COPELAND, E. B. 1947. Genera filicum. Chronica Botanica, Waltham, Massachusetts.
DIELS, L. 1899. Polypodiaceae. *In* A. Engler and K. Prantl, Die natürlichen Pflanzen Familien I. (Abt. 4): 139–339.
DIXON, W. J., AND F. J. MASSEY. 1957. Introduction to statistical analysis. McGraw-Hill, New York.
ECHOLS, R. M. 1955. Linear relation of fibrillar angle to tracheid length, and genetic control of tracheid length in slash pine. Trop. Woods 102: 11–22.
FROST, F. H. 1930a. Specialization in secondary xylem of dicotyledons. I. Origins of vessels. Bot. Gaz. 89: 67–94.
———. 1930b. Specialization in secondary xylem of dicotyledons. II. Evolution of end wall of vessel segment. Bot. Gaz. 90: 198–212.
GOULD, F. W. 1957. Pollen grain size as related to polyploidy and speciation in the *Andropogon saccharoides–A. barbinodes* complex. Brittonia 9: 71–75.
HOLTTUM, R. E. 1949. The classification of ferns. Biol. Rev. 24: 267–296.
———. 1954. Flora of Malaya. II. Ferns of Malaya. Govt. Printing Office, Singapore.
JACKSON, I. W., AND J. T. GREENE. 1958. Tracheid length variation and inheritance in slash and loblolly pine. Forest Sci. 4: 316–318.
JEFFREY, E. C. 1917. The anatomy of woody plants. Univ. of Chicago Press, Chicago.
JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill, New York.
MORZENTI, V. M., AND W. H. WAGNER. 1962. Southeastern American "Blackstem spleenworts" of the *Asplenium heterochroum-resiliens* complex. Assoc. Southeastern Biol. Bull. 9(2): 40–41.
SIERP, H. 1914. Über die Beziehungen zwischen Individuengrösse mit besonderer Berucksichtingung des erblichen Zweigwuchses. Jahrb. Wiss. Bot. 53: 55–124.
TIPPO, O. 1946. The role of wood anatomy in phylogeny. Amer. Midl. Nat. 36: 362–372.
WAGNER, W. H., JR. 1954. The evidence used in recent classifications of the ferns. Huitieme Congr. Internatl. Bot. Rapp. et Commun. au sect. 6: 9–15.
———. 1961. Problems in the classification of ferns. Rec. Adv. in Bot. Sec. 9: 841–844.
———. 1962. The endemic Botrychiums of the southeastern United States. Assoc. Southeastern Biol. Bull. 9: 40.
WALKER, S. 1961. Cytogenetic studies in the *Dryopteris spinulosa* complex. II. Amer. Jour. Bot. 48: 607–614.
WHITE, R. A. 1961. Vessels in roots of *Marsilea*. Science 133: 1073–1074.
———. 1962. A comparative study of the tracheary elements of the ferns. Thesis. Univ. of Michigan.
WHERRY, E. T. 1961. The fern guide. Doubleday, New York.

OBSERVATIONS ON THE FINE STRUCTURE OF OEDOGONIUM. II. THE SPERMATOZOID OF O. CARDIACUM[1]

LARRY R. HOFFMAN[2] AND IRENE MANTON
Department of Botany, University of Illinois, Urbana, Illinois
and
Department of Botany, The University, Leeds, England

ABSTRACT

HOFFMAN, L. R., and IRENE MANTON. (U. Leeds, England.) Observations on the fine structure of Oedogonium. II. The spermatozoid of O. cardiacum. Amer. Jour. Bot. 50(5): 455–463. Illus. 1963.—Salient features of the fine structure of the spermatozoid of *Oedogonium cardiacum* are described and illustrated as they appear in whole mounts and in sections. There is a close resemblance to the zoospore of the same species (Hoffman and Manton, 1962) though the gamete is smaller and in some respects simpler. The flagella, though similar in length to those on the zoospore, are fewer (ca. 30 instead of ca. 120 per cell). The construction of the flagellar ring is similar though there is less mechanical material associated with the flagellar bases in the gamete. Compound "roots" alternating with the flagellar bases are identical in structure and relative position in both types of motile cells; there is no direct connection with the nucleus. Other details of resemblance and difference between the spermatozoid and the zoospore are discussed.

IN A PREVIOUS communication (Hoffman and Manton, 1962) the fine structure of zoospores of *Oedogonium cardiacum* (Hass.) Wittr. was investigated with special reference to salient features of the flagellar apparatus. In the present paper we propose to extend the inquiry to spermatozoids of the same species.

[1] Received for publication September 11, 1962.
[2] This work was carried out during tenure of a National Science Foundation Fellowship for the session 1961–62.

