

Resumen por el autor, Robert H. Bowen

Sobre ciertos rasgos de la espermatogénesis de los anfibios e insectos

Un estudio comparativo de las espermátidas de *Plethodon*, *Lixus*, *Rhomaleum* y *Ceutophilus* ha confirmado la opinión de que el acrosoma del espermatozoide se origina en relación con el aparato de Golgi. En *Plethodon* se ha demostrado que el aparato citado y el idiozoma, después de producir el acrosoma, son expulsados y pasan a la base de la cabeza del espermatozoide donde puede observarse durante algún tiempo. Nunca presenta una conexión orgánica con los centriolos situados en su vecindad, ni tampoco juega ningún papel en la formación del segmento intermedio del espermatozoide, conforme se ha supuesto. En *Lixus* y *Ceutophilus* el acrosoma se origina de un modo muy semejante al de los hemípteros. En el saltamontes *Rhomaleum*, por otra parte, los corpúsculos de Golgi de la espermátida nunca se fusionan para formar un solo acroblasto como sucede en las formas precedentes, sino que cada elemento de Golgi parece contribuir una porción individual y separada, formándose el acrosoma en virtud de la fusión de estas numerosas partes.

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## ON CERTAIN FEATURES OF SPERMATOGENESIS IN AMPHIBIA AND INSECTS

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TWO PLATES (FORTY-SIX FIGURES)

In a recent paper (Bowen, '20) on insect spermatogenesis I was able to demonstrate a relationship between the acrosome and the Golgi apparatus, and from this and facts previously published by other workers, I suggested that "The acrosome of the animal sperm is probably universally formed in connection with the Golgi apparatus, and has nothing to do with spindle fibers or other cell parts." At that time the evidence from other groups was very scanty indeed, the work of Sjoevall, Schitz, Gatenby, and Duesberg on various molluscs and mammals furnishing almost the only indications of the correctness of this suggestion. On the other hand, there were several well-known cases in which the current descriptions seemed to present serious difficulties, if not a direct contradiction. I decided, therefore, to examine some of these cases more carefully, together with material from other groups in which the Golgi apparatus had not yet been studied. In working up the material, a number of unexpected features were brought to light and it seemed of value to look more carefully into some of the cytoplasmic constituents which had not been accurately followed heretofore. This, however, called for the collection of much more material and further experimenting with fixation and staining—tasks which could not be immediately undertaken. I have accordingly decided to publish the major facts relating to acrosome formation, together with certain other features of interest in the spermatogenesis of several Amphibia and Insecta, leaving the details for a later study. A review of technical methods and acknowledgment of much assistance in the collection and identification of material will be for the present postponed.

## AMPHIBIA

Accounts of the formation of the acrosome and the middle-piece in the urodele sperm have long presented a most puzzling contradiction. According to Meves ('97), in *Salamandra*, the acrosome ('Sphaerenblaeschen') arises by the fusion of many small, clear vesicles which appear in the idiosome ('Sphaeren-substanz') of the spermatid. The vesicular acrosome thus formed becomes the apical body of the sperm, while the remnant ('der kleine Rest') of the sphere substance disappears completely. The 'middle-piece,' on the other hand, is formed by the proximal centriole alone, which penetrates the nuclear membrane and thus comes to lie inside the nucleus. But McGregor ('99), who has made a similar study of *Amphiuma*, gives a quite different interpretation of these details. According to McGregor, the acrosome arises from the sphere (idiosome) in a manner essentially similar to that described by Meves, except that the remnant of the sphere does not disintegrate. Instead it migrates with the centrioles to the opposite pole of the nucleus, where it forms the major part of the middle-piece, in which the small, spherical proximal centriole becomes embedded. McGregor also describes the penetration of the middle-piece into the nucleus, both authors thus agreeing that the middle-piece is intranuclear. With respect to the fate of the sphere remnant (Golgi remnant of my nomenclature) and the origin of the middle-piece, there was thus a discrepancy which has never been cleared up. It was with the idea of ascertaining the true relations of the Golgi apparatus (plus idiosome), acrosome, and centrioles that this work was undertaken. The material for this study has been drawn from a single species, *Plethodon cinereus* Green, though I hope eventually to examine other urodeles in a comparative way.

The cytoplasmic structures so far made out in the primary spermatocytes are of at least three categories, the Golgi apparatus-idiosome complex, the mitochondria, and a number of darkly stained granules of doubtful nature, but possibly to be considered as chromatoid bodies. The mitochondria, present in rather limited amount, occur as more or less scattered rodlets of very small diameter, similar to those figured in *Geotriton* by

Terni ('14). The idiosome and Golgi apparatus have been often described by earlier workers. Together they form much the most conspicuous object in the spermatocytes. The idiosome appears as a more or less irregularly spherical mass, staining rather more darkly than the general cytoplasm. To its surface are applied a large number of small, separate Golgi rodlets, each shaped somewhat like a banana (fig. 1), and seemingly never interconnected to form a 'network.' These rodlets, now definitely shown to be Golgi elements, have of course been long known under a variety of names, e.g., the 'Archoplasmaschleifen' of Hermann, the 'Centralkapsel' and 'Pseudochromosomen' of Heidenhain, and the 'formazioni periidiozomiche' of Terni.

