that observed by HENKING (25) for Lasius and by DICKEL (14) for Apis.

A Keimhautblastem is present in the egg of *Polistes* and has been previously observed in other *Hymenoptera*, a number of workers have found it present by *Apis*, HENKING (25) by *Lasius*, and CAR-RIÈRE and BÜRGER (11) for *Chalicodoma*. The earliest stages of *Polistes* that we studied showed it to be already present, a thin finely granulated and vacuolated layer, covering the entire surface of the egg just within the egg membranes. Externally it has a distinct boundary but is internally very irregular, sending numerous processes into the mass of yolk. As the egg develops it increases in thickness, becoming widest just previous to the entrance of the cleavage nuclei to form the blastoderm. It is noticeably thicker on the ventral than on the dorsal surface. We at first thought the thickness of the Keimhautblastem could be used in distinguishing the different surfaces of the egg and in judging of its comparative age. This it is impossible to do, the thickness varying nearly as much in eggs of the same age as those in different stages of development,

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there also being a variation in its thickness over different parts of the same egg. It is true that in general the ventral surface has a thicker layer than the dorsal, but this is not always so, it being often more marked at other parts.

Having now arrived at a stage in the development of the egg just preceeding the arrival of the cleavage nuclei at the egg's surface and their entrance into the Keimhautblastem, we will proceed to a study of the nuclei we have seen are present within the egg. In any stage during the pre-blastodermic development of Polistes we find, as already stated, that the difference between the many nuclei within the egg is one of position. The similarity between the cleavage and the yolk nuclei was, we believe, first pointed out by CHOLOD-KOWSKY (13) and WHEELER (60), and has later been referred to by DICKEL (14). Much support was formerly given to the view that the yolk nuclei divide amitotically and are thus distinguished from the mitotically dividing cleavage nuclei. CHOLODKOWSKY (13), HEIDER (23), WHEELER (60), TSCHUPROFF (55) and SCHWARTZE (51). We cannot agree with those who take this view; the statement we made that all nuclei, previous to blastoderm formation, are, in the egg of Polistes similar, except in position, includes also the more minute structure of either resting or dividing nuclei. We find that not only do all nuclei divide similarly but that the resting nuclei are alike at from whatever point within the egg they are selected.

We find in Polistes that all eggs, previous to blastoderm formation, show in their dividing nuclei an abundance of those in the equatorial plate stage, this stage not only being more abundant than any other, but it is found oftener than all other stages of mitosis combined. Considerable differences were shown between the various eggs examined; some with many nuclei would have them all in a resting stage, others would have part resting, and part in various stages of mitosis. Eggs containing many dividing nuclei would generally show them in different stages of division, exceptions to this being found however in some eggs in which all dividing nuclei were in the equatorial plate stage; this is the only stage of mitosis ever found exclusively within an egg. It was also noted that late anaphase stages were very prevalent. HEIDER (23) noticed that in an egg of Hydrophilus most of the many nuclei would be in the same stage of mitosis. The same thing was noticed by WHEELER (60) in Doryphora. He also called attention to the great preponderance of resting nuclei. A somewhat different condition was noticed by

HEYMONS (26) for *Forficula* where, after the two groups of nuclei have been formed, the inner group, yolk nuclei, are nearly all resting, the outer group, cleavage nuclei dividing.

Each nucleus, in any of the stages of development already described, is surrounded by a mass of cytoplasm the amount of which varies but little around the different nuclei. This mass of cytoplasm is amoeboid in shape, the pseudopod-like processes extending for varying distances, some anastomosing with similar processes from neighboring nuclei. Each nucleus with its surrounding cytoplasm is in this way connected with several of those near it, there presumedly being a connection between the great part, if not all, of the nuclei within the egg. To what degree this connection extends we are unable to say, it being impossible to follow the fine connecting strands to any great distance between the yolk globules. Any stage of pre-blastodermic development will by careful search reveal some of the nuclei connected in this way (Figs. 11 and 12). Upon entrance of the cleavage nuclei and their surrounding cytoplasm these connections are undoubtedly lost; even at a stage just before this the strands could not be seen, although we incline to the belief that the connection remains until the Keimhautblastem is reached. Even after the cleavage nuclei have entered the Keimhautblastem connecting strands can still be seen joining the cytoplasm of the yolk nuclei which have remained within the egg. These, the yolk nuclei and surrounding cytoplasm, later fuse with each other, forming large multinucleate masses, from which connecting strands have disappeared.

A resting nucleus (Figs. 8, 9 and 10), taken from either cleavage or the yolk group, contains a number of fairly large chromatin granules of various sizes and without any definite shape. These granules do not show any regular arrangement within the nucleus other than that most appear restricted to the peripheral part: each granule is enclosed in the net-like strands of a reticulum. They are often collected in small groups and around each group the network gathers more abundantly than around a single granule. This network, besides surrounding the granules, or groups of the same, connects them with each other, passing from one to another in narrow fibrils or more commonly as wide strands of varying thickness. We were unable to distinguish a nucleole, as such, within the nuclei. An examination of individual nuclei often led to the belief that a nucleole was present, but, what we at first considered a nucleole, did not appear constantly in any number of nuclei. We were unable to see

any nuclei in the spirem stage such as HENKING (25) found and figured for Pyrrhocoris. The cytoplasm is distributed evenly around each nucleus which is often excentric in position, although oftener central. We did not find that the nucleus occupied any regular position within its surrounding cytoplasm relative to the center or periphery of the egg; the regular movement of the nuclei towards the surface of the egg and their frequent division would tend to greatly disturb any regularity of position, if such were present. These statements regarding the position of the nucleus within its surrounding cytoplasm hold good only until the Keimhautblastem is reached after which, as we shall show later, a change takes place. The general outline of the cytoplasm has often been described as amoeboid, and this undoubtedly gives the best comparative description. When we examine the many irregular branching processes (Fig. 11) we find that they resemble much more closely the pseudopodia of the reticulosa than the lobopods of an Amoeba. The processes form a delicate branching network which it is impossible to follow at any great distance. The shape of the cytoplasmic mass around each nucleus is greatly influenced by the surrounding yolk globules.