The source of motile cells has been exactly as in our previous investigation for which zoospores from male plants grown in soil-water culture were used. The culture (LB39) came originally from the Culture Collection of Algae at Indiana University (Starr, 1960) and the optimum treatment required to produce large numbers of spermatozoids instead of zoospores is as follows. With an old culture (several months old), it is sufficient to transfer a few filaments into a shallow layer

(ca. 4 mm) of distilled water in a Petri dish and illuminate continuously. With a younger culture it is necessary to bubble a mixture of air and CO_2 (0.5–1%) through the medium (distilled water or soil-water extract) in continuous light. With a light intensity of 300 ft-c at room temperature, spermatozoids may be expected within 2–4 days.

The methods of study have been as in our previous paper. We have worked with a combination of light and electron microscopy, examples of both being reproduced in the illustrations. Where it is desired to study complete flagella or the flagellar apparatus as a whole, it is necessary to kill spermatozoids directly on prepared carbon films or glass slides, after which they may be shadow-cast for electron microscopy or examined at once by light microscopy.

For the study of internal structure, standard osmic fixation was used (2% osmium tetroxide in distilled water buffered to pH 7 with acetate veronal and used for ½ hr). This was sometimes followed by bulk staining with 1% phosphotungstic acid in absolute alcohol used for 1–6 hr before embedding in either n-butyl methacrylate or a mixture of butyl and methyl methacrylate.

Sections were cut on a Porter-Blum microtome using a glass knife. They were examined in a Siemens Elmiskop I. The light microscopy was