During the maturation divisions the idiosome goes to pieces and its exact behavior during the division period is unknown. The Golgi rodlets undergo a great loss in staining capacity, and appear to be simultaneously fragmented, so that with the usual methods they cannot be satisfactorily followed in the division stages. It is certain, however, that the Golgi pieces (dictyosomes) are collected about the poles of the spindle (fig. 2) as described in invertebrates by several workers, although the exact method by which this distribution is achieved has not yet been clearly made out. At the close of the first maturation division the idiosome is reconstituted with the Golgi rodlets applied to its surface as before (fig. 3). Similarly, in the spermatids the same structure is built up soon after the second maturation division is completed. In my preparations, however, the Golgi rodlets, as distinct elements, are soon lost, and the outer part of the acroblast (as the Golgi apparatus plus idiosome may now be called) tends to impregnate rather uniformly with the various Golgi methods (figs. 5 and 6).

The acroblast lies at first some little distance from the nucleus; but presently it moves over into contact with the nuclear membrane and a small clear vesicle makes its appearance on one side of the acroblast (fig. 5). This is the 'Sphaerenblaeschen' of Meves and the acrosome of McGregor. In general appearance it closely resembles the homologous structure which I have described in the hemipteran spermatid (Bowen, '20), and for which I have

adopted the term acrosome. The acrosome increases in size, becoming a large, clear vesicle, to the surface of which the remains of the acroblast are attached (figs. 6 and 7). The centrioles presently become conspicuous, being sharply stained with Fe-hematoxylin. They are always located in the neighborhood of the acroblast (figs. 6, 7, and 8); indeed, they seem often to be resting on its surface. The relation seems, however, to be purely a topographical one.

When the acrosome has reached a certain stage, it becomes applied closely to the nuclear membrane, and gradually the cell wall is drawn down over it, creating the impression that the acrosome has actually protruded through the cell wall (fig. 8). Throughout this period of fixation, the acroblast remains close to the acrosome (figs. 8 and 9), but the connection between the two has obviously become very loose. In fact, the acroblast or, as it may now be called, the Golgi remnant, becomes more or less spread out or broken up into separate masses (fig. 9), and in Fe-hematoxylin preparations is usually not to be made out at all. This loss in staining capacity is obviously the source of Meves' erroneous description of the disappearance of the 'Sphaerensubstanz.'

The Golgi remnant, together with the centrioles, lingers for a time in the immediate vicinity of the acrosome, and then both are shifted to the opposite pole of the nucleus establishing the future long axis of the sperm. Everything indicates that McGregor was correct in holding that the separation of acrosome and centrioles is due to the active migration of the centrioles, rather than of the acrosome, as Meves contended.

The spermatid nucleus meanwhile becomes very irregular in shape and then begins to draw out to form the head of the sperm with the acrosome at one end and the centrioles at the other (fig. 11). The Golgi remnant lies in the cytoplasm at the base of the head close to the centrioles (figs. 11 and 12), becoming oftentimes more or less broken up and diffuse. It is easily demonstrated by the special methods for the Golgi apparatus, but not by the usual Fe-hematoxylin techniques. The Golgi remnant remains in this same position for a long time; indeed,

long after the stage shown by McGregor in his figure 32, in which it has supposedly been incorporated with the proximal centriole to form the middle-piece of the sperm. Its ultimate fate is unknown, preparations of late stages in sperm formation not yet having been successfully impregnated. Meanwhile the proximal centriole (in particular) increases progressively in size and staining capacity (compare figs. 11, 12, and 13) and becomes applied very closely to the nuclear membrane. At first it is spherical, but as the head elongates and becomes narrower, the sphere begins to draw out (fig. 14) and it becomes eventually a long rod (fig. 19)—the (centrosomal) middle-piece of the sperm. In this process I have been quite unable to find any evidence of an actual penetration of the nuclear wall by the 'middle-piece,' as described by both Meves and McGregor. The centriole seems rather to be merely in close contact with the nucleus, as is the case in many other animal sperms.

With respect to the origin of the middle-piece, I am, therefore, in general agreement with Meves. The unusual topographical relation existing between the Golgi remnant and the centrioles was doubtless the cause of McGregor's error in the derivation of the middle-piece, coupled also with the poor staining conditions at this period. There is also a possibility that some large, darkly staining granules of doubtful relations, which occur in the spermatids, may have been confused by him as derivatives of the cast-off 'sphere.'