A considerable variation is noticeable in the size of the nuclei from different eggs as well as of the nuclei within the same egg. By measuring a number of nuclei from each of several eggs we find that they are as a whole apt to be larger, or smaller, in some eggs than in others. We find that in early stages of segmentation the nuclei within an egg are nearly all of the same size, the differences, if any, being slight. After the nuclei have wandered as far as the Keimhautblastem, and even in stages a little earlier, the yolkbecomes noticeably larger than the cleavage-nuclei, the difference becoming more pronounced as soon as the blastoderm is formed. KULAGIN (33) noticed that, previous to blastoderm formation, nuclei of different sizes were found within the egg, although the difference in size was apparently not general. In later stages he noticed that the nuclei of the blastoderm cells were decidedly smaller than the yolk nuclei. HEYMONS (26) describes the cleavage nuclei as smaller in Forficula than the yolk nuclei, and many others have called attention to this and other differences in size which they noticed in the eggs of the insects they studied. The difference in size which we found between the yolk and the cleavage nuclei, would naturally tend to disprove what we have held regarding the exact similarity

of the nuclei in all parts of the egg. We find, however, that only in the late stages is this noticeable and then not more so than the differences in size between nuclei of different eggs. It must be borne in mind, when we meet with these and other slight variations, that we are working with eggs collected indiscriminately from the nest, which were destined to develop into wasps of different sexes. Until proved to the contrary, we see no reason why the fate, as to its sex, of an egg could not influence certain minute differences in the structure of parts of the egg, more especially in the nuclei.

Returning to the account of the resting nuclei we find many of them showing a distinct centrosome (Figs. 9 and 10); this appears as a small, darkly stained granule, situated but a short distance without the nuclear membrane. We could not find that it had any definite position relative to that of the nucleus. NOACK (42) has shown that the centrosome present in the cleavage nuclei of Calliphora is in front of the nucleus; that later it divides, the halves separating and going to either side. In Polistes a single centrosome surrounded by a mass of archoplasm was present, a single one dividing or the two which would result from such a division not being observed. The boundary between the archoplasm and the surrounding cytoplasm was not such as could be represented by a distinct line; the former stained darker and passed rather abruptly into the lighter colored cytoplasm surrounding it. The archoplasm had in some instances a regular outline (Figs. 8 and 10); in others a distinct radiation was observed (Fig. 9) which penetrated to different depths the surrounding cytoplasm. While not common, two nuclei have been observed within the same mass of cytoplasm (Fig. 11). What we have said concern-ing the resting nuclei holds good for those taken from early or late pre-blastodermic stages, and also for either cleavage or yolk nuclei.

A study of the dividing nuclei shows that the chromosomes are small irregular bodies the minuteness of which makes a determination of their exact size and number difficult. In an equatorial plate stage we find spindle fibres passing towards each centrosome and converging within the surrounding archoplasm. In this stage a centrosome is clearly seen at each pole surrounded by archoplasm from which radiates a number of distinct astral fibres (Figs. 14 and 15). The distance between each centrosome and the adjacent margin of the cytoplasm is generally short and in this direction, away from the plate, the astral fibres are short, often hardly perceptable. The fibres all remain within the cytoplasm and their length is often

limited by the amount of it which extends beyond the margin of the archoplasm. Those astral fibres lying nearest the spindle fibres are the longest, often extending so far that they meet similar fibres from the other pole. Just outside of the spindle fibres a clear space was sometimes noticed, widest at the equatorial plate and from here narrowing towards the poles (Figs. 13 and 15). Its general appearance was as if the cytoplasm lying nearest the connecting fibres was shrunken and pulled away from them.

In many eggs in which dividing nuclei were abundant nearly all stages of mitosis could be found within a single egg. A stage in which the chromosomes have left the equatorial plate and started towards the poles is seen in Fig. 14. Excepting the slight divergence of the chromosomes towards the poles, the figure is similar to the one just described. The chromosomes are quite distinct, centrosomes, archoplasm and astral fibres present, the spindle fibres slightly shortened. Connecting fibres are not yet visible; they appear at a little later stage (Fig. 23) and remain distinct until the daughter nuclei begin to form. This last figure mentioned is the earliest we could find in which connecting fibres were present. After the arrival of the chromosomes at the poles they still remain distinct from each other (Figs. 17 and 20); in some specimens they apparently group closer together than in others and do not then remain distinct but overlap and appear like a single, irregular, darkly stained mass (Fig. 18). Each group of chromosomes now lies near the archoplasm which is still present, showing, as earlier, a distinct radiation. The centrosomes, as easily seen as during the earlier stages, are still present near the center of the archoplasm.