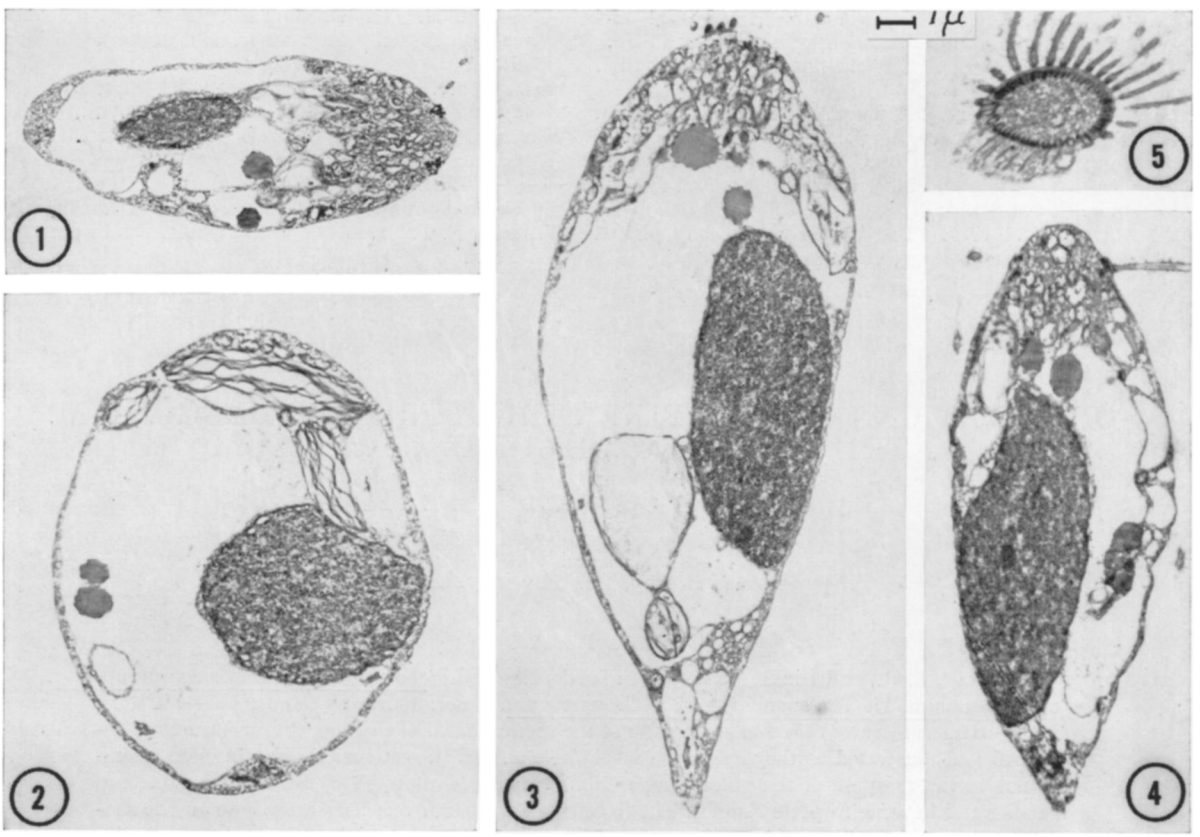
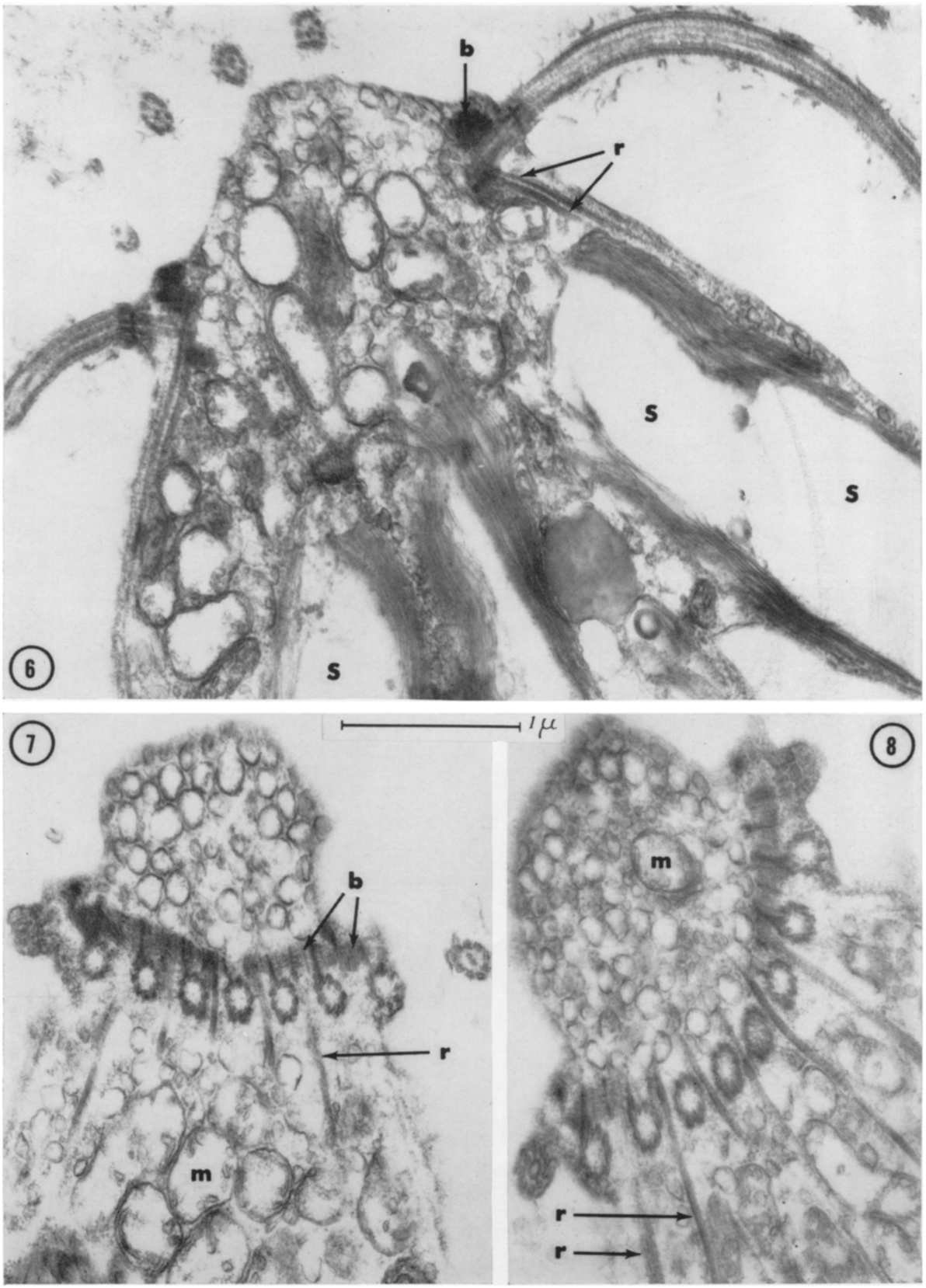
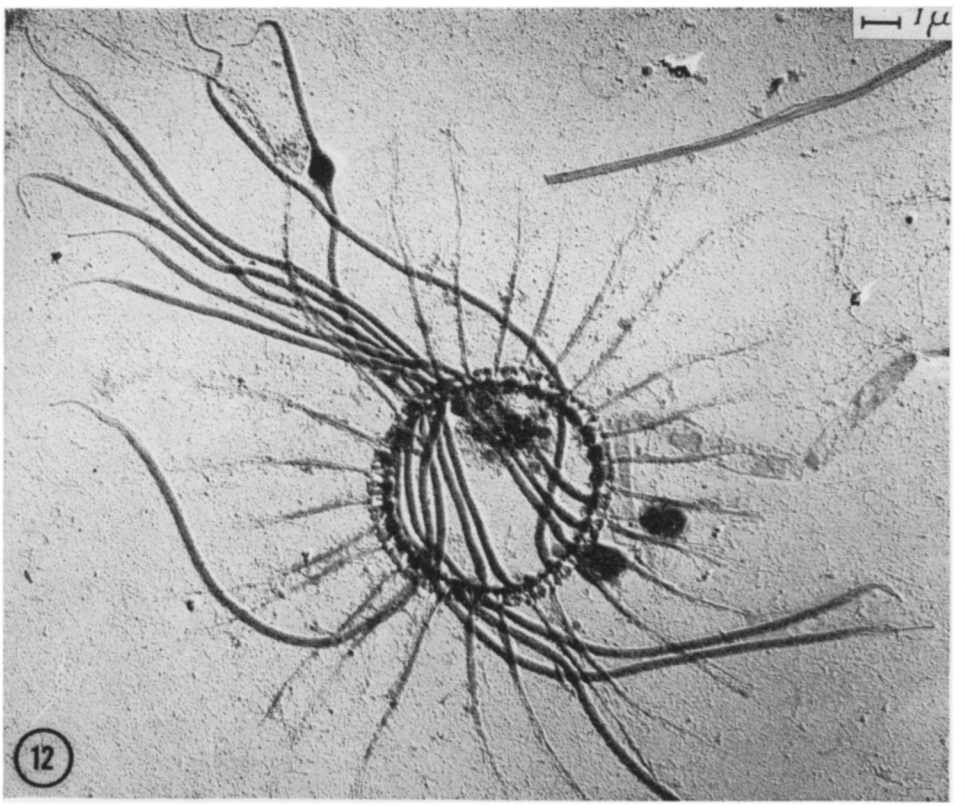