Finally, the conditions in the completed middle-piece described by McGregor remain to be explained. Since, according to this author, in the formation of the middle-piece the proximal centriole merely becomes embedded in a mass of sphere material, it should be possible to separate these two components by differential staining. This was in fact apparently accomplished (McGregor's fig. 32), the sphere material staining less heavily than the centriole. My own observations of the proximal centriole (so-called) suggest, however, a quite different interpretation of this appearance. As was noted by McGregor, the proximal centriole in the earlier spermatid stages often appears

bipartite or dumb-bell shaped,<sup>1</sup> the two parts being typically equal in size. Meves figures much the same thing, though he described the formation as a curved rod. This same division of the proximal centriole occurs in *Plethodon* (figs. 7 and 10), the separation of the two parts being sometimes very marked. To one of the halves the axial filament is attached. When the nucleus begins to draw out, this division of the proximal centriole is apparently lost in many cases—sometimes, no doubt, by the recombination or fusion of the two parts and at other times by the orientation of the centrioles. At the same time, the part to which the axial filament is attached increases very rapidly in size, while the other part remains relatively small and inconspicuous. However, with a little searching, examples can be found at any of the intermediate stages in which the two parts of the proximal centriole can be clearly separated (figs. 13, 14, and 15). That the two bodies in these cases are actually the equivalent of the apparently single proximal centrioles of other cases can be demonstrated by comparison of adjacent spermatids in which the parts can or cannot be separated (figs. 13 and 14). It seems probable, as Professor Wilson has suggested to me, that in McGregor's preparations the large centriole was extracted more than the smaller one, giving rise to the interpretation already noted. The

<sup>1</sup> It should be pointed out that the origin of this bipartite condition has not yet been adequately worked out. I have followed the usual custom in considering the two parts as formed by the division of the proximal centriole, my own preparations not permitting a detailed study of the very early spermatid centrioles. Doctor Wilson has, however, called my attention to the fact that generally in the differentiation of the sperm a ring arises from only a portion of the distal centriole, while the remainder later comes into close relation with the proximal centriole. It is possible that something of the same kind obtains in the case of the urodele. Thus we might consider the portion of the so-called 'proximal' centriole to which the axial filament is directly connected as the central part of the ring centriole, while the other portion would actually be the proximal centriole itself. This would explain the apparent transfer of the insertion of the axial filament from the distal to the proximal centriole as required by McGregor's description, and would fall in line with the usual accounts of sperm formation which derive the axial filament from the distal centriole. Further study of this possibility is contemplated. However, in view of the uncertainty attaching to these speculations, I have thought it best to follow the traditional accounts for the present purely as a matter of convenience in description.

variability in staining behavior of the centrioles in *Plethodon* leads me to look upon this as a very plausible explanation.

In the late stages this small centriole seems to fuse with the free end of the much elongated middle-piece proper, from one end of which the axial filament arises (compare fig. 15). The function of this small centriole is not yet understood, but a possible explanation has suggested itself, which I hope eventually to prove or disprove definitely. It will be recalled that the tail of the urodele sperm bears a long, undulating membrane, the base of which is inserted along the axial filament of the tail, while the free edge is marked by a definite filament somewhat smaller in size than the main tail filament. The axial filament proper undoubtedly arises from the large centrosomal middle-piece. The exact origin of the membrane filament has not, however, been satisfactorily explained. I would like to suggest, on the basis of observations already made, the possibility of the origin of this filament from the second, eventually much the smaller, of the original halves of the proximal (?) centriole. Unfortunately, this filament is so delicate that its free portion can be made out in its earlier stages only with some difficulty, while the insertion can never be satisfactorily followed. It frequently happens, however, that the developing sperms are cut obliquely through the base of the head, and in such sections one can sometimes see what appears to be a fine filament arising from the small centriole and passing backward through the ring centriole (figs. 16 and 17). (See also fig. 18, in which the ring was not included in the section.) Should further study bear out such an interpretation, the origin of the membrane filament would fall in line with the facts already known as to the origin in general of vibratile filaments. It is possible, nevertheless, that the small centriole plays a rôle in the late stages during the drawing out of the ring centriole; for I have constantly observed a small darkly staining mass at the base of the tail filament which seems to originate from the centrosomal middle-piece (fig. 19). This mass subsequently moves out along the filament toward the free end of the elongating ring centriole, but its fate and function are alike unknown.



I have also found in the spermatids a collection of granules which seem to be preserved best in the absence of acetic acid. During the formation of the acrosome these granules are arranged in a circle around the acroblast and lie close to the nuclear membrane. This arrangement is a constant and striking one (fig. 4). The nature and fate of these granules are not known. Possibly they are related to the chromatoid bodies of other animals. They may be related to the granules previously mentioned as occurring in the spermatocytes. A more critical study of these granules will be undertaken on new material.

### *Summary*

1. The acrosome of the urodele sperm arises as in many other animals from the Golgi apparatus plus idiosome (acroblast), which is later cast off and remains for some time in the cytoplasm near the base of the sperm nucleus.

2. The middle-piece of the sperm is derived from the so-called proximal centriole, and is not, as McGregor claimed, "chiefly derived from *the remnant of the sphere*."

3. The origin of the filament on the free edge of the undulating membrane of the sperm tail from one part of the originally bipartite proximal (?) centriole is suggested as a possibility.