From their first appearance the connecting fibres are in all dividing nuclei very distinct, remaining so until the two nuclei resultant from the division have been formed. We observed in *Polistes* that the connecting fibres often appeared peculiar in that a number of irregular thickenings occurred on them, appearing without any regularity as to position or number (Figs. 17 and 19). Generally more than one swelling was present on a fibre, and, if so, they might be near together or far apart. In one instance they were observed directly in the equator (Fig. 18), but were smaller than usually seen, and only two present. Somewhat similar structures are those found by HOFFMANN (26a) in the Hydroids but were always along or near the equator, and do not occur so early in mitosis as we found them in *Polistes*. CARNOY (12) has figured similar thickenings

on the connecting fibres in *Steropus* which differ, however, from those we find in being much thinner and longer. That which, as far as we can ascertain, approaches nearest to the condition we find in *Polistes* occurs on the connecting fibres of dividing cartilage cells figured by SCHLEICHER (50), although he has represented the thickenings as occurring regularly, a condition not found by us. Another peculiarity we wish to mention was the occurrence in some dividing nuclei of one or more large irregular bodies found most frequently surrounded by the connecting fibres (Figs. 20 and 21), but which were also seen just outside of them (Fig. 23). As many as five of these peculiar bodies were seen together, the number two or three being, however, much more prevalent. They were of a light brown color, failed in any of our preparation to stain, and occurred oftener in the mitosis of the yolk nuclei.

After the divergence of the chromosomes is completed the centrosome, archoplasm and astral fibres can yet be seen. When the nuclear membrane starts to form, the astral fibres begin first to disappear, followed by the centrosome and archoplasm, the last disappearing somewhat later than either of the others. Even after the nuclear membrane can be distinguished, a small mass of archoplasm is often discernable (Fig. 25). After completed mitosis the resultant nuclei are small, less than one-half the size of the nucleus before division; they apparently increase rapidly in size, very few of the smaller ones being seen. Just previous to, and often after, the formation of the nuclear membrane, connecting fibres are distinctly seen (Fig. 25). The same thing has been figured by LECAILLON (35) where, after the mitosis is completed, in the egg of *Clythra*, and the nuclear membranes formed, connecting fibres still remain.

We have shown that in the egg of *Polistes* the nuclei arrange themselves into two groups and, either resting or dividing, are similar to each other, their only difference being their position within the egg. The inner group, the yolk nuclei, lie scattered irregularly throughout the greater part of the egg; the other group, the cleavage nuclei, have arranged themselves in a zone which is at nearly all parts equidistant from the eggs surface. On the surface of the egg, just within the membranes, lies the Keimhautblastem. The nuclei forming the zone increase their number by mitosis and gradually approach the surface. The last stage we have described was one in which the cleavage nuclei were near the surface of the egg. We © Biodiversity Heritage Library, http://www.biodiversitylibrary.org/; www.zobodat.at

#### William S. Marshall and Paul H. Dernehl,

will now endeavour to trace their progress into the Keimhauthlastem and the formation by them of the blastoderm.

But little seems to be known concerning the entrance of the cleavage nuclei into the Keimhautblastem and the part played in the formation of the blastoderm cells by the cytoplasm which surrounds each of these nuclei. METCZNIKOW (40) thought that in Aphis, and earlier the same for Cecidomyia, that the protoplasm of the blastoderm cells was formed »aus dem Dotterplasma«. BLOCH-MANN (2) found that in the eggs of Musca, when the cleavage nuclei and surrounding cytoplasm reached the Keimhautblastem, the forward end of each fused with it. CARRIÈRE and BÜRGER (11) for Chalicodoma, found that the protoplasm surrounding the cleavage nuclei fuses with the Keimhautblastem as soon as it reaches it, and NOACK (42) for Calliphora, describes these nuclei as carrying their surrounding cytoplasm with them when they reach the outer layer, but fusing with it. The best figures on this subject, much fuller than the text, are those by CARRIÈRE and BÜRGER for Anthophora. In Fig. 16 we show a resting nucleus lying just inside the Keimhautblastem, the inner boundary of which is seen to be indistinct, passing gradually into the protoplasm surrounding the nucleus. For the first time the nuclei have now taken a definite position within the surrounding cytoplasm, being near the forward margin, that which is nearest the egg's surface. The whole thus assumes a comet-like appearance, the nucleus the head, and the cytoplasm the tail of the comet following behind. Reaching the nearest part of the Keimhautblastem the nucleus with its surrounding cytoplasm enters it, not however at first fusing, but remaining distinct. In many of our preparations there is no division between the cytoplasm of the cleavage nuclei and the Keimhautblastem; a number of other slides showed that there was undoubtedly, for a time, no union between the two. In Fig. 27 we have a view of three cleavage nuclei entering the Keimhautblastem, the upper one the least advanced of the three. It is evident that no fusion has as yet taken place, the cytoplasm around the nucleus can, in these preparations, be distinguished from the surrounding Keimhautblastem by its darker shade. Such a separation was not seen in all our preparations. The ultimate fate of the cytoplasm brought with the cleavage nucleus is a fusion with the Keimhautblastem, the two forming with the nucleus the contents of a blastoderm cell. GRABER (18) holds that by Lucilia the blastoderm cells are formed from the cytoplasm the cleavage nuclei bring with them;

a great difference is here noticeable in the fact that GRABER (18) found, in the eggs of this *Dipteron*, the Keimhautblastem to be absent. If we compare the figures we have just described with any of the blastoderm cells, it will be seen that the amount of protoplasm surrounding the nucleus is much smaller than the amount within the cell. An inner Keimhautblastem is not present immediately after the formation of the blastoderm, and in no sections did we notice a continuous layer just within the blastoderm. We do find, after completed formation of the blastoderm, irregular patches of a rather finely granulated mass at the base of some of the blastoderm cells. We will shortly show that the cleavage nuclei, after reaching the inner surface of the Keimhautblastem, push on through it until they reach a position near its outer surface. The migration of these nuclei to the egg's surface is undoubtedly due, at least the active part of it, to the cytoplasm which surrounds them. The pseudopodial-like processes possessed by these masses of cytoplasm, are used to partially envelope the yolk globules and expose a greater amount of their surface to them, and they are also used in locomotion. When the nuclei reach the Keimhautblastem they move into it, and this locomotion, it seems to us, is easier explained if the two, nucleus and cytoplasm, continue intact. The immediate fusion of the cyto-plasm with the Keimhautblastem would compel the nuclei to be themselves the active agency in their further progress. The nuclei pass on into the Keimhautblastem until about two-