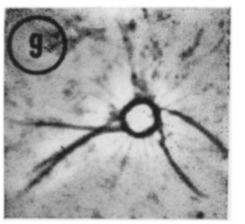
Fig. 1–5. *Oedogonium cardiacum* spermatozoid; electron micrographs of sections, for general views at low magnifications.—Fig. 1. Approximately median longitudinal section at the magnification used for the zoospore (Hoffman and Manton, 1962); micrograph B5890. ×3000.—Fig. 2. Transverse section showing the nucleus projecting into the main vacuole and with parts of the plastid and other smaller inclusions in the cytoplasm; micrograph S752.23. ×5000.—Fig. 3–4. Longitudinal sections from 2 cells of different size; micrographs B5882 and B8197. ×5000.—Fig. 5. Slightly oblique transverse section through the base of an apical dome showing the flagellar ring; micrograph B8252. ×5000.

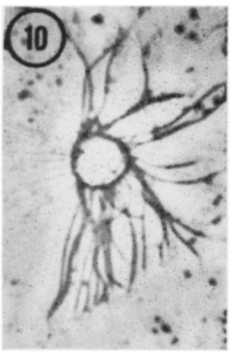
Fig. 6–8. Details of the apical dome from longitudinal sections. ×30,000.—Fig. 6. Median view showing flagellar bases and "roots," the former cut longitudinally on each side of the cell and with the fibrous band above them cut transversely; elsewhere in the cytoplasm residual plastids with starch, a fat body and other small inclusions; micrograph B6397.—Fig. 7. Tangential section transecting the flagellar bases at the level of the fibrous band; parts of "roots" visible between and below them; a transected flagellum outside the cell but belonging to it included on the right to show the vertical orientation of the 2 central fibers with respect to the ring; micrograph B7685.—Fig. 8. Tangential section cut more deeply in the cell than that of Fig. 7 showing the anterior terminations of "roots" passing between the flagellar bases in the center of the profile; micrograph B7690.

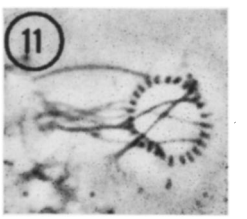
CODE: b = fibrous band; m = mitochondrion; r = "root"; S = starch.

