STUDIES OF SPERMATOGENESIS IN THE HEPATICAE

II. Blepharoplast Structure in the Spermatid of *Marchantia*

ZANE B. CAROTHERS and GERALD L. KREITNER

From the Department of Botany, University of Illinois, Urbana, Illinois 61801

ABSTRACT

The blepharoplast in a young, developing spermatid of *Marchantia polymorpha*, is a composite structure consisting of two basal bodies and a subjacent narrow band of axonemal-type tubules that we have termed the "spline." For most of its length, the spline consists of six long parallel tubules that nearly encircle the cell. The spline anterior is asymmetrically widened for about 2 μ by shorter tubules of the same kind. The lateral displacement of three long, adjacent marginal tubules by three short intervening tubules at the spline tip produces a long narrow aperture. Distally, the aperture is closed by the convergence of the displaced tubules with another trio of long tubules. Together, these form the six-membered cell-encircling portion. The expanded spline anterior has, at this stage of development, the four-layered (*Vierergruppe*) structure, of which the aforementioned tubules constitute the uppermost layer. The lower three strata consist of diagonal fins, elongated chambers, and fine tubules, respectively. The two flagellar bases lie close above the spline tip—one slightly anterior to the other—and diverge unequally from the spline axis. A few triplets extend proximally from the basal bodies, but do not connect with the spline. The anterior basal body is longer than the posterior one.

INTRODUCTION

In our earlier report (2), we showed that the *Dreiergruppe* organelle first observed by Heitz (9, 10) was actually a four-layered structure of unsuspected complexity. At that time, our work had progressed far enough to permit only a tentative statement regarding gross morphology of this cytoplasmic structure which we called a *Vierergruppe*. We suggested that it bore a superficial resemblance to a curved sewing needle, the eye-containing portion of which was expanded for a short distance. Subsequent comparison of our findings with those of earlier studies based on light microscopy has shown that this stratified organelle corresponds in surface view to the blepharoplast and that it is, in fact, the same or part of the same structure. In many of the previous investigations dealing with spermatogenesis in bryophytes—see for example the literature reviews of Wilson (28), Sharp (20), Lepper (12), and Vazart (25)—the blepharoplast received rather special emphasis, a fact which is partially attributable to the investigators' interest in determining whether blepharoplast and centrosome were homologous structures. While considerable controversy surrounded the ontogenetic and phylogenetic origin and affinities of the blepharoplast, there seems to have been substantial agreement that, in the spermatids of many embryophytes, it first appeared as a small,

dark-staining granular body which developed into an elongated, cilia-bearing band or thread; see Wilson (27), pp. 389–390. This development has been illustrated and described for *Marchantia* (cf. Figs. 26–33 of Ikeno, 11, Figs. 38–43 of Woodburn, 29, and Plate 5 of Gavaudan, 6) and other liverworts too numerous to mention here. Understandably, the early reports gave little information on the structure and relationships of blepharoplast components. Nevertheless, it is clear that the *Vierergruppe*, and particularly its uppermost tubular layer, corresponds to the aforementioned cilia-bearing band. Our attention was directed to a reconsideration of organelle terminology both by correlated light and electron microscope data and by the recent discovery in this laboratory that the *Vierergruppe* structure itself is transitory. At its morphological maturity, the spermatozoid of *Marchantia* was found to lack all or nearly all of the underlying three strata, although the upper layer of tubules persists. It seemed, therefore, that a less specific term—one unaffected by this transitory condition—would be preferable to "*Vierergruppe*." A survey of the early literature shows that the elongated subtending portion of the blepharoplast was frequently described as bandlike (21, 26) or cordlike (1, 3, 4); it was also variously compared to a ribbon, rod, or thread. More recently, investigators using electron microscope techniques have referred to it as a filamentous appendage (17), fibrous band (13), and as a *Vierergruppe* appendage (2). In light of present knowledge, we consider "appendage" to be incorrect and misleading and will discontinue its use in this context. Our information indicates that the term "band" has been in use the longest and, unlike the other terms, has had its appropriateness confirmed at the ultrastructural level. The major weakness of this term lies in the need to increase its specificity by adding modifiers such as blepharoplastic (30), ciliferous (26), or fibrous (13). We believe that a better designation is the word "spline," a noun which suggests a long, thin, flexible or inflexible strip of unspecified composition (24). Insofar as we are aware, this term has not been previously used in a biological sense and could be applied without ambiguity to this particular structure.

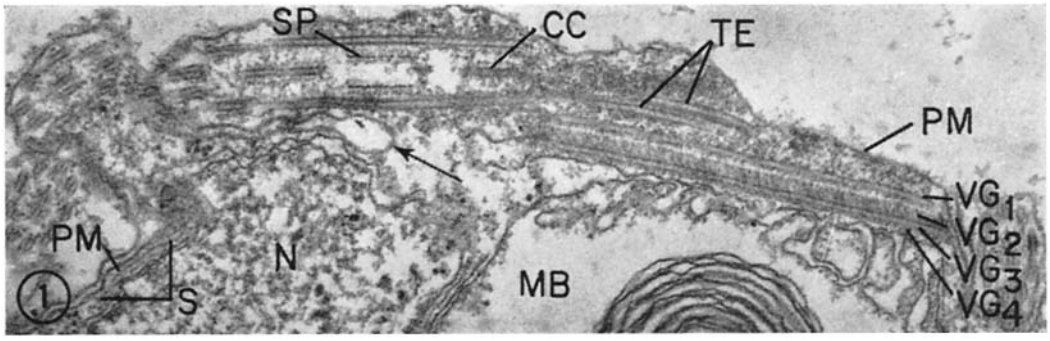
In the present work, we will use the term blepharoplast in its prior meaning to include the two basal bodies and the subtending, bandlike structure. For the latter, we have adopted the word spline. The term *Vierergruppe* will be used only as a topographic designation to refer to the four-layered anterior region of the spline as it appears in the early stages of spermatozoid development.

MATERIALS AND METHODS

The materials and methods used in this investigation are virtually the same as those described in our earlier paper (2), except that we have frequently found it necessary to base dimensions on fewer individual measurements. In order to achieve consistency in illustrations showing rotational direction and left- or right-handedness, we have inverted some of the transverse and tangential micrographs. Thus all transverse-section micrographs are arranged to correspond to Gibbons and Grimstone's "clockwise" configuration seen when one is looking along the flagellar base from the proximal end (7). While we have not yet conclusively shown the clockwise condition to be characteristic for *Marchantia polymorpha*, we assume here that it is. Throughout the paper, all mention of "right" or "left" will refer to that side of the picture as normally viewed by the reader, rather than intrinsically to the organelle under discussion.