The nuclei pass on into the Keimhautblastem until about twothirds of the distance from the inner to the outer surface has been traversed; here their migration ceases. We have often noticed that many nuclei, after they have entered the Keimhautblastem, lengthen, their longitudinal axis then being at right angles to the eggs surface; this is not constant, and is seen only in a few nuclei. A somewhat similar elongated nucleus has been observed by BLOCHMANN (2), HENKING (25), KOWALEVSKY (31) and NOACK (42) as occurring either in an early or late pre-blastodermic stage. All these observers, however, figured the nucleus as cuneiform with the truncated end towards the surface of the egg. When in *Polistes* the nuclei have pushed partly through the Keimhautblastem there follows a period during which it recedes from the egg membranes, not at all points, but between the nuclei, giving the surface an undulating appearance. The egg in surface view appears very similar to a blastula of *Amphi*oxus, each protruding part enclosing a nucleus, but no cells have as yet formed. A similar appearance has been described and figured

for numerous insect eggs. There are never in this stage as many nuclei at the surface as later there are blastoderm cells. This increase in number occurs by the division of the nuclei within the Keimhautblastem and also later by a division of the blastoderm cells, the former method lasting but a short time and resulting in the nuclei being evenly distributed throughout the Keimhautblastem (Fig. 33).

The arrival of the cleavage nuclei at the periphery of the egg has been described very many times, it has been found that, among different insects, there is a great variation as to that part of the egg at which the nuclei first arrive. To give an idea of this variation we tabulate most of the results.

	BLOCHMANN,	for	Diptera (Musca).
Nuclei arrive at all	Bobretzky,	for	Lepidoptera (Porthesia)
parts of egg's sur-	HEYMONS,	for	Forficula.
face at the same	NOACK,	for	Diptera (Calliphora).
time.	Voeltzkow,	for	Coleoptera (Melolontha).
	WEISMANN,	for	Diptera (Chironomus).
At the posterior end first.	GANIN,	for	Hymenoptera (Formica).
	GRABER,	for	Diptera (Lucilia).
	GRIMM,	for	Diptera (Chironomus).
	Heider,	for	Coleoptera (Hydrophilus).
	Heymons,	for	Orthoptera (Gryllus).
	Kowalevsky,	for	Diptera (Musca).
	WEISMANN,	for	Hymenoptera.
At the anterior end first.	(AYERS,	for	Orthoptera (Oecanthus).
	Bobretzky,	for	Lepidoptera (Pieris).
	Bütschli,	for	Hymenoptera (Apis).
	CARRIÈRE and		
	Bürger,	for	Hymenoptera (Chalicodoma).
	DICKEL,	for	Hymenoptera (Apis).
	Grassi,	for	Hymenoptera (Apis).
	KOWALEVSKY,	for	Hymenoptera (Apis).
	WEISMANN,	for	Diptera (Musca).
At the equator first.	Kulagin,	for	Hymenoptera (Platygaster).
	SCHWARTZE,	for	Lepidoptera (Lasiocampa).
At the ventral surface	(Heymons,	for	Orthoptera (Gryllotalpa).
first.	MELNIKOW,	for	Coleoptera (Donacia).
Appearing at surface in groups.	BRANDT,	for	Odonata (Callopteryx).

In Polistes the cleavage nuclei reach the surface first at the anterior end, but just which part is not clear to us. They appear to arrive first near the equator, the blastoderm cells being first formed at the poles, especially at the anterior. GRASSI (19) and DICKEL (14) both observed that in Apis the blastoderm first forms at the anterior pole and from here extends to the posterior. In Fig. 7 we show a sagittal section of an egg shortly after the cleavage nuclei have reached the surface. The nuclei along the ventral and dorsal surfaces have entered the Keimhautblastem. At the two poles a slightly advanced stage is seen and this extends over the egg for a short distance, further on the ventral than on the dorsal surface. Near the posterior end, and also ventrally, a few nuclei are seen which have only begun to enter the Keimhautblastem. CARRIÈRE and BÜRGER (11) have shown that in *Chalicodoma* the same thing occurs, the entrance of the nuclei into the Keimhautblastem not being the same over the entire surface of the egg.

The blastoderm cells are formed by cell boundaries appearing which cut off the nuclei from each other, resulting in a continuous layer of cells over the egg. The basal boundary is the last formed, the cells being separated from each other before they are cut off from the yolk. In general, it can be said that the nuclei at the poles are separated before those on the rest of the surface. We have observed in eggs not yet showing a distinct layer of blastoderm cells, certain peculiarities in the Keimhautblastem between the nuclei. These are found equidistant between the nuclei; occupying a position corresponding to that at which a little later cell boundaries will occur. In this space between two nuclei rows of small vacuoles could be seen, these occupying a position corresponding to that at which the boundary will later appear and place the two nuclei in adjacent cells. These rows of vacuoles were always at right angles to the egg's surface (Fig. 34). Sometimes there were a number of small vacuoles; these might in other places be represented by one or two large ones; or both large and small would be found in the same row. Again is was noticed that there would be one or two elongated vacuoles, and through the center of each, a gathering of slightly darker granules than found in the surrounding Keimhautblastem. These central granules were connected with the edge of the vacuole by finer rows of granules (Fig. 35). Both the elongated vacuoles and the rows of granules extended entirely, or in part, between the yolk and the surface of the egg. Again we noticed