### COLEOPTERA

My observations on the Coleoptera are confined to a single species, *Lixus concavus* Lec., which was examined merely to check up the origin of the acrosome in the light of my previous work on Hemiptera. In this beetle, the Golgi apparatus is present in the spermatocytes in the form of numerous scattered Golgi bodies (fig. 27) similar in a general way to those which I have described in the pentatomids. I have not followed out the preliminary history of these Golgi bodies or their distribution in the maturation divisions. In the early spermatids, however, before the mitochondria have condensed to form the nebenkern, the Golgi bodies are easily demonstrated, scattered irregularly in the cytoplasm (fig. 28). They gradually draw together (fig. 29), and eventually fuse to form a single mass (fig. 30), the acro-

blast, exactly as in Hemiptera. This lies at first in the angle between nucleus and nebenkern, whence it moves to an anterior position (fig. 30). Later it appears again at the base of the head (figs. 31 and 34), so that it probably undergoes a migration similar to that which occurs in Hemiptera. The acroblast in this beetle impregnates in a very peculiar manner. Instead of forming a thimble-shaped mass with a heavily impregnated periphery as in the pentatomids (Bowen, '20), it is apparently shaped like a disc, only the periphery of which is blackened by osmic acid. That this is a ring, not an optical section of some solid surface, is easily demonstrated by viewing the acroblast from different angles (compare figs. 30 and 31).

From the acroblast the acrosome arises by a process of differentiation similar in all probability to that in the Hemiptera and Amphibia. In this case, however, the acrosome is so very small that it cannot be satisfactorily demonstrated until the time approaches for the casting off of the acroblast, when it can be made out as a small vesicle applied closely to the nuclear membrane (fig. 36). Then the acroblast separates from the acrosome proper (figs. 37 and 38), and passing rapidly back along the tail (fig. 39), remains visible as a conspicuous blackened ring for a considerable period. Its fate is doubtless similar to that in the Hemiptera. At the time the acroblast is cast off, the head of the sperm is usually bent rather sharply over, so that its major axis is nearly at right angles to that of the tail filament. It is accordingly difficult to say whether the acrosome is deposited directly in place and becomes subsequently anterior by the straightening out of the head; or whether there is an active migration of the acrosome to its definitive position, as in the pentatomids. Probably both factors play a part. At all events, the head eventually straightens out, and the acrosome can be made out as a minute body applied to its tip (fig. 40). Subsequently the head becomes much drawn out, as in other insect sperms.

I have also been able to make some observations on the nebenkern which form an interesting supplement to my recent discussion of this subject (Bowen, '22 b). In *Lixus* the nebenkern goes through a process of condensation quite similar to that

which I have described in *Brochymena*, and already outlined in part by Shaffer ('17) in *Passalus*, and Holmgren ('02) in *Silpha*. The chromophilic substance (Bowen, '22 b) seems to form a plate-work very like that in the Hemiptera, and I can find no trace whatever of a 'spireme' such as Gatenby ('18) claims to have demonstrated in *Tenebrio*. At all events, in the later stages of condensation, the chromophilic substance is arranged exactly as in the pentatomids, and cross-sections of the nebenkern (figs. 32 and 33A and B) could hardly be distinguished from similar sections of *Brochymena*. (Compare Bowen, '22 b, figs. 21A and B, also fig. 13.) In one respect, however, there is a fundamental difference in the behavior of the nebenkern. In the Hemiptera the chromophilic substance disappears completely and the nebenkern is divided into two parts during the initial stage in its drawing out; while in the Coleoptera the drawing out of the nebenkern has progressed much farther before these same results are attained. Thus, in figure 34, a stage in the final condensation of the chromophilic substance is shown, which is quite comparable to my figure 16 (Bowen, '22 b) of *Brochymena*; while in an adjacent spermatid (fig. 35) the chromophilic substance has completely (?) disappeared.

In the paper already referred to, I attempted to show that the division of the nebenkern always waits on the final disappearance of the chromophilic substance. The observations of Holmgren on *Silpha* indicated that this was the case in the beetles, but Shaffer's account of *Passalus* left the point somewhat in doubt. I have, therefore, reexamined this point more carefully in *Lixus*, and I find the facts exactly as described by me in the Hemiptera. Cross-sections of the nebenkern through the chromophilic substance and also above and below it demonstrate conclusively that the nebenkern is divided only in the region from which the chromophilic substance has been withdrawn (figs. 33A and B, cross-sections of the nebenkern at a stage approximately like fig. 34. Compare these cross-sections with figs. 21A and B from *Brochymena*, Bowen, '22 b).

The halves of the nebenkern ultimately spin out to form the tail sheaths so characteristic of insect sperms, and I can find no

evidence of a degeneration of the mitochondrial elements such as Holmgren described in *Silpha*. The central substance (Bowen, '22 b) was not demonstrable in my preparations, so that I have been unable to check Holmgren's account of this feature.

### *Summary*

1. The acrosome arises in connection with the Golgi apparatus plus idiosome, in a manner similar to that described in several other animals.

2. The nebenkern passes through a series of condensation phenomena similar to those of the Hemiptera, and is completely divided only with the disappearance of the chromophilic substance.

### ORTHOPTERA

The development of the acrosome in various groups of Orthoptera has been studied by many workers with very discordant results, a short résumé of which I have given in another place (Bowen, '22 a). Most of these cases, however, have been easily interpreted on the basis of conditions found in the Hemiptera, but in the case of the grasshopper the facts are as yet by no means clear. It was with the hope of clearing up the whole problem of the acrosome in Orthoptera that this study was undertaken. Thus far I have had opportunity to examine only a few species from the families Acrididae and Tettigoniidae.