OBSERVATIONS

The spline found in a young spermatid of *Marchantia polymorpha* is composed largely of a few long tubules which lie parallel and close to each other to form a narrow band that nearly encircles the cell. This cytoplasmic structure occupies a superficial position immediately beneath the plasma membrane. Our material shows that at this stage of development the spline is usually six tubules wide for most of its length. With each tubule about 265 A in outside diameter and adjacent tubules nearly 55 A apart, this region of the spline is calculated to be about 1865 A wide. As viewed in longitudinal section, the anterior or proximal end of the spline is seen to have the complex, four-layered, or *Vierergruppe* structure and to be subtended by a conspicuous mitochondrial body (Fig. 1). The long, encircling tubules are continuous with the VG_1 or uppermost *Vierergruppe* layer. Viewed tangentially, the spline anterior is nearly spatulate in outline, the widened portion extending longitudinally for almost 2 μ. Most of this increased breadth is brought about through the addition of a series of eight successively longer tubules on the left side of the long tubular band. To a lesser extent, it is widened by the intercalation of three short tubules near the middle of the band at the spline tip (Figs. 6 and 8). Thus, the spline anterior has a somewhat asymmetric outline and an elongated, tapered aperture



Key to Symbols

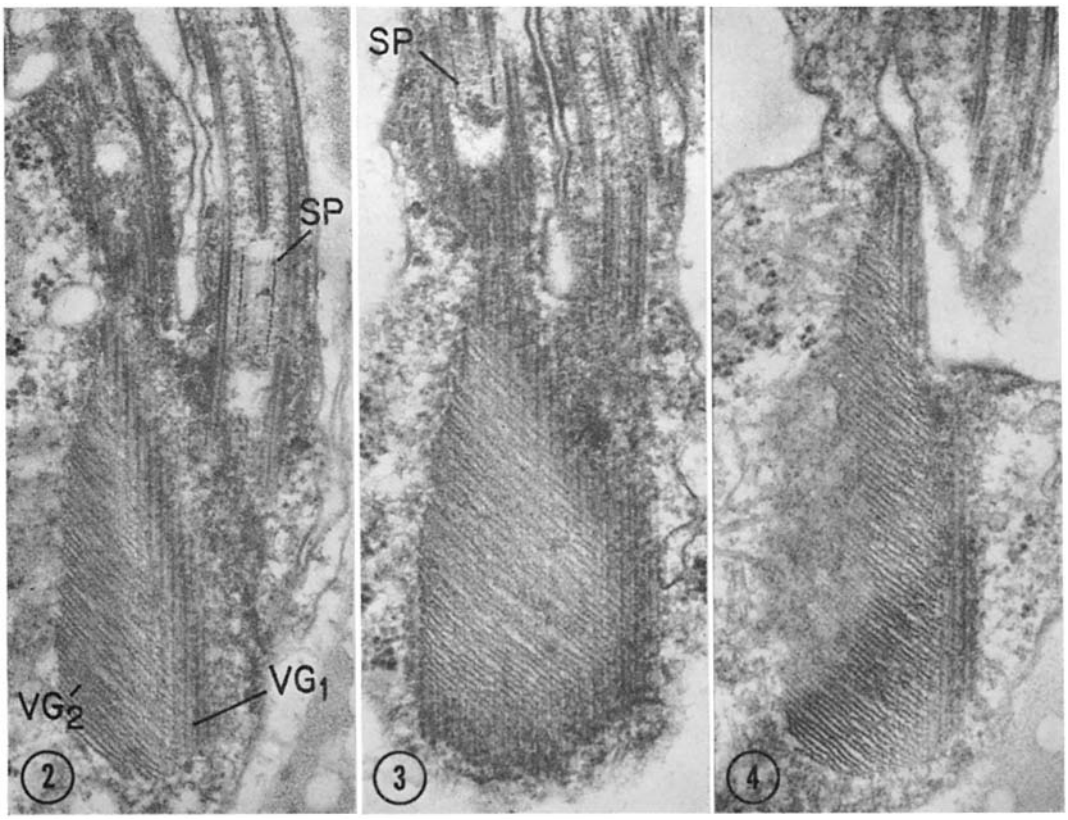
ABB, anterior basal body
AF, anterior flagellum
CC, cylindrical core
MB, mitochondrial body
N, nucleus
PBB, posterior basal body
PF, posterior flagellum
PM, plasma membrane
S, spline
SA, spline aperture
SH, shaft
SP, stellate pattern
TE, triplet extension
TZ, transition zone
VG, *Vierergruppe* (subscript indicates layer number)

FIGURE 1 Longitudinal section showing near-median view of flagellar base at upper left and the obliquely sectioned *Vierergruppe* region and subjacent mitochondrial body at right. Triplet extensions lie nearly parallel to and close above the VG_1 layer. Part of the spline may be seen in oblique section extending back over the nucleus at lower left. Arrow indicates point at which flagellum projects above the cell surface. × 44,000.

formed by the separation of the single band of six long tubules into two three-tubule branches (Figs. 5 and 6). This slitlike aperture in the VG_1 layer is roughly 1.5 μ long. As may readily be seen in transverse section, the spline anterior at its widest region comprises 17 parallel tubules (Figs. 10 and 11). Confirmation of this fact is obtained with difficulty from tangential sections, however, because the spline curves in correspondence with the cell surface. All 17 tubules may be seen in Fig. 3 if the micrograph is viewed from a low angle parallel to its long axis. The seemingly marginal position of the VG_1 tubules reflects the organelle's curvature, the central area belonging to the underlying stratum of diagonal fins (the VG_2 layer). With 17 tubules and 16 spaces, the spline's maximum width is about 5400 A, or about 0.5 μ.

In the *Vierergruppe* region of the spline, there is a relatively close correspondence between the tangential outline of the uppermost or VG_1 tubules and that of the underlying three layers. Superimposed above one another, the lower three strata bear some resemblance to a thickened comma that has been inverted by a 180° rotation. Looking distally from the spline tip and beginning at the most distal point of the VG_2 layer, we can follow its outline as the left-hand margin curves gently downward on a radius of about 3 μ (Fig. 2). This boundary tends to straighten at some nearly halfway point and then descends to the spline apex where the margin recurves on a radius of 3000 A (Fig. 4). The right-hand margin now ascends in a straight line for slightly more than a micron and then it recurves sharply to form a shallow, offset lobe (Fig. 6). Again it recurves sharply, having, in effect, indented the upper portion of the right-hand margin which runs nearly straight up to the distal extremity of the VG_2 layer (cf. the diagonally striated VG_2 in Figs. 2–6 with the composite representation shown in Fig. 8). Over-all, the *Vierergruppe* region is about 1.75–2.0 μ long. The underlying three VG layers are only slightly narrower than the superposed VG_1 layer (e.g., Fig. 10).