(Fig. 36) the appearance of a number of dark granules either separated, or much oftener appearing partially fused, and then taking the form of a plate, not straight, but of a wavy appearance. At either side of this traces of one or more vacuoles were seen giving the plate the appearance of having been enclosed in a vacuole, or vacuoles, which had nearly disappeared. Despite the fact that the boundaries between the blastoderm cells have been described as cutting in between the nuclei from the outside, and as appearing in a similar way in Polistes, we have here to do with the formation of boundaries, which, separating the nuclei from each other, give rise to at least some of the blastoderm cells. In what order the figures we give occur we could not say, it appearing to us probable that the vacuoles are first to appear, and they are followed by the granules and the cell boundaries. We call attention to the fact that the boundaries are in the Keimhautblastem most of them formed between resting nuclei, CARNOY (12) has figured the formation of cell boundaries between resting nuclei in a Hymenopteron, Bombus, noting rows of granules, very similar to what we find by Polistes, but without the appearance of the vacuoles which we have described.

The undulating outline of the egg has already been noted and explained by the withdrawal of the Keimhautblastem from the egg membranes at certain points. This appearance does not persist for any length of time, the outer margin becoming again even and a layer of nearly equal thickness is formed over the surface of the egg. Cell boundaries are formed between the nuclei in this layer, and the egg becomes covered by a single layer of cells, the blastoderm. These cells are at first very much alike, differing in a slight variation in size and shape. In reality such a stage is probably never present in *Polistes*, or, if present, has an exceedingly short duration. The reason for this is found in the fact that the cleavage nuclei reach the anterior surface before the posterior, and also that the blastoderm formation does not go on similarly over the entire surface of the egg.

Most of the earlier students of insect embryology, in fact until the last few years, described the division of the blastoderm cells as occurring amitotically; this has, however, been changed, mitosis having been observed in the blastoderm cells of a number of different insects. In *Polistes* a mitotic division is the only one found, different stages of which are easily seen and have been observed from the different parts of the egg's surface. We have figured a

number of these dividing blastoderm cells, and would call attention to two points, namely, the entire absence of direct division and to the fact that the divisions do not occur in any particular direction relative to the surface of the egg. PETRUNKEWITSCH (45) figures a portion of the blastoderm of the honey-bee, showing the nuclei dividing in different planes. Many observers have, however, recorded observations directly opposed to these, and held that the blastoderm cells do all divide in some one definite direction. GRIMM (20) says that in the eggs of Chironomus the blastoderm cells divide »in der Richtung der Eiradien«. Tangential division of these cells has been recorded by BLOCHMANN (2), WHEELER (60) and HEYMONS (26). Here and there in the blastoderm, bi-nucleate cells were observed, although never occurring abundantly. BÜTSCHLI (9) and WHEELER (60) have recorded a similar occurrence. To gain some idea of the direction in which blastoderm cells divide we selected an egg in an early blastoderm stage, one cut transversly, and, examining one-third of the sections, marked the direction of division in each dividing cell. In Fig. 43 we give the result, the longer lines representing each a longitudinal axis of a mitotic figure, the small transverse lines the position of the chromosomes, the long line above these the surface of the egg. It will be noticed that the equatorial plate stage occurs much oftener than any other.

We have now reached a stage in the development of the egg of *Polistes* in which the blastoderm cells have been formed as a single continuous layer over the surface of the egg. What changes next take place, while they may have, by a further division of the cells and an increase in their number, something to do in altering the blastoderm, yet really have more to do with the first arrangement of the cells in the formation of the germinal band.

# The first arrangement of the blastoderm cells.

The various shapes and sizes assumed by the blastoderm cells on the different parts of the eggs surface have been noted by a great many observers, and to give all the variations in shape and size which each one has recorded, would require a citation from nearly all of the works noted in the bibliography as well as several which have been omitted. We can say in general that the blastoderm cells, after being formed, soon change their shape and their size, the surface of the egg becomes covered by a layer of cells showing inequalities in their form and in their mass. A final result

is a difference between the blastoderm on part of the ventral surface where the cells become cylindrical, making the blastoderm thicker here than at any other place. Dorsally, the cells become flattened, extending over the sides of the egg and passing by a gradual transition into the ventral cylindrical cells. This thickening of the blastoderm along part of the ventral surface was known before eggs were sectioned. The dorsal cells may become so flat and narrow that they are with difficulty seen.

In the egg of *Polistes* a stage occurs in which the blastoderm cells covering most of the surface are of nearly the same shape and size. Fig. 44 is a transverse section of such an egg, cut in a region nearly equidistant from either end and showing all the blastoderm cells to be very similar in outline. An examination of the entire series of this egg gives us near the anterior pole a part of the egg covered with cells which are much more rounded. These shortly give place to others which are different, a section showing a variation in the shape of the cells surrounding it. At one side of the section the cells are cylindrical and opposite these we find them to be cuboidal; between these two groups the cells are more flattened. Passing further down the egg we soon come to sections in which these variations in the shape of the blastoderm cells give place to the much more regular appearance seen in the figure. Near the other end of the egg we find the cells again becoming somewhat rounded but not so marked as at the first end observed. We see in this stage, which we hold to be an early one in blastoderm formation, that the cells are not the same over the entire surface of the egg. At and near both poles they are different from the remaining surface of the egg, and even the two ends, while covered with cells which are somewhat similar, yet show a marked contrast.