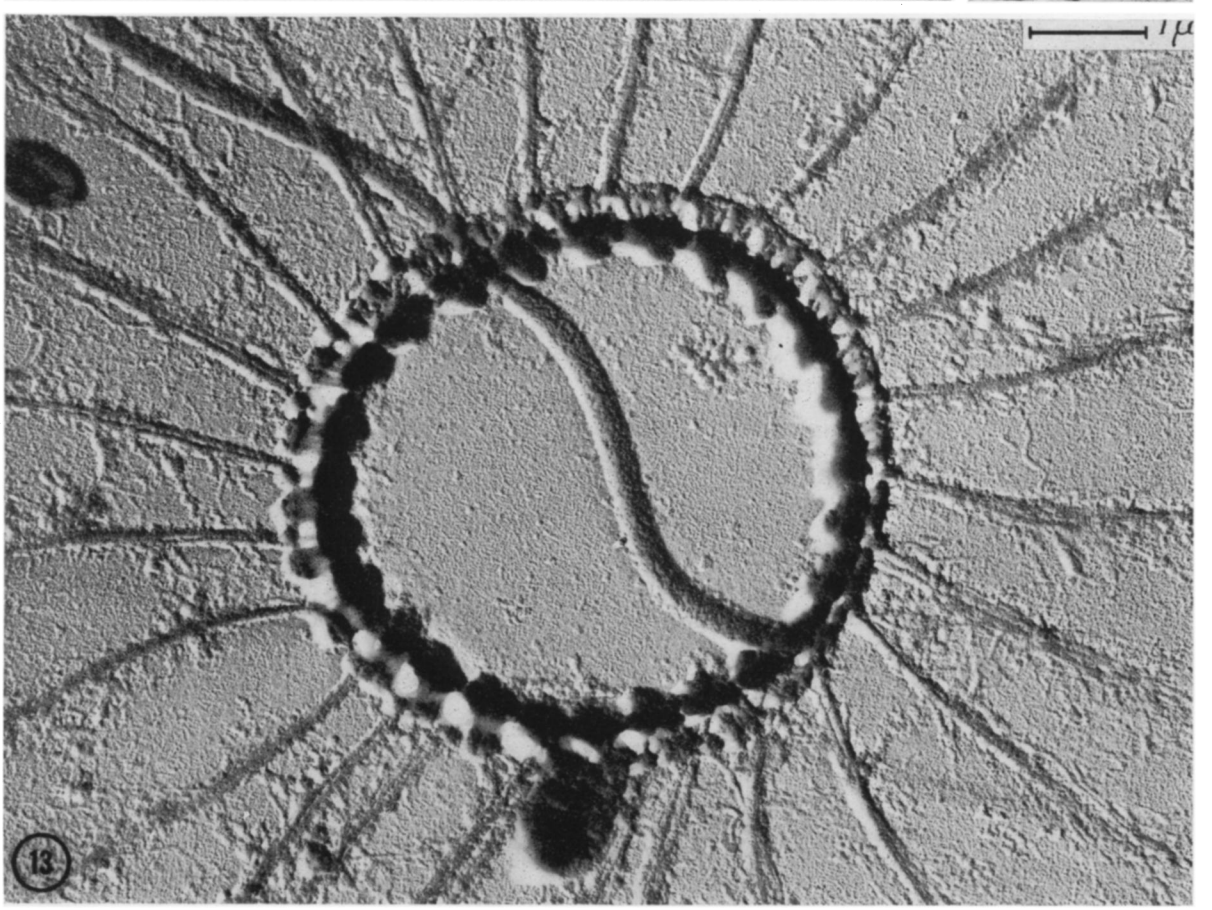












carried out with a Reichert Zetopan microscope using phase contrast, a dry lens 40 × and a 12½× ocular. The work was carried out in the Botany Department, Leeds University, England.

GENERAL FEATURES OF THE SPERMATOZOIDS—The spermatozoid is so much smaller than the zoospore that it is difficult to compare the two on the same scale. Figure 1, reproduced for this purpose, represents the maximum degree of enlargement (i.e., × 3000) which could be used to permit a complete longitudinal section of a zoospore to be reproduced within the width of a printed page (Hoffman and Manton, 1962, Fig. 6); but for convenient demonstration of even major features