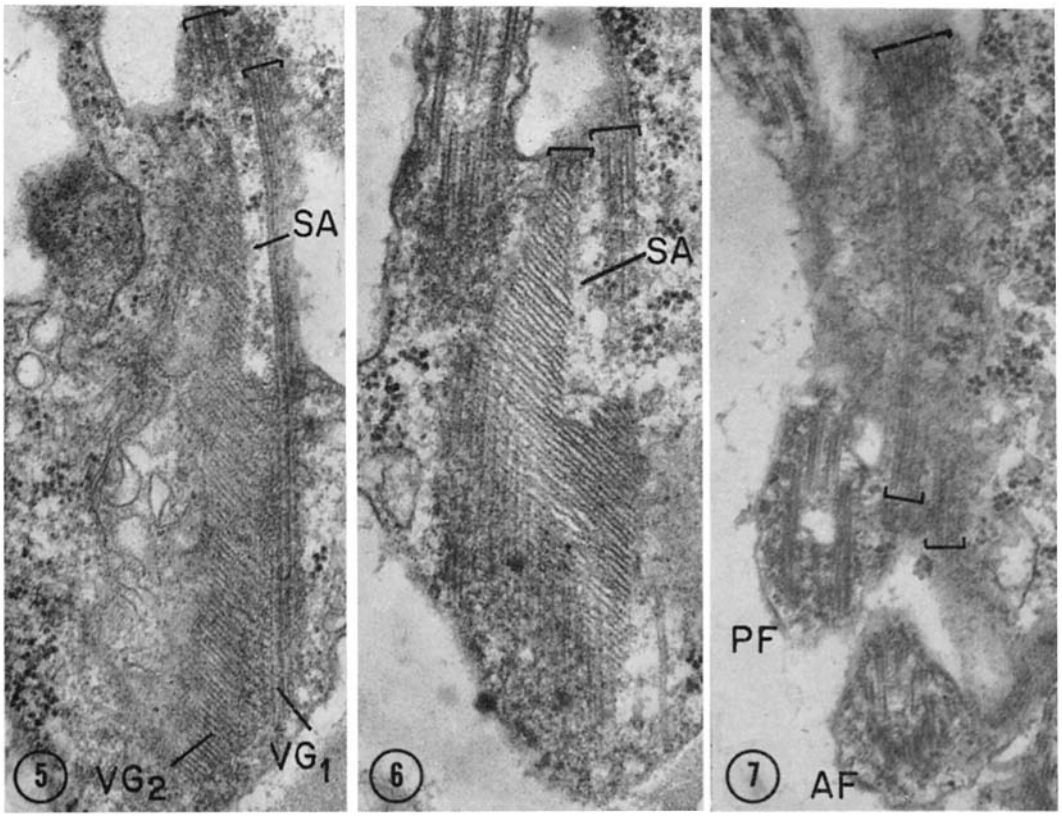
The structural relationship between the spline's aperture in the VG_1 layer and the underlying strata was determined by analysis of both tangential and transverse sections. This information



FIGURES 2–4 Slightly inclined tangential sections at successively lower levels showing the curved spline anterior at bottom and some of its component layers. The anterior flagellum is shown at upper right; in Figs. 2 and 3, the posterior flagellum may be seen at upper left. Fig. 4 shows the offset distal prolongation of the *Vierergruppe* region. × 51,000; 59,500; and 51,000, respectively.

has been graphically summarized in the composite line drawing shown in Fig. 8. Numbering the VG_1 tubules from left to right, we see that the first eight constitute a series of successively longer tubules. These account for approximately half the width of the organelle's spatulate anterior. The next three tubules (Nos. 9–11) are very long and make up the left half of the tubular band which encircles the cell. Of these, No. 9 is the median tubule in the *Vierergruppe* region, while No. 11 is the one lying superposed over the indented distal portion of the right-hand VG_2 margin. Tubules 12–14 are intercalated between the two groups of long tubules and thus form an aperture having a maximum width of about 1000 A. Transverse sections show that the three intercalated tubules are only about half as long as the *Vierergruppe* region; therefore, the VG_2 fins are uncovered by VG_1 tubules for a distance of about 3400 A (see also Figs. 12–14). The last three tubules (Nos. 15–17) are also very long and collectively represent the right half of the long, cell-encircling portion of the spline. These pass directly over the shallow lobe on the right-hand margin and then gradually converge with the trio comprising the left group at a point about 2 μ behind the apex (see also Figs. 5–7). They then continue around the cell as a single group of six parallel tubules (Fig. 20).

The two flagella emerge from the cell surface at a very low angle (Fig. 1), and extend side by side partway around the developing spermatid. At their proximal ends, the flagella lie appressed to the widened anterior of the subjacent spline. A reconstruction from tangential and transverse



FIGURES 5 and 6 Tangential sections at successively lower levels having the same orientation shown in Figs. 2–4. Brackets indicate two groups of three tubules each extending posteriorly and converging to form the spline aperture. Fig. 6 shows clearly the shallow lobe and offset distal margin of the *Vierergruppe* region. × 45,000 and 51,000, respectively.

FIGURE 7 Tangential section showing area just distal to *Vierergruppe* region in which the two groups of tubules converge to form the long, six-membered portion of the spline. × 46,000.

sections shows that the basal extremity of the right flagellum is closer to the spline apex than that of the left flagellum (Figs. 2, 9). The same reconstruction also shows that the flagellar bases extend posteriorly from the spline anterior at unequal divergent angles. Our measurements indicate that the anterior flagellar base diverges about 8° right from the spline axis, while the posterior base is directed about 3° to the left of the axis. The anterior base lies partially superposed above the aperture and overlaps the right side of the widened spline tip by nearly a micron. The posterior base overlaps the left side for about 0 5 μ.

Both flagella have similar axial structure except for a few minor differences in their proximal extremities. In each flagellum may be recognized the three topographic regions delineated by Sleigh (23), viz., basal body, transition zone, and shaft. The basal body has the usual outline of a partially fluted cylinder formed of nine peripheral triplets. At the distal end of the basal body the plane of each triplet lies tangential to the cylindrical surface (Fig. 13). Proximally, each plane is rotated up to 45° on its long axis (Fig. 12) and corresponds to the clockwise orientation of triplets described by Gibbons and Grimstone (7). Some triplet strands are longer than others and extend asymmetrically beyond the cylindrical region toward the spline apex (Fig. 1). Figs. 10 and 11 show a single triplet of the anterior basal body (ABB) lying perpendicu-