The section we next figure (Fig. 45) is from an egg which we think is a later stage and which shows the blastoderm cells as having changed their shape over the entire surface of the egg. The cells are not so regular over any large area as we found them in the preceeding stage, their boundaries being no longer represented by straight, but by curved, lines. No section from this egg will show cells so nearly equal in size and shape as we found in Fig. 44. At one part of the egg, along upper part of drawing, a space is seen which is without any definite layer of blastoderm cells; nuclei are present at the surface but in a small area no cell boundaries were observed. To select with certainty stages which immediately

follow each other is extremely difficult, and we can only give what it seems to us, after studying all our sections, is the correct sequence. Longitudinal sagittal sections would show which were the early and which the late stages of development, but when we add to these longitudinal sections, cut in other than a sagittal plane, and also transverse sections, the task of selection becomes difficult.

Following the two stages we have briefly described we find another change taking place which is best seen in a transverse section. Here (Fig. 46), we notice the first definite arrangement of the cells, in that, along one part of the section there is a grouping of a number of columnar cells. These are found only at one place. The cells covering the remainder of the section are flattened against the egg. This arrangement is not shown in a section near either end of the egg (Fig. 47), a longitudinal sagittal section of such an egg, shows that the cylindrical cells are grouped along one surface, beginning nearer one end than the other. We are now able to determine that the surface bearing the cylindrical cells is ventral, and that the end, nearest which they occur is anterior. This formation, part of the ventral surface of these cylindrical cells, we find persists. To follow this would lead to a study of the germinal band which is beyond the scope of this paper.

# The yolk nuclei.

Regarding the origin and fate of the nuclei which are found within the egg after the blastoderm is formed there has been much dispute; at present no general statement can be made, if different insects are considered. Concerning the ultimate fate of these nuclei we here have nothing to say. Our observations have extended as yet but a short time after the blastoderm is formed, and, at this stage, the nuclei are still present within the egg, being easily seen in any of the pre-blastodermic stages we have described. We are, however, at present interested in the yolk nuclei during the stages we have herein recorded for *Polistes*, and will give their history only so far.

In regard to the origin of the yolk nuclei there are two distinct views advanced; one accounts for their origin from the dividing nuclei within the egg and follows in general what we have so far described as occurring in *Polistes*; the other derives the yolk nuclei from the cells of the blastoderm, holding that their origin is in the blastoderm, and that they wander back into the egg. Mention might Zeitschrift f. wissensch. Zoologie. LXXX. Bd. 10

also be made of a view between these two in which they are formed in part by each method; also of eggs like those of Campodea and Platygaster in which no nuclei are present within the yolk after the blastoderm is formed. Those who have held that part of the dividing nuclei remain within the egg, that is, the nuclei do not all go to the periphery to take part in blastoderm formation, are: AVERS (1), BLOCHMANN (2), BOBRETZKY (3), CARRIÈRE and BÜRGER (11), CHOLOD-KOVSKY (13), DICKEL (14), GRABER (18) for Lina etc., HEIDER (23), HEYMONS (26) for Forficula, KNOWER (28), NUSSBAUM (43), SCHWARTZE (51), TICHOMIROFF (52), UZEL (56) for Lepisma, VOELTZKOW (58), WHEELER (60) for Doryphora, and WITLACZIL (62). Against this view and deriving the vitellophags from the blastoderm are: BRUCE (6) for Meloë, BRUES (7) (origin late and unknown, but no cleavage nuclei remain in egg), GRABER (18a) for Melolontha, HEYMONS (26) for Periplaneta and Gryllotalpa, PATTEN (44), UZEL (56) for Macrotoma, WEISMANN (59), WHEELER (60) for Periplaneta, and WILL (61). It does not necessarily follow that the authors here cited believe but one way of vitellophag derivation is present among insects. Contrary to this, some have worked with more than one insect and found this origin different in the insects studied. As an example we would call attention to UZEL's (56) paper in which the eggs of Campodea are found to be without yolk nuclei, Lepisma retains part of the nuclei within the center of the egg, and in Macrotoma all the nuclei go to the surface, the yolk nuclei then arise from the blastoderm cells. There are also views deriving the yolk nuclei from peculiar thickenings in the Keimhautblastem; also earlier ones not here given. We hold that in Polistes the yolk nuclei are derived from the dividing nuclei within the egg and not from the blastoderm.

We have already called attention to the similarity of the cleavage and the yolk nuclei, holding that while resting and dividing the two are similar except in their position in the egg, and also a slight one in size. We have described these nuclei both resting and dividing, and have nothing more to say concerning the yolk nuclei previous to the stage in which the cleavage nuclei have reached the Keimhautblastem. Coming to this stage we find that the nuclei which remain within the egg and take no active part in blastoderm formation change in their method of dividing, not in any one stage or in an abrupt manner.

In Fig. 7 we have shown an egg of Polistes in which the cleavage

nuclei have entered the Keimhautblastem and the blastoderm is soon to be formed. From an examination of the sections of this egg we find the yolk nuclei evenly distributed throughout it, and the masses of cytoplasm containing as yet each but a single nucleus. A number of these nuclei are seen in mitosis, similar to that we have already described, and no changes are noticed in their structure which would separate the two kinds of nuclei from each other. One decided change is, however, found, and that is the presence, in the yolk, of nuclei which are dividing amitotically. This has not as yet been recorded by us in any of the earlier stages, and we get here two methods of nuclear division occurring among the yolk nuclei in the same egg, the mitotic predominating; the amitotic was found only a few times in all the sections of the egg.