### *Family Acrididae*

Although the grasshoppers have been studied by many workers there has been a uniform failure to make out any details whatever concerning the acrosome. Indeed, Buchner seems to have been the only one who even noted its occurrence. According to his latest account (Buchner, '15), the acrosome is derived from the 'proximal' centriole by a process of division—an origin which is obviously quite different from that to be expected on the basis of my hypothesis. As a matter of fact, Buchner failed to make out most of the essential stages, due to the fact that the cyto-

plasmic elements in the grasshopper seem to be most difficult to preserve and stain satisfactorily. My observations thus far are based on two species, *Rhomaleum micropterum* Beauv., and *Dissosteira carolina* Linn.

The Golgi apparatus in the grasshoppers is present in the primary spermatocytes in the form of many scattered Golgi bodies not essentially unlike those which I have described in the Hemiptera. The division stages have not yet been worked out, but at the close of the second maturation division the Golgi elements can be demonstrated clearly, being scattered about in the cytoplasm around the nucleus and mitochondria (fig. 20). The latter consist of a group of rather imperfect threads which have been derived from the second maturation division in a manner somewhat similar to that noted in Hemiptera. These threads soon condense to form the typical spherical nebenkern of insects.

The separate Golgi bodies are not easily demonstrated with clearness, but it is certain, especially from later stages, that each one is made up of two substances, one of which is more readily stained than the other which it partially encloses. (Compare with the Hemiptera which I have described fully (Bowen, '22 a).) In one respect, however, the Golgi bodies differ in behavior very decidedly from other cases which have been described. They remain separate for the most part as distinct bodies (figs. 23 and 24), and never fuse to form the massive acroblast so characteristic of the animal spermatid. Occasionally, it would appear, two or three fuse together to form a larger aggregate, but in general this does not occur. These Golgi bodies tend now to collect near the nuclear membrane, particularly on one side of the nebenkern (figs. 21 and 23), and they remain, generally speaking, in this vicinity until the nebenkern begins to elongate (fig. 24). Then they begin to migrate back along the tail (fig. 25), and are probably cast out in the protoplasmic mass sloughed off the tail at the close of sperm formation.

In the later spermatid stages and especially just prior to the backward migration of the Golgi bodies, an intensely staining globule can be made out in contact with the nuclear membrane not far from the centriole (figs. 23 and 24). This is at first difficult to

distinguish with certainty, but it seems to increase in size, and when the Golgi bodies clear away from the nucleus it is a very conspicuous object. This globule is the acrosome. It now migrates around to the opposite side of the head (nucleus) (fig. 25), always retaining its close contact with the nuclear wall. As it moves it becomes differentiated into a basal, plate-like portion which rests on the nucleus, and a small knob directed toward the cell wall (fig. 25), the whole reminding one of a collar-button. As the head begins to elongate in the manner characteristic of the insect sperm, the basal portion of the acrosome becomes drawn out into two rod-like lugs which extend back over the surface of the nucleus for a short distance and apparently serve as a means of anchorage for the acrosome (fig. 26). The distal portion becomes drawn out as a little ball on the end of a short rodlet. Very frequently this can be divided into two—a duplex condition which is possibly the characteristic one, though visible only when the sperm head is favorably turned. As the head draws out, the acrosome retains, for a time, this rod-and-ball structure; its ultimate history has not been studied.

Unfortunately, the exact method by which the acrosome is produced has not been made out. The separate Golgi bodies are themselves so small that in my preparations I was not able to follow the history of each individual. It seems probable, however, that from each one is differentiated its small proportionate share, and by the deposition of many such parts the acrosome is gradually built up. Instead of the Golgi bodies' fusing to form a single acroblast from which the acrosome is differentiated in toto as in most animals, each Golgi body is an acroblast in itself and the acrosome arises as a fusion product of the portions contributed by each such 'acroblast.' This all fits in with the facts which I have made out in the Hemiptera. In the pentatomids I was able to show that occasionally the Golgi elements fail to fuse on time, and in such cases each partial acroblast produces its own acrosome of a size proportionate to the available material (Bowen, '22 a, fig. 54). Thus, what may be an occasional accident in the bug becomes in the grasshopper the customary procedure.

In addition to these observations on the acrosome, I have been able also to add some facts corroborative of the nebenkern history which I have described in the Hemiptera (Bowen, '22 b). Thus I have found that the nebenkern passes through a process of differentiation, which, in the beginning at least, is strikingly like that in *Brochymena*, and I have been unable to make out the thread-like formation figured by Giglio-Tos and Granata ('08). As in the Hemiptera, the chromophilic substance gradually condenses and eventually disappears entirely, figure 21 being a cross-section of the nebenkern just before its final disappearance. Following this, the nebenkern divides. The condensation of the chromophilic substance in the grasshopper nebenkern takes place so rapidly that the nebenkern divides some time before it begins to elongate, the two halves rounding up in a very characteristic manner (fig. 23). Another case is thus added to those already described, which show that the complete division of the nebenkern follows immediately upon the disappearance of the chromophilic substance. With respect to the condition of the chromophilic substance at the time the nebenkern begins to elongate, an interesting series is thus formed by the grasshopper, the bug, and the beetle (and possibly the butterfly).