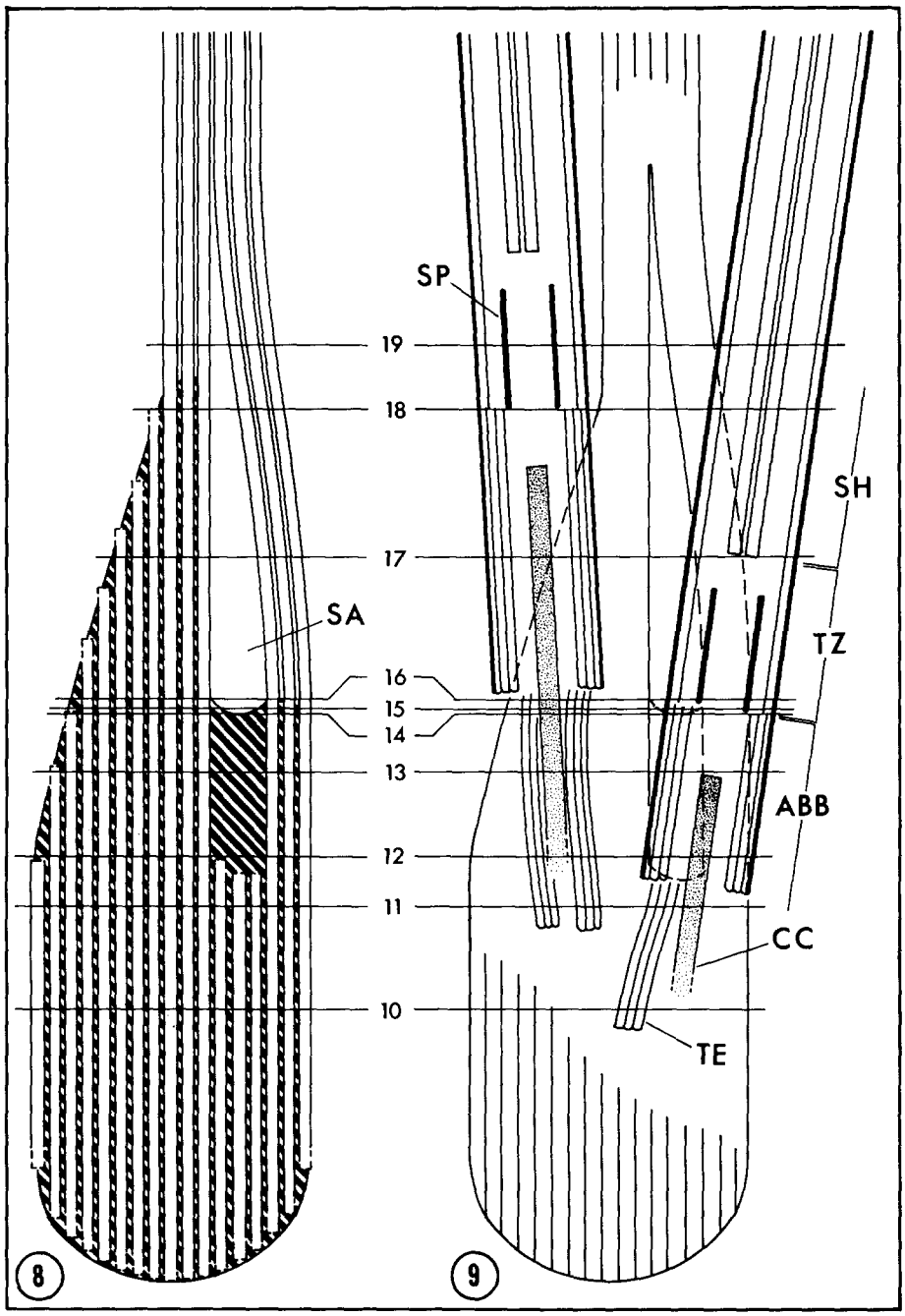


FIGURE 8 A diagrammatic, composite representation of the spline anterior as seen in tangential section at the level of the upper two layers. The outline of the lower *Vierergruppe* strata follows that of the diagonally striped VG_2 layer. The numbers in a vertical row refer to figures of corresponding transverse sections. × 69,000.

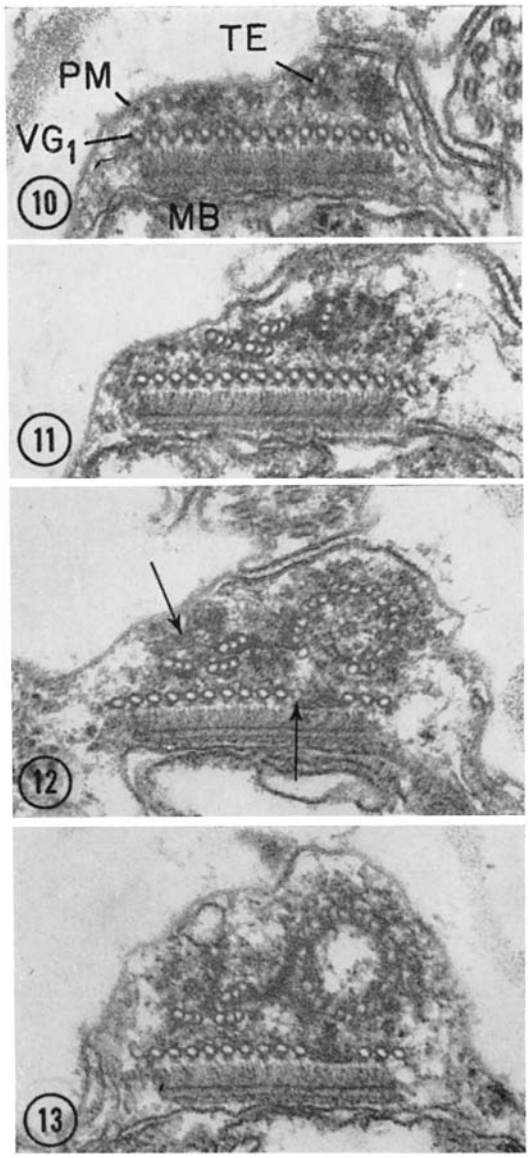
FIGURE 9 A composite representation similar to that shown in Fig. 8, but at a slightly higher level to show the relationship between the flagellar bases and the spline anterior. The radial links between cylindrical cores and basal body triplets have been omitted, as have the plasma membranes and cytoplasm external to the axonemes. × 69,000.

lar to the plane of the spline. This triplet appears to be about twice as long as the other eight. (In order to facilitate its identification, the same strand is represented in Fig. 9 as if it lay parallel to the spline.) A comparison of Figs. 11 and 12 shows that, as counted in a counterclockwise direction, the next two strands lying beside the single extended triplet are shorter than all the others. This condition contrasts with that in the posterior basal body (PBB) where the longest strands are three triplet extensions associated with the lower half of the nine-membered ring (Figs. 11–16). These three are approximately twice as long as their six neighbors. The tubules that compose the triplet strands lie closely parallel to, but are not confluent with those of the spline. Fig. 9 shows diagrammatically that the two basal bodies are of different lengths. Excluding the triplet extensions, we calculate ABB to be about 3500 A long and PBB to be about 5500 A. Each basal body is 2200 A in diameter. When seen in transverse section, the basal bodies have, for part of their lengths, the familiar cartwheel configuration, including a cylindrical core or hub and fine, spokelike radial filaments. The exact linear extent of the cylindrical core is difficult to determine. Figs. 1–3 and 13 indicate that at least part of the distal portion of the basal body has a relatively structureless interior. This region is 1250–1650 A long. Proximally, the core projects beyond the ring of nine triplets and continues in association with the triplet extensions (Figs. 9, 11–16). We estimate the ABB cylindrical core to be about 0.4 μ and the PBB core about 0.8 μ in over-all length. Transverse sections suggest that the cylindrical core has a structured interior (e.g., Figs. 13 and 16); however, our material has not yet yielded sufficient detail to permit its elucidation. Another questionable detail concerns the occasional appearance of an indistinct dark mark located on the axis at the extreme distal end of the basal body (Figs. 1, 3, 14). While this feature does not appear to represent a discrete segment of the cylindrical core, we are presently unable to identify it.