Passing to a somewhat later stage, one in which the blastoderm cells are nearly all formed, we find that the yolk nuclei have not as yet formed multinucleate masses and many regular mitotic figures are still seen. This formation of multinucleate masses or »nests« has been noted by different observers, CARRIÈRE and BÜRGER (11), HEIDER (23), SCHWARTZE (51) and others. These, in the early phases of mitosis, appear similar to those we have described, but we note that often when division is completed, the resultant nuclei and their membranes formed, the two nuclei are still joined by the connecting fibres. The fibres have already been described as very pronounced, but we here find what we have previously not observed, a persistence of the fibres after completed division. Another egg having the appearance of being in nearly the same stage of development, having, however, the cells at the ends of the egg formed and partly rounded, failed to show any yolk nuclei in mitosis, but they had begin to form multinucleate masses. Amitotic division was seen in this egg. The relative stage of development of these two eggs is hard to determine, one having been cut longitudinally the other transversely.

A study of the later stages in the development of *Polistes* shows a complete absence of mitosis in yolk nuclei, although this form of division can often be seen in the blastoderm cells. Direct division is also rare, appearing to be scarce in any of the late stages, and disappearing when the blastoderm cells have become arranged to show the earliest suggestion of a germinal band. When the blastoderm cells are formed, and even just before this, the yolk nuclei begin to arrange themselves into multinucleate bodies so that each mass of cytoplasm will contain from two, to six or eight, nuclei.

We do not hold that these multinucleate masses are formed by division of the nuclei alone, but that more active in their formation appears to be a joining of uninucleated masses we have earlier described. The number of the multinucleate masses is much less than were the single nuclei present just before blastoderm formation. We find, also, that the outline of each multinucleate mass is much more regular and clearly defined than in the yolk nuclei. Finally we find that the nuclei of the two groups, cleavage and yolk nuclei, have each an entirely different fate. The first, after forming the blastoderm cells, continue to divide mitotically and take part actively in the development of the insect. The yolk nuclei apparently reach a stage in which active division ceases, but before this takes place true mitotic division is lost or greatly changed. As already mentioned, true mitosis does not cease all at once and amitosis take its place, but both kinds of division may be present in the same egg. Amitosis was found but rarely, no egg being observed which contained many yolk nuclei so dividing. In the last stages of development which were here described, we found, fairly abundantly, nuclei which showed a completed division quite different from what we earlier described. Whether these were the result of a direct or an indirect division we cannot say; the presence of more darkly stained strands between the nuclei might point to a mitotic division, but the fact that these figures were never found in pre-blastodermic stages would point, we think, to their being the result of amitotic division. Another view, which we hold as most likely, is that the method of mitosis has changed and these figures (Figs. 56, 57, 58, 59 and 60) are the result of a very greatly changed mitosis resulting in the separation of the daughter nuclei, but in a method which, probably, differs somewhat from what we have hitherto recorded.

The nuclei which were amitotic in their division at first elongate (Figs. 50 and 51), their outline later resembling that of a figure 8 (Fig. 52). The chromatin granules are larger than in those nuclei seen in earlier stages, but become finally arranged so that the resultant nuclei will each contain approximately one-half of the chromatin. We find here, what has often before been noted, the fact that these nuclei are somewhat larger than normal.

The statement of SCHWARTZE (51) that the yolk nuclei, when they become such, degenerate, would appear to us to be, in part at least, incorrect. In the early stages of cleavage, after the two groups of nuclei have become separated from each other, the inner group

we think can rightly be called yolk nuclei, although the blastoderm has not as yet been formed. These nuclei remain within the yolk, never actively taking part in the formation of the blastoderm. Previous to the beginning of blastoderm formation they do not, as we have shown, differ from the cleavage nuclei, and it would be hard to find at this stage anything in them which could be looked upon as degeneration. If the term yolk nucleus is restricted to those nuclei which remain within the egg after the blastoderm is formed then degeneration may at once occur in the yolk nuclei, but we see no good reason for this late separation of the two kinds of nuclei from each other. If, on the contrary, we call yolk nuclei those which, long before the blastoderm is formed, become separated by their position from the cleavage nuclei, we cannot say that degeneration does at once occur, for these we have shown are not as yet in any sense degenerate.

#### Methods.

Many different methods were used in hardening and staining our specimens, but it would be of apparently little use to call attention to them all, and we give but a few methods which were used more successfully than most of the others. Eggs were killed in hot water, and in a few seconds an equal amount of a saturated aqueous solution of sublimate was added. The eggs were allowed to remain here for twenty to forty minutes, were washed and placed in  $70^{\circ}/_{0}$ alcohol. Another method was to heat a saturated aqueous solution of sublimate to near the boiling point and then add to this an equal amount of alcohol. This was then poured directly over the eggs and allowed to stand for ten to twenty minutes. The two methods of staining which we used oftenest were iron-haematoxylin, generally followed by Bordeaux red, and the safranin-methylen-violet, orange G, triple stain.

Zoological Laboratory, University of Wisconsin, Madison, December 1904.

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# Explanation of Plates X and XI.

All figures drawn with a camera-lucida.

Fig. 1. Sagittal section through a young egg showing a few nuclei near the anterior pole. Vergr. 62.

In this and the five following figures, the resting nuclei are drawn with a circle, the dividing nuclei with a cross.

Fig. 2. A later stage. Vergr. 62.

Fig. 3. A still later stage, the zone of cleavage nuclei is beginning to show plainly. Vergr. 62.

Fig. 4. Sagittal section through an egg after the cleavage nuclei have wandered well towards the posterior pole. Vergr. 62.

Fig. 5. Tansverse section through an egg at a little later stage. The nuclei are all resting, and, in the drawing, have been combined from two neighboring sections. Vergr. 62.

Fig. 6. Longitudinal section through an older egg; the difference in position between the cleavage and the yolk nuclei is very marked. Vergr. 62.