Finally, in Cajal preparations, I was able to demonstrate in the nebenkern (as in the Hemiptera) the occurrence of a material which I have called the 'central substance.' This stuff in the grasshopper nebenkern is by no means as regular in its arrangement as in *Euschistus*, for example. Instead, it forms a very indefinite mixture of granular (and thread-like?) elements which combine to make an exceedingly complicated picture (fig. 22). When the nebenkern halves elongate this central substance is drawn out also, and seems eventually to form a delicate thread-like core for the mitochondrial tail sheaths. The vesicles which I have described on the tail sheaths in Hemiptera appear likewise in the grasshoppers, but are by no means so conspicuous. Their general history seems not unlike that in the Hemiptera.

## FAMILY TETTIGONIIDAE

In contrast to the unusual method of acrosome formation found in the grasshoppers, among many of their relatives the process would appear to be a very simple one, quite like that in the Hemiptera. Thus far I have examined carefully only *Ceuthophilus maculatus* Harris, but the condition in this form is probably similar to that in other crickets and the locustids, where the published accounts are very unsatisfactory indeed (see Bowen, '22 a, for a short résumé). In *Ceuthophilus* I have not yet obtained satisfactory Golgi preparations of the spermatocytes and earliest spermatid stages. However, during the early steps in the condensation of the chromophilic substance, the acroblast, in the form of a single compact sphere, appears in the cytoplasm in its accustomed location in the angle between nucleus and nebenkern (fig. 41), indicating an origin from the fusion of scattered Golgi elements as in the beetle and the bug.

The differentiation of the acrosome begins immediately, and a small vesicle soon appears in connection with the acroblast quite as in the Hemiptera (fig. 42). The vesicle is sometimes clear and transparent (fig. 43), but very frequently it takes the stain intensely (fig. 42), by which it is rendered more conspicuous. I have noticed a similar tendency in *Murgantia* after certain staining technique. The position of the acroblast-acrosome complex with reference to the nucleus is not constant as in the Hemiptera, and the acrosome often does not touch the nuclear wall at all.

Shortly before the nucleus begins to elongate to form the sperm head, the acrosomal vesicle becomes applied to the anterior surface of the nucleus (fig. 43), the acroblast still maintaining its original attachment to the acrosome. Presently the acrosome flattens out on the surface of the nuclear membrane (fig. 44), and its connection with the acroblast is severed (fig. 45). The latter quickly migrates to the base of the head (fig. 46) and thence moves backward along the tail exactly as in other insects, in all of which its ultimate fate is probably the same. The acrosome, already in its definitive position, rounds out to form a



knob-like apical piece (figs. 45 and 46), the further history of which has not been studied.

Aside from the mitochondria, the spermatids of *Ceuthophilus* contain two cytoplasmic components of interest. The first of these appears in the early spermatids as a small flocculent mass, more or less granular in texture and blackening with osmic acid (modified Kopsch method) (fig. 41). Later this mass seems to move backward along the tail (fig. 42), but it no longer impregnates clearly and cannot be followed satisfactorily. It seems to correspond to the granular mass of similar behavior which I have described in the Hemiptera under the name of spermatid remnant (Bowen, '22 a). In *Ceuthophilus*, however, this material seems to be traceable at least back to the maturation divisions, and a more complete study of its history and significance is now being attempted.

The other cytoplasmic component referred to above is a body apparently homologous with the 'formation juxta-nucléaire' described in *Grylotalpa* by Voinov ('16). According to this author, the late primary spermatocytes contain four of these bodies which are distributed to the spermatids by a regular division process, each spermatid always receiving one juxtannuclear body. The last contention seems to hold true in *Ceuthophilus*, but I have been unable thus far to substantiate the earlier history as outlined by Voinov, and in my preparations this body first becomes clearly demonstrable in the differentiating spermatids. In shape, Voinov describes it as a flattened, oval body the periphery of which stains very darkly. In *Ceuthophilus* also it has the same disc-like shape, but whether the whole periphery stains heavily (after Benda and various Fe-hematoxylin methods) or only a portion of it, giving rise to a crescentic appearance, is not certain (figs. 43 and 45). It is clear from my figures, and in agreement with Voinov, that the juxtannuclear body has nothing to do with the formation of the acrosome. It finally passes out along the tail together with the Golgi remnant, with which it probably shares a similar fate. As to the nature of this body, nothing definite can be stated. Its fate recalls that of the chromatoid body, but in other respects there is little ground for com-

parison. Possibly it is related to the micro-mitosome described by Gatenby ('17) in Lepidoptera. Although we lack any definite knowledge as to what this juxtannuclear body may be, attention may nevertheless be called to the fact that it seems to be in no way related to the Golgi apparatus. The suggestion of Duesberg ('20) (based on Voinov's account), that these bodies represent Golgi elements, is, therefore, almost certainly incorrect.