The proximal limit of the transition zone, sensu Gibbons and Grimstone (7) and others, coincides with the distal limit of the basal body, i.e., where triplet structure ends and doublet structure begins. Its distal boundary corresponds to that transectional level in which the familiar 9 + 2 axonemal configuration begins. Within this zone in *Marchantia* flagella lie the stellate pattern of interconnections among the nine peripheral doublets and, more distally, another region with an apparently structureless interior. The stellate pattern extends longitudinally for about 2300 A and, in transverse section, may be seen to contact each of the nine doublets (Fig. 16). The proximal limit of the stellate pattern coincides rather precisely with the transition from triplets to doublets, so that a slightly oblique transection of that level shows the stellate pattern associated with doublets and triplets, as illustrated in Figs. 15 and 18. It, therefore, serves as an excellent reference point for orientation. Figs. 14–16 show three closely spaced sections illustrating, respectively, triplet structure associated with the extreme upper edge of the stellate pattern, the stellate pattern associated with both triplets and doublets, and, finally, the stellate pattern-doublet configuration characteristic of most of the transition zone. The fluted interior boundary of the stellate figure is approximately 950 A in diameter and appears "empty" (Figs. 1, 2, 16, 19). In our material, the transition zone is about 3000 A long, including the distal region of about 800 A that separates the stellate figure from the 9 + 2 pattern. The outside diameter of the cylinder formed by the peripheral doublets in the transition zone, like that in the basal body and in the distal shaft, is 2200 A. The flagellar shaft extends for an undetermined distance. Its nine doublets lack the arms frequently encountered in flagella of other organisms (Figs. 17, 20). No attempt has been made in this portion of the investigation to elucidate the details of structure in the axonemal matrix or in the shaft's distal tip. A three-dimensional representation depicting the salient structural interrelationships of spline and basal bodies is shown in Fig. 21. The dotted lines in this figure indicate flagellar segments from which peripheral doublets or triplets have been omitted in order that certain details could be made more evident.

DISCUSSION

The earliest report of individual spline tubules occurs in a 1952 paper by Manton and Clarke (15) who described, from electron micrographs of *Sphagnum* spermatozoids, a "curious little appendage of five fine fibrils" that extended distally beyond the cell body. However, working with intact or nearly intact wholemounts, these investigators were unable to determine the structural nature or functional significance of the fibrils.



FIGURES 10–16 Transverse sections corresponding to the reference numbers shown between Figs. 8 and 9. The viewer is looking distally from the spline apex. In each micrograph, the anterior flagellum is on the right, the posterior on the left.

FIGURE 10 A single triplet of ABB is nearly perpendicular to the underlying spline which here is 17 tubules wide. × 75,500.

FIGURE 11 Three triplet extensions of PBB lie nearly parallel to the spline surface. × 72,500.

FIGURE 12 The VG_1 consists of 10 tubules on the left and three on the right, the two groups separated by a space equivalent to the three short intercalated tubules. The lower arrow points to the extreme proximal tip of one of the three intercalary tubules. The ABB cartwheel lacks two lower triplets at this level. A cylindrical core indicated by the upper arrow is associated with the three PBB triplets. × 70,500.

FIGURE 13 The VG_1 consists of 10 + 3 tubules. ABB shows a circle of nine triplets around a seemingly structureless interior. × 60,000.

Satô (17) was first to recognize and report the tapelike fibrillar form of the spline which he termed a "filamentous appendage." He used wholemount preparations of mature *Conocephalum conicum* spermatozoids which had been pretreated with hot water, a procedure which dispersed the nuclear contents and revealed the tapelike structure extending lengthwise along one side of the spermatozoid body. He also used ultrasonic pretreatment and was able to show the 9–10 fibrils, each about 20 mμ in diameter, which comprised the band. In a later paper, Satô (19) confirmed the presence of 10 constituent fibrils in this species. Manton (13), in a second, more detailed study, using thin sections to analyze *Sphagnum* spermatozoids, also demonstrated the ribbon-like nature of the spline and its proximity to the elongated nucleus. Discontinuing the use of "appendage," she referred to the spline as a fibrous band and as a fibrous nuclear band. It is noteworthy that Manton did not adopt Satô's terminology. She commented that in *Sphagnum* at least, the ribbon-shape somewhat invalidates his description as "filamentous," a point which is equally applicable to *Marchantia*.

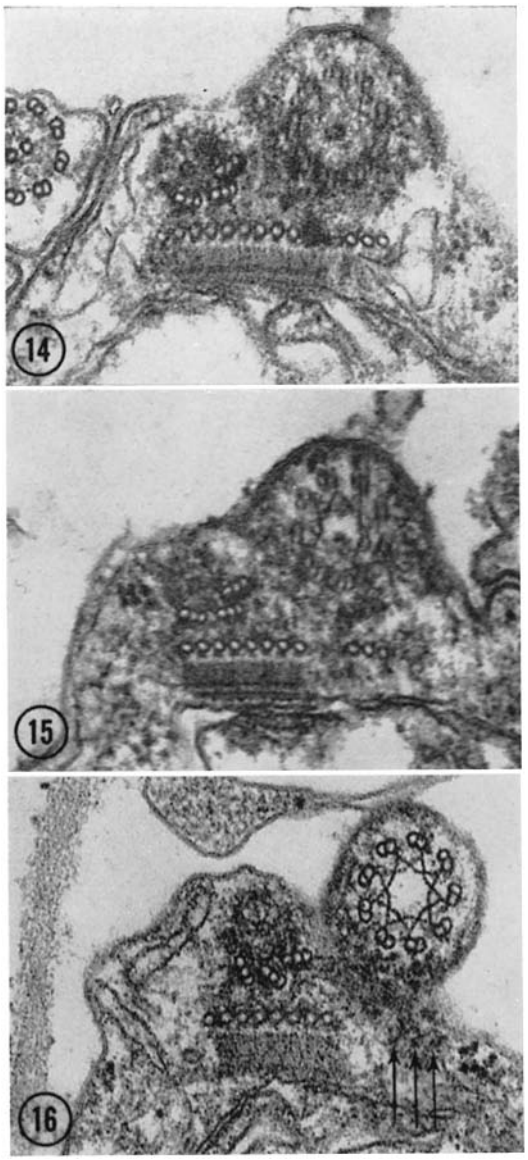


FIGURE 14 The VG_1 layer is here interpreted to consist of nine plus three tubules. The lower VG layers are nearly gone from beneath the three tubules on the right. The anterior flagellum is shown at the demarcation between basal body and transition zone. Triplet structure is evident, and the stellate pattern is just discernible. × 71,000.