Fig. 7. Longitudinal section of an egg in which the cleavage nuclei have entered the Keimhautblastem. It will be noticed that at the poles the undulating outline has appeared. Below and to the right will be noticed a place where the nuclei have not entirely entered into the Keimhautblastem. Vergr. 62.

Fig. 8. Resting nucleus, from an egg about as far developed as Fig. 2. Centrosome and archoplasm are seen. Vergr. 800.

Fig. 9. Resting yolk nucleus, from an egg in which the cleavage nuclei have just entered the Keimhautblastem. Archoplasm with astral fibres. Vergr. 800. Fig. 10. Resting yolk nucleus, from an egg in which the cleavage nuclei

have entered the Keimhautblastem, but blastoderm cells not yet formed. Vergr, 800.

Fig. 11. Showing connections between the cytoplasmic masses surrounding the nuclei; one binucleate. Vergr. 800.

Fig. 12. Showing connections of three such masses. Vergr. 500.

Fig. 13. Dividing nucleus, from an egg at a stage between Figs. 1 and 2. Vergr. 800.

Fig. 14. Slightly later stage in division of a yolk nucleus. From an egg in about the same stage as Fig. 7. Vergr. 800.

Fig. 15. Dividing nucleus, from same egg as Fig. 13. Vergr. 800.

Figs. 16, 17, 18 and 19. Dividing yolk nuclei, from an egg in which the cleavage nuclei have all reached the Keimhautblastem. Figs. 17 and 19 show peculiar thickenings on connecting fibres. Vergr. 800.

Figs. 20 and 21. Dividing yolk nuclei, from an egg slightly younger than preceding. Both show the peculiar bodies surrounded by the connecting fibres. Vergr. 800.

Fig. 22. Dividing nucleus, from a late pre-blastodermic stage. Vergr. 800.

Fig. 23. Dividing nucleus, the earliest stage in which the peculiar large bodies seen in Figs. 20 and 21 were noticed. Here they are outside of the connecting fibres. Vergr. 800.

Fig. 24 and 25. Nuclei showing division nearly completed. From a late pre-blastodermic stage. Vergr. 800.

Fig. 26. Cleavage nucleus near the Keimhautblastem, which is to the right. The line to the right of this and following figures represents the egg membranes. Vergr. 800.

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Fig. 27. Three cleavage nuclei which, with their surrounding cytoplasm, have entered the Keimhautblastem. Vergr. 800.

Figs. 28. 29 and 30. Later stages of the same showing the cytoplasm of the cleavage nuclei still separate from Keimhautblastem. Vergr. 800.

Fig. 31. Two cleavage nuclei in the Keimhautblastem, between them, has begun to recede from the egg membranes. Vergr. 800.

Fig. 32. The same at a slightly later stage. Vergr. 800.

Fig. 33. Two cleavage nuclei dividing within the Keimhautblastem, before the boundaries have formed cutting off the blastoderm cells. Vergr. 800.

Fig. 34. Three nuclei in the Keimhautblastem showing rows of vacuoles between them which may be stages in formation of the boundaries separating the nuclei each into a blastoderm cell. Vergr. 800.

Fig. 35. A single nucleus in the Keimhautblastem, showing a large vacuole at either side within each of which is seen the row of granules in the form of an irregular line. Vergr. 800.

Fig. 36. Similar to preceding, but the granules are darker and the vacuoles can, with difficulty, be seen as clear spaces at either side. Vergr. 800.

Fig. 37. Two blastoderm cells from an egg similar to Fig. 46. Vergr. 800.

Fig. 38 and 39. Dividing blastoderm cells. The straight line in this and the three following figures represents the surface of the egg. Vergr. 1050.

Figs. 40, 41 and 42. Dividing blastoderm cells. Vergr. 800.

Fig. 43. Diagram showing plane of nuclear division in blastoderm cells. The long straight line represents the surface of the egg; near this, the other lines show axis of mitosis in cells, the short cross markings the position of the chromosomes in each division. Combined from sections through one-third of an egg.

Fig. 44. Transverse section through middle of egg showing shape of blastoderm cells shortly after their formation. Vergr. 110.

Fig. 45. Transverse section through slightly older egg. Vergr. 110.

Fig. 46. Transverse section of an egg in which the cells on the upper (dorsal) surface have begun to flatten. Vergr. 110.

Fig. 47. Sagittal section through an egg which shows difference in the blastoderm between ventral (to the left) and dorsal (to the right) surfaces; also between the anterior (upper) and posterior (lower) poles. To the right the cylindrical cells are shown which have begun to form the germinal band. Vergr. 62.

Fig. 48. Enlarged view of seven blastoderm cells taken from space — a — in preceding figure. Vergr. 800.

Fig. 49. Two blastoderm cells taken from space -b — in figure 48. Here in each nucleus a nucleole is distinctly seen. Vergr. 800.

Fig. 50. Amitotically dividing yolk nucleus, from an egg very similar to Fig. 44. Vergr. 800.

Figs. 51, 52 and 53. Three such nuclei, from an egg slightly older than preceding. Vergr. 800.

Fig. 54. Yolk nucleus having completed amitotic division. From an egg a little older than Fig. 46. Vergr. 800.

Fig. 55. Division completed. From same egg as Fig. 50. Vergr. 800.

Fig. 56, 57 and 58. Yolk nuclei which have divided and show the remaining strands (connecting fibres?) and the darkened cytoplasm between the nuclei. From an egg slightly older than Fig. 44. Vergr. 800.

Fig. 59. Similar nucleus from an egg in which only three true mitotic figures could be found within the yolk. Vergr. 800.

Fig. 60. Similar nucleus from an egg nearly the same age as Fig. 45. Vergr. 800.



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