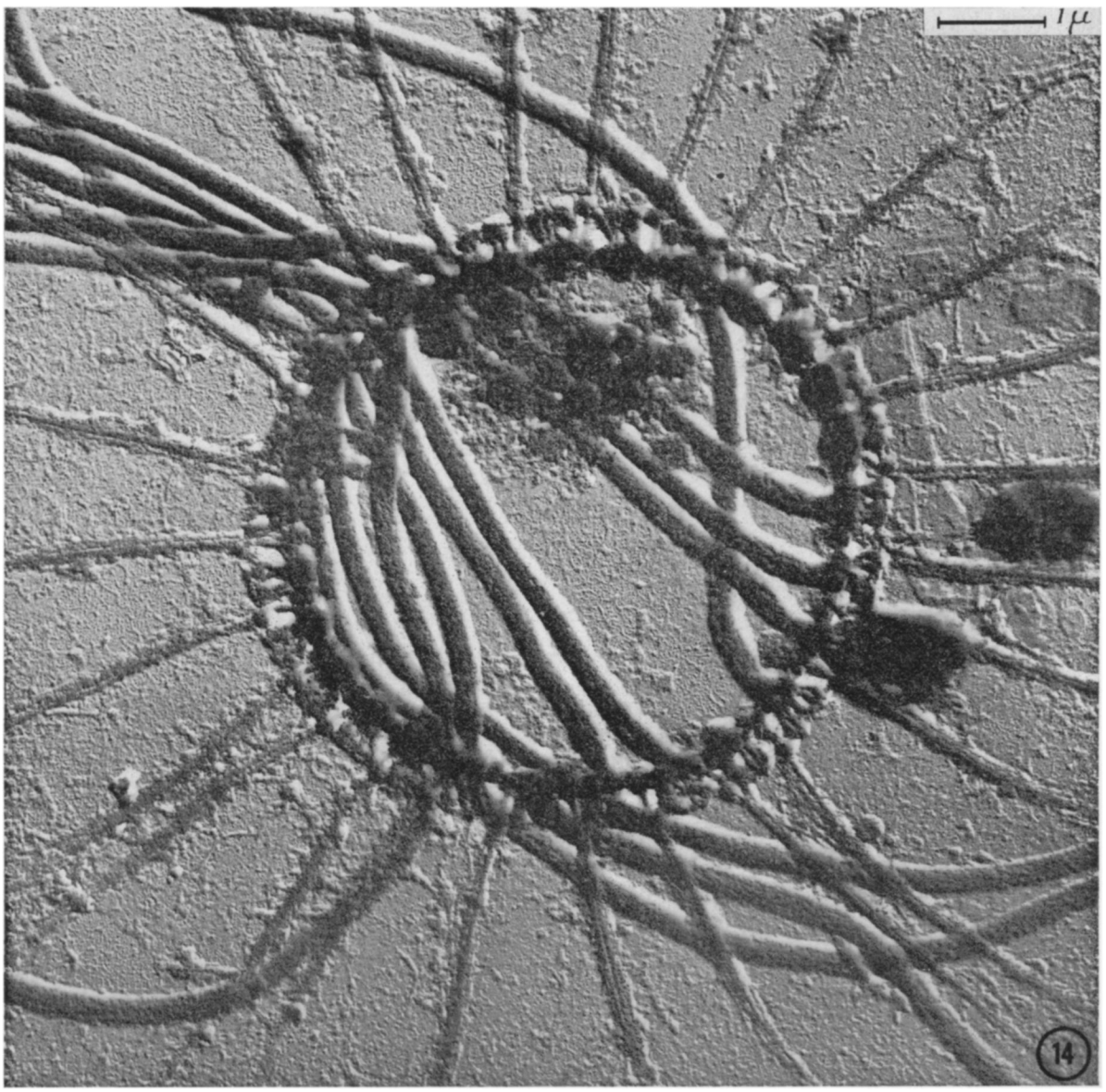


Fig. 14. More highly magnified view of the center of Fig. 12; micrograph B6470. ×15,000.

Fig. 9–13. *Oedogonium cardiacum* spermatozoid; examples of detached flagellar rings.—Fig. 9–11. Photographs with the light microscope of dried specimens under phase contrast × 1000 showing fibrous rings in different states of post-mortem expansion, Fig. 9 least expanded, Fig. 11 most expanded, with flagella or flagellar bases visible, and in some places signs of the much thinner fibrous "roots" (see especially Fig. 10 left).—Fig. 12. A moderately expanded ring (contrast with Fig. 5) with many flagella still attached and with the fibrous "roots" spread on the field; micrograph B4669. ×5000; for further details see Fig. 14.—Fig. 13. A flagellar ring with a full complement of roots but with only 1 intact flagellum and a total of 26 flagellar bases; micrograph B4661. ×15,000.

in the spermatozoid, a magnification of not less than × 5000 (Fig. 2–5) is greatly to be preferred. The over-all cell dimensions, expressed to the nearest half micron and in each case as a mean of 100 measurements made with the light microscope on material killed with iodine, are 26 × 37 μ for the zoospore and 13 × 18 μ for the gamete. These measurements do not exactly express the shapes in life, since dead cells always tend to become less fusiform and more rounded even after iodine fixation. As a rough indication of comparative volumes the measurements are nevertheless significant.

Compared with the zoospore, a living spermatozoid is not only smaller but is normally more elongated relative to its width. Furthermore, the crown of flagella is placed further forward, so that an apical dome, as seen in the zoospore, cannot be distinguished with the light microscope. The color of the cell as a whole is yellowish, or very pale green, and there is no eyespot.

When seen in section, the most conspicuous organelles are the relatively dense nucleus and the vacuole which occupies a much larger proportion of the total volume than is usual in the zoospore (see especially Fig. 2). The plastid, on the other hand, which dominates the whole zoospore except in the apical dome, is greatly reduced both in size and in internal complexity in the gamete. It is, therefore, relatively inconspicuous, although some starch is usually still present.

The other cytoplasmic components, notably Golgi bodies, mitochondria and vesicles of various kinds, need little comment except perhaps to note that the mitochondria are consistently more transparent in sections than they were in the zoospore. This may, perhaps, be a significant difference and correlated with the more limited length of active life of the gamete, but it is difficult to exclude entirely the possibility of differential solubility of some opaque substance during fixation of cells of very dissimilar size. The meaning of this feature must, therefore, at present be left uncertain. Another detail is the complete absence, from the spermatozoid, of the vesicles with dense contents which are crowded near the surface of the apical dome in the zoospore and which we believe to be the source of the adhesive material secreted from the anterior end when the spore settles down prior to germinating. This difference is, therefore, almost certainly to be correlated with the very different subsequent behavior of the gamete.