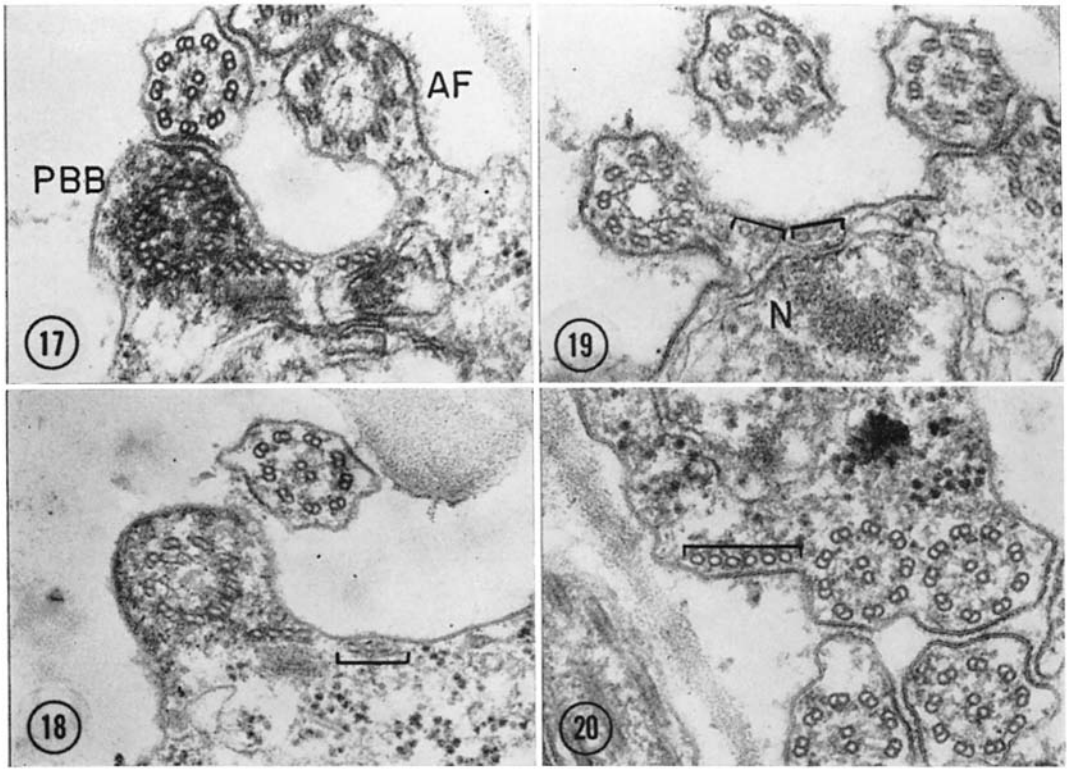
FIGURE 15 The VG_1 layer is here reduced in width to eight plus three tubules. Four lower triplets still persist in the anterior flagellum; the other five are now doublets. × 77,000.

FIGURE 16 The VG_1 layer still consists of eight plus three tubules, the latter indicated by arrows. Above the three tubules lies the stellate pattern of the anterior flagellum transition zone. × 75,000.

In her analysis of thin sections, Manton also showed that the spline begins to widen at the anterior end of the cell near the posterior basal body. That finding is similar to the condition shown for *Marchantia* in the present investigation.

Little is presently known regarding intra- and interspecific variation in width of the elongated medial region of the spline, and in the number of parallel tubules comprising this region. Satô (18) analyzed nine species representing eight genera and three orders and found the width to range from 0.08 to 1.2 μ. The high value of 1.2 μ occurred in *Makinoa crispata*, a plant that produces the largest spermatozoids known among the liverworts. His measurement for *Marchantia polymorpha* was 0.2 μ, a value that corresponds closely to that obtained by the present authors from transverse sections of a six-membered medial region of the spline. Unfortunately, Satô did not specify the number of constituent tubules in each of the species he studied. For the present then, it seems reasonable to assume that the variability in width is more closely correlated with the number of parallel, medial region tubules rather than with their diameter or center-to-center spacing.

The discovery of a spline aperture—the needle's eye in our earlier analogy (2)—has doubtless raised more questions than it has answered. At present, we know virtually nothing about the occurrence of this feature in other liverworts, or about its ontogenetic and phylogenetic development. In referring to the intercalation of the three short VG_1 tubules which in effect produces the aperture, we do not mean to imply that they were formed ontogenetically or phylogenetically later than the other tubules on either side. Spline morphology could suggest that those three tubules (Nos. 12–14) were foreshortened and that the outer three (Nos. 15–17) later converged with Nos. 9–11 to form the medial band of long tubules. It would be most interesting to know whether the sperms of other species lack the aperture altogether. In later stages of spermatozoid development, the anterior tip of the elongated nucleus lies close beneath the spline aperture, but, at present, the functional significance of this relationship, if any, remains obscure. The disposition of VG_1 tubules at the spline anterior is closely correlated with and may, perhaps, be determined by the underlying three *Vierergruppe* layers. In the earliest stages of spline development we have seen so far, the underlying three layers have always been present, although some young splines may lack the subjacent mitochondrial body. It is clear also that, to varying extent, the VG_1 tubules lengthen progressively with sper-



FIGURES 17–19 continue the sequence and have the same orientation as shown in Figs. 10–16.

FIGURE 17 The VG_1 here consists of six plus three tubules. *PBB* shows the cartwheel configuration. The anterior flagellum is shown at the demarcation between transition zone and shaft region, with the two central tubules just becoming evident. The upper two shafts shown in transection represent continuations of the two flagella around the cell. × 66,500.

FIGURE 18 The VG_1 consists of four plus three tubules, the latter indicated by a bracket. The change from basal body to transition zone is shown in the posterior flagellum. × 54,500.

FIGURE 19 The spline aperture is nearly closed as indicated by the small space separating the two groups of three tubules marked by brackets. The stellate pattern is shown in the posterior flagellum. × 59,300.

FIGURE 20 A transverse section showing the spline (bracketed) halfway around the cell from the *Vierergruppe* region. × 68,500.

matozoid maturation. Further, the splines seen in fully developed spermatozoids have lost the underlying VG layers or have had them greatly altered. It seems plausible, therefore, that some or all of the lower layers might serve as a focal point for the initiation of VG_1 tubule synthesis. On the other hand, a study of the earlier stages of spline ontogeny may show the underlying VG layers to appear after VG_1 tubules have begun their development.