Finally, a word may be added concerning the mitochondria in the spermatid, especially in view of the wide divergence of Vejdovský's account (in a related genus, *Diestrammena*) from more recent work on other insects. In the first place, the development of the vacuoles on the periphery of the nebenkern (fig. 41), as described by Vejdovský, is obviously the first step in the condensation of the chromophilic substance as already noted in the Hemiptera, etc. (Bowen, '22). The chromophilic substance gradually condenses (fig. 42), though always in a somewhat irregular way, and eventually disappears during the early stages in the elongation of the nebenkern. The exact nature of the 'pattern' which it forms is not clear in my preparations.

The central substance, correctly described by Vejdovský in the intermediate stages (see his figures 182, 183, and 184), seems not to arise from the chromophilic material as he believed, but to be differentiated *de novo* in the unstained area of the nebenkern, as I have described in the pentatomids and the grasshopper.

The formation of vesicles on the mitochondrial tail sheaths is very striking in *Ceuthophilus*, and due to the general failure of the chromophobic substance to stain, it is not easy to follow their differentiation. Hence the error of Vejdovský in describing them as fatty degeneration products of the central substance, and I can find no support whatever for his assertion that the mitochondrial structures are entirely lost from the sperm. The vesicles in *Ceuthophilus* are, it is true, very confusing if one has not become familiar with them in more typical cases, for the intervening portions of the sheaths are often not demonstrated (fig. 43), and one would be readily misled as to the actual meaning of the vesicles themselves. Somewhat later stages leave little doubt that the sheaths actually spin out along the tail filament

as in Hemiptera (Bowen, '22 a, fig. 132). During the early development of the tail vesicles the central substance can be demonstrated with Fe-hematoxylin and it can be seen to retain its threadlike distribution even in the region of the vesicles. It would accordingly seem that the central substance cannot play the rôle in the development of the vesicles which the conditions in Hemiptera led me to suggest as a possibility (Bowen, '22 b).

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## EXPLANATION OF PLATES

All of the figures have been outlined as far as possible with the camera lucida, those of plate 1 at an initial enlargement of approximately 2875 diameters, those of plate 2 at approximately 3800 diameters. At so great enlargements it has, of course, been necessary to correct the outlines extensively and to add much of the finer detail free hand. In reproducing, the figures have been reduced uniformly, those of plate 1 to an enlargement of 2300 diameters, those of plate 2 to 3350 diameters. In every case the method employed in the preparation of the original object has been indicated.

### ABBREVIATIONS

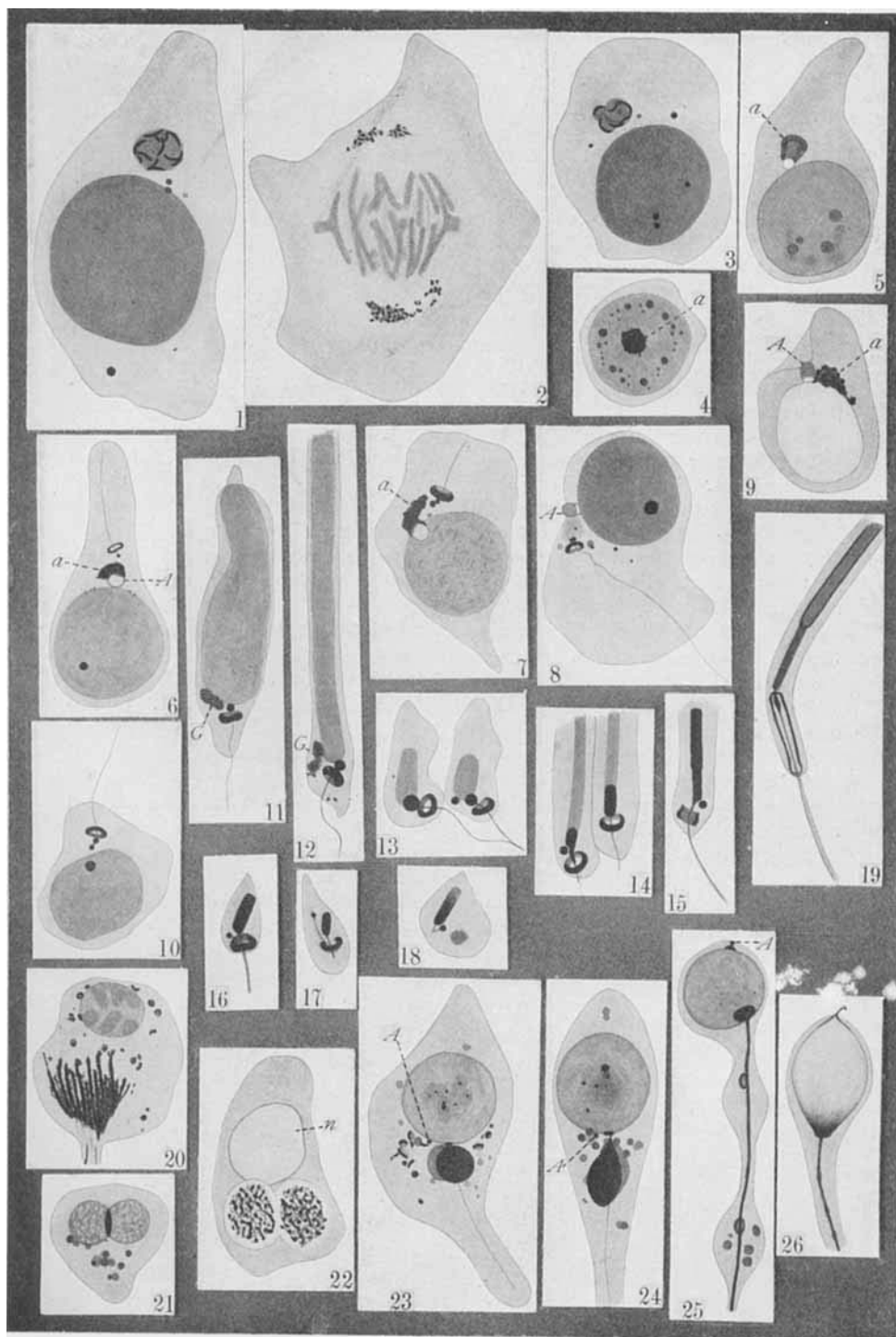
<i>A</i> , acrosome	<i>j</i> , juxtannuclear body
<i>a</i> , acroblast	<i>n</i> , nucleus
<i>G</i> , Golgi remnant	<i>s</i> , spermatid remnant