THE FLAGELLAR APPARATUS—There is no ascertainable difference in size or structure between individual flagella from zoospores and gametes. In the former (Hoffman and Manton, 1962) we found an average length of 17 μ in a sample of 56 measurements from 12 different cells. Measurements of 20 intact flagella from 5 spermatozoids gave an average length of 16.5 μ, a difference which is not significant in view of the small size of the samples.

In contrast, there is a conspicuous difference in flagellar number on the 2 types of cells. Whereas in the zoospore we encountered an average of about 120 flagella per cell, in the spermatozoids there are only about 30. Counts, to a high degree of accuracy, can be made by utilizing the flagellar bases or flagellar "roots" in slightly expanded whole mounts (Fig. 11, 13), the flagella themselves being rarely, if ever, completely retained after the preparative treatment. Slight individual differences from cell to cell undoubtedly exist. Sample counts from 5 complete specimens examined with the light microscope in the condition of Fig. 11 gave counts ranging from 27 to 34, with uncertainty of not more than ±1 flagellum in any one count. Several weeks later, 5 similar specimens were prepared and examined with the electron microscope (Fig. 13, 14), giving results ranging from 24 to 27 with no uncertainty. We have not attempted to explore the effects of different conditions in greater detail.

The construction of the flagellar ring is essentially as in the zoospore except for minor differences in relative proportions. Examples of 2 complete specimens are illustrated in Fig. 12–14, each showing breakage of the flagella themselves to a different extent, but both possessing the whole array of bases, "roots" and other ancillary structures. As in the zoospore, the fibrous "roots" are conspicuous and regular features, alternating with the flagellar bases and sometimes visible in comparable preparations with the light microscope (Fig. 10). Further details about their composition and arrangement are obtainable only from sections.

When examined in section, the most conspicuous feature of the flagellar ring is a complex band of fibrous and other mechanical materials which circumscribes the cell immediately below the surface, touching, and doubtless attached to the whole array of flagellar bases on their anterior side (Fig. 7, 15). As in the zoospore, this band is marked off into segments by dense cross-bands of amorphous material corresponding with the position of each flagellum. These segments are then further subdivided by smaller cross-bands arranged in a characteristic repeating pattern (Fig. 15). The details of this minor pattern (Fig. 15) were at first thought to be simpler than in the zoospore, but in specimens cut as in Fig. 18 (compare also Fig. 5) the presence of 2 different patterns in superposed layers is revealed, thereby indicating that there are probably 2 separate layers in both types of cells, but that the outer layer carrying the pattern previously seen in the zoospore is greatly reduced in thickness in the gamete.

On the lower side of the flagellar bases (Fig. 15) there is only a very small amount of dense amorphous material closely investing each base, instead of the substantial gutter-shaped masses seen in the zoospore. The ancillary material between