Of the several functions that have been suggested for the spline, a supportive function has been mentioned most frequently in the more recent literature. Satô (18, 19), Manton (13), and Vazart (25) have called attention to the likelihood that the spline contributes substantially to the over-all shape of the spermatozoid. The organelle seems well suited to the purpose. The resistance of its component tubules to the disruptive physical and chemical treatments employed by Satô (18, 19) and the infrequent sharp bends in the tubules fol-

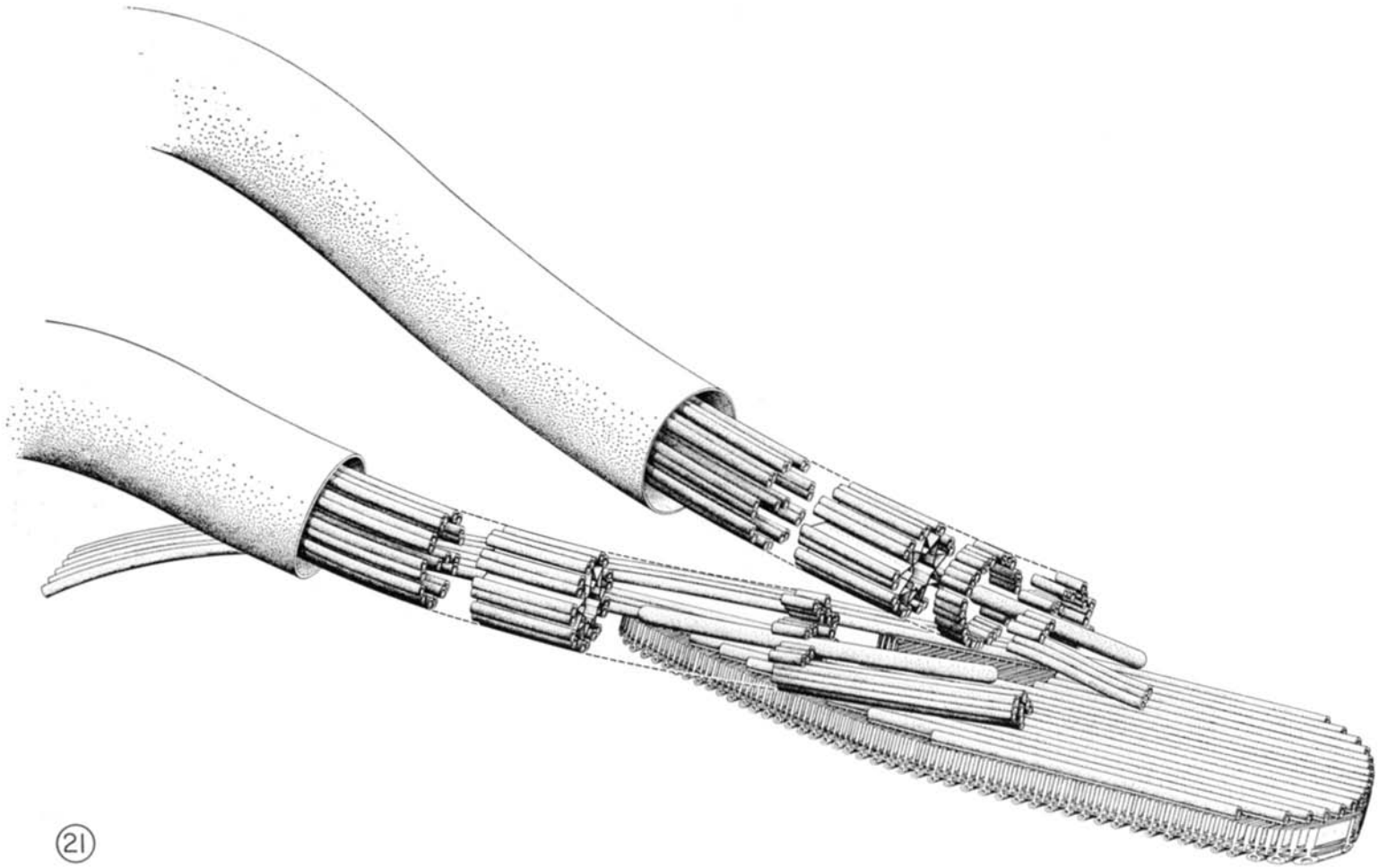


FIGURE 21 A three-dimensional, interpretive drawing showing salient structural relationships among flagellar bases and subtending spline. The dotted lines do not imply an "exploded" view; rather, the parts are shown in situ with segments of peripheral doublets or triplets removed so that underlying components might be seen. No attempt has been made to represent the radial connections between triplets and the cylindrical core or to show the internal structure of the core.

lowing these treatments indicate their considerable strength and rigidity. Another indication of innate elastic rigidity may be found in the consistently peripheral position occupied by the spline in developing spermatids, assuming, of course, that spline tubule synthesis can occur in other than just subsurface cytoplasm. Correlative evidence can be drawn from organisms other than bryophytes. In flatworm spermatozoids, for example, the cortical tubules, which are essentially identical in structure to axonemal tubules, are presumed to play a major supportive role in the maintenance of highly asymmetric cell form (22). Another supporting role has been suggested by Paolillo (16) who considers the spline to be a functional analogue of flagellar roots. Satô (19) suggested that the spline might in some way serve the movement of the spermatozoid in concert with the flagella. Although no demonstration of this function has yet been made, the suggestion warrants consideration. If spline tubules were contractile, the organelle might be capable of slight undulating or flexing motion. Presumably, the energy source for such movement would be the mitochondrial body which, in the mature *Marchantia* spermatozoid, lies appressed to the spline's under surface at its anterior end. An interesting comparison can be drawn between the spline and the contractile axostyle found in some flagellates. Grimstone and Cleveland (8) have shown that axostyles, too, consist of tubules similar to those traversing the centers of cilia and flagella. In several flagellate species, the axostyles are long and ribbon-shaped and undulate actively. At their anterior end, the axostyle tubules are joined to one or more basal bodies, while posteriorly they are attached to the plasma membrane. The morphology of the spline and its in situ relationships within the mature spermatozoid are certainly not identical with those of the axostyle, nor are we attempting to equate the two structures. Nevertheless, the axostyle does represent a possible functional analogy worth consideration. For additional examples of nonaxonemal tubules that are known or presumed to have an undulatory capacity, we refer the reader to the work of Silveira and Porter on the microtubular systems of flatworm spermatozoids (22). Given some capacity for movement, the spline could, perhaps, contribute to locomotion after spermatozoid release and to egg penetration. Diers (5) has reported that in spermatozoids of *Sphaerocarpus donnellii* both headpiece and nucleus enter the archegonium and reach the egg whereas the distal cytoplasmic portion becomes separated and lost. It seems possible that the blunt anterior edge of the spline could serve as a relatively inflexible rim that could facilitate egg penetration by the male nucleus. It is not yet certain whether the headpiece itself enters the egg just prior to fertilization. Finally, it seems possible that the spline tubules could serve as a "guide" which might determine or influence the path of elongation of the nucleus.