## PLATE 1

### EXPLANATION OF FIGURES

Figures 1 to 19 are from *Plethodon cinereus*; figures 20 and 22 are from *Disosteira carolina*; the others are from *Rhomaleum micropterum*.

- 1 Late primary spermatocyte. (Flemming without acetic-hematoxylin.)
- 2 Metaphase first spermatocyte division. (Cajal's formol-uranium nitrate.)
- 3 Secondary spermatocyte. (Flemming without acetic-hematoxylin.)
- 4 Spermatid nucleus showing group of granules encircling the acroblast. (Cajal-gold chloride-hematoxylin.)
- 5 Spermatid. Early formation of acrosome from acroblast. (Modified Kopsch.)
- 6 Spermatid. Later stage in formation of acrosome. (Cajal-gold chloride-hematoxylin.)
- 7 Spermatid. Final stage in formation of acrosome. (Cajal-gold chloride-hematoxylin.)
- 8 Spermatid. Fixation of acrosome. (Champy-hematoxylin.)
- 9 Spermatid. Casting off of the acroblast (Golgi remnant). (Cajal.)
- 10 Spermatid. Showing proximal (?) centriole divided into two equal parts. (Champy-hematoxylin.)
- 11 and 12 Spermatids. Progressive stages in the elongation of the nucleus. Figure 12 shows only a portion of the nucleus. (Cajal-gold chloride-hematoxylin.)
- 13 and 14 Basal end of two adjacent spermatid heads in successive stages of differentiation, to show separation and fusion of the components of the proximal (?) centriole. (Champy-hematoxylin.)
- 15 Basal end of spermatid nucleus, to show continued separation of the components of the proximal (?) centriole. (Champy-hematoxylin.)
- 16 to 18 Oblique sections through the basal end of the spermatid head, to show possible origin of the membrane filament. (Champy-hematoxylin.)
- 19 Late stage in the differentiation of the spermatid. Elongation of the ring centriole. (Champy-hematoxylin.)
- 20 Second maturation division. Late telophase. (Cajal.)
- 21 Cross-section through the nebenkern just prior to the complete disappearance of the chromophilic substance. (Flemming without acetic-hematoxylin.)
- 22 Spermatid. Development of the central substance in the nebenkern. (Cajal.)
- 23 and 24 Spermatids. Stages in the development of the acrosome. (Flemming without acetic-hematoxylin.)
- 25 and 26 Spermatids. Later stages in the transformation of the acrosome. (Flemming without acetic-hematoxylin.)



## PLATE 2

### EXPLANATION OF FIGURES

Figures 27 to 40 are from *Lixus concavus*; figures 41 to 46 are from *Ceuthophilus maculatus*.

27 Spermatocyte (or late spermatogonium). (Modified Kopsch.)

28 to 30 Spermatids. Successive stages in the fusion of Golgi bodies to form the acroblast. (Modified Kopsch.)

31 Spermatid. (Modified Kopsch.)

32 Cross-section of slightly elongated nebenkern. (Benda.)

33 Cross-sections of the nebenkern at approximately the stage of figure 34. *A*, through the chromophilic substance; *B*, above (or below) the chromophilic substance. (Benda.)

34 and 35 Spermatids, just prior to, and at the time of, disappearance of the chromophilic substance. (Flemming-hematoxylin.)

36 to 38 Late spermatids. Casting off of the acroblast. (Benda.)

39 Late spermatid. Golgi remnant passing backward along the tail. (Modified Kopsch.)

40 Spermatid, showing acrosome in final position. (Benda.)

41 Early spermatid. (Modified Kopsch.)

42 and 43 Spermatids. Development of the acrosome. (Flemming without acetic-hematoxylin.)

44 Spermatid. Fixation of the acrosome. (Benda.)

45 Spermatid. Casting off of the acroblast. (Benda.)

46 Spermatid. Backward migration of the Golgi remnant and the juxta-nuclear body. (Flemming without acetic-hematoxylin.)