Flagellar structure, as revealed in this investigation, presents few differences from that described previously for many other species. Not surprisingly, in *Marchantia* the flagellar organization resembles very closely that reported for *Sphagnum* (13). We find, among their many similarities, that the flagellum, in each species, lacks a transverse plate of electron-opaque material in the vicinity of the transition zone and that, in each species, the central pair of flagellar tubules does not extend proximally as far as the stellate pattern, as has been reported for certain plant flagella (14). Noteworthy among the details of structure presented here are the unequal lengths of the two basal bodies and the apparent constancy in the asymmetric nature of the triplet extensions. Sections through blepharoplasts in many spermatids showed a single triplet extension associated with the anterior basal body and three with the posterior one. Moreover, as seen in transverse sections, their relative positions in the basal body were also constant, the three triplet extensions of PBB typically lying immediately above the VG_1 layer, and the one of ABB being usually perpendicular to the spline. To some extent, this pattern of triplet extensions appears to apply to the blepharoplast of *Polytrichum*. A comparison of our Fig. 13 with Fig. 10 in Paolillo's paper (16) reveals nearly exact transectional correspondence of basal bodies and spline. In both micrographs, three triplet extensions may be seen on the left, whereas on the right there appears to be a circle of nine triplets that has a structureless interior. Below the basal bodies lies the spline which, in each micrograph, has a gap in its uppermost layer. In *Marchantia* at least, this gap corresponds to the spline aperture. Although the number of spline tubules on each side of the gap differs—10 and 3 for *Marchantia*; seven and four for *Polytrichum*—the general organization of blepharoplasts in both species must be very similar.

The structural relationship between the spline and the pair of basal bodies is not yet fully under

stood. So far as we are aware, no study to date has shown tubular connections between VG_1 and the triplet extensions or any other part of the basal body. In 1957, Manton (13) expressed the possibility that the tubules of the flagellar base might become united with the spline, and at that time she was inclined to think that they did (p. 397). Our own investigation leads us to conclude that in *Marchantia* there is no such tubular continuity. Instead, the cytoplasmic material that lies between the flagellar bases and the subtending spline perhaps serves as a cementing matrix which stabilizes the position of the two basal bodies. More information regarding early stages in blepharoplast ontogeny might shed considerable light on the interrelationships among these organelles. Such an investigation is in progress in this laboratory.

We wish to express our thanks to Mr. Stanley L. Jones, staff illustrator in the Botany Department, for his rendition of Fig. 21.

This investigation was supported by the National Science Foundation (Grant No. GB 2813).

Received for publication 28 August 1967.

REFERENCES

1. BLACK, C. A. 1913. The morphology of *Riccia Frostii*, Aust. *Ann. Botany (London)*. **27**:511.
2. CAROTHERS, Z. B., and G. L. KREITNER. 1967. Studies of spermatogenesis in the Hepaticae I. Ultrastructure of the *Vierergruppe* in *Marchantia*. *J. Cell Biol.* **33**:43.
3. CHALAUD, G. 1930. Le cycle évolutif de *Fossombronia pusilla*. *Rev. Gen. Botan.* **42**:99; 159; 219.
4. CHALAUD, G. 1930. Les derniers stades de la spermatogénése chez les Hépatiques. *Ann. Bryol.* **3**:41.
5. DIERS, L. 1967. Der Feinbau des Spermatozoids von *Sphaerocarpus Donnellii* Aust. (Hepaticae). *Planta*. **72**:119.
6. GAVAUDAN, P. 1930. Recherches sur la cellule des Hépatiques. *Le Botaniste*. **22**:105.
7. GIBBONS, I. R., and A. V. GRIMSTONE. 1960. On flagellar structure in certain flagellates. *J. Biophys. Biochem. Cytol.* **7**:697.
8. GRIMSTONE, A. V., and L. R. CLEVELAND. 1965. The fine structure and function of the contractile axostyles of certain flagellates. *J. Cell Biol.* **24**:387.
9. HEITZ, E. 1959. Elektronenmikroskopische Untersuchungen über zwei auffallende Strukturen an der Geisselbasis der Spermatiden von *Marchantia polymorpha, Preissia quadrata, Sphaerocarpus Donnellii, Pellia Fabroniana* (Hepaticae). *Z. Naturforsch.* **14b**:399.
10. HEITZ, E. 1961. Über die Geisselstruktur sowie die Dreiergruppe in den Spermatiden der Leber-und Laubmoose. *Proc. European Regional Conf. Electron Microscopy, Delft, 1960*. **2**:934.
11. IKENO, S. 1903. Beiträge zur Kenntnis der pflanzlichen Spermatogenese: Die Spermatogenese von *Marchantia polymorpha*. *Botan. Centr.* **15**:65.
12. LEPPER, R., JR. 1956. The plant centrosome and the centrosome-blepharoplast homology. *Botan. Rev.* **22**:375.
13. MANTON, I. 1957. Observations with the electron microscope on the cell structure of the antheridium and spermatozoid of *Sphagnum*. *J. Exptl. Botany*. **8**:382.
14. MANTON, I. 1964. The possible significance of some details of flagellar bases in plants. *J. Roy. Microscop. Soc.* **82**:279.
15. MANTON, I., and B. CLARKE. 1952. An electron microscope study of the spermatozoid of *Sphagnum*. *J. Exptl. Botany* **3**:265.
16. PAOLILLO, D. J., JR. 1965. On the androcyte of *Polytrichum*, with special reference to the *Dreiergruppe* and the limosphere (Nebenkern). *Can. J. Botany*. **43**:669.
17. SATÔ, S. 1954. On the filamentous appendage, a new structure of the spermatozoid of *Conocephalum conicum* disclosed by means of the electron microscope. *J. Hattori Botan. Lab.* **12**:113.
18. SATÔ, S. 1956. The filamentous appendage in the spermatozoids of Hepaticae as revealed by the electron microscope. *Botan. Mag. (Tokyo)*. **69**:435.
19. SATÔ, S. 1958. Electron microscopical and cytochemical studies on the spermatozoid of *Conocephalum conicum*. *J. Fac. Sci. Univ. Tokyo, Sect. III.* **7**:257.
20. SHARP, L. W. 1912. Spermatogenesis in *Equisetum*. *Botan. Gaz.* **54**:89.
21. SHARP, L. W. 1914. Spermatogenesis in *Marsilea*. *Botan. Gaz.* **58**:419.
22. SILVEIRA, M., and K. R. PORTER. 1964. The spermatozoids of flatworms and their microtubular systems. *Protoplasma*. **59**:240.
23. SLEIGH, M. A. 1962. The Biology of Cilia and Flagella, The Macmillan Company, New York. 242.

24. STEIN, J., editor. 1967. Random House Dictionary of the English Language. Unabridged edition. Random House, New York. 2059.
25. VAZART, B. 1963. Différenciation des cellules sexuelles et fécondation chez les Cryptogames. *Protoplasmatologia*. **7**:273.
26. WEBBER, H. J. 1901. Spermatogenesis and fecundation in *Zamia*. *Bur. Plant Sci. Industries (USDA), Bull. No. 2*. 1.
27. WILSON, E. B. 1925. The Cell in Development and Heredity. The Macmillan Company, New York. 3rd edition. 1232.
28. WILSON, M. 1911. Spermatogenesis in the Bryophyta. *Ann. Botany (London)*. **25**:415.
29. WOODBURN, W. L. 1911. Spermatogenesis in certain Hepaticae. *Ann. Botany (London)*. **25**:299.
30. WOODBURN, W. L. 1915. Spermatogenesis in *Mnium affine* var. *ciliaris* (Grev.), C. M. *Ann. Botany (London)*. **29**:441.