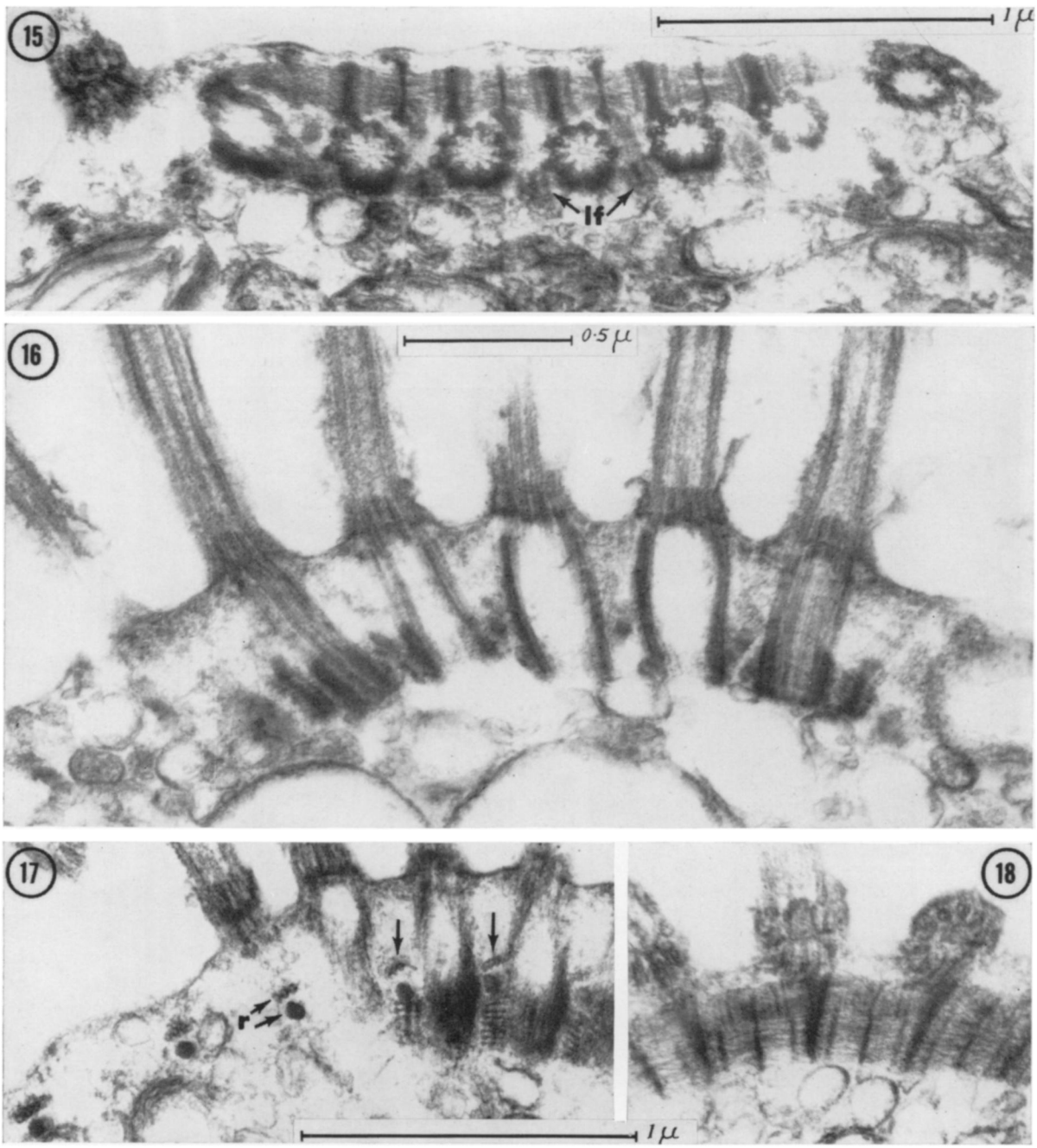


Fig. 15–18. *Oedogonium cardiacum* spermatozoid; details of the fine structure of the flagellar ring as seen in various planes of section. ×50,000.—Fig. 15. Tangential longitudinal section of a cell at the level of the fibrous ring showing transversely cut flagellar bases below it with additional fibrous material (lf) between them on their inner side; micrograph B5868.—Fig. 16. Oblique transverse section of a cell showing the basal parts of 5 flagella; the flagellar membranes imperfectly preserved (compare Fig. 6); micrograph B7695.—Fig. 17. Oblique transverse section of a cell near the flagellar ring and accurately transecting both components of several "roots" (for further explanation see text). On the right of the figure, "roots" (arrows) are seen passing between the flagellar bases above the lower fibrous material; micrograph B7588.—Fig. 18. Part of the fibrous ring above the flagellar bases in an oblique transverse section as in Fig. 5. Two different patterns of cross-banding detectable on the fibers between the flagella indicating the presence of 2 layers (for further explanation see text); micrograph B7593.

the bases on the lower side is also less. In the zoospore we encountered a stout wedge of amorphous dense material, appearing triangular in cross section and occupying most of the remaining space between the flagellar bases on their lower side. In the spermatozoid the triangle is replaced by 3 parallel rods (Fig. 15) mutually joined by fibers, the latter more clearly seen when cut in another plane (Fig. 16 left, Fig. 17 right).

This degree of reduction in the mechanical arrangements for the flagellar ring does not extend to the "roots", which appear to be identical in structure and position (though not of course in number) in the 2 types of cells. Characteristic views of "roots" are contained in Fig. 9, 10, 12–14 for whole mounts or Fig. 6–8 for low-power longitudinal sections. When cut transversely (Fig. 17), each "root" can be seen to contain 2 radially superposed components, namely an outer ribbon of 3 parallel fibers, each appearing circular in cross section, and a stouter inner component, also isodiametric in cross section but more solid. As in the zoospore, the outer fibrous component starts between the flagella at the level of the fibrous ring to which it is almost certainly attached and passes thence backwards close to the plasmalemma (Fig. 6, 19) for an unknown but considerable distance. The more solid inner component is not directly attached to the fibrous ring but passes beneath and beyond it in both directions, ending anteriorly at the base of the apical dome (Fig. 8) as in the zoospore. The 2 ends of this component are distinctly tapered (Fig. 20) and when seen in tangential view at higher magnification (Fig. 21) a delicate cross-banding of alternating thinner and thicker lines can, in favorable cases, be resolved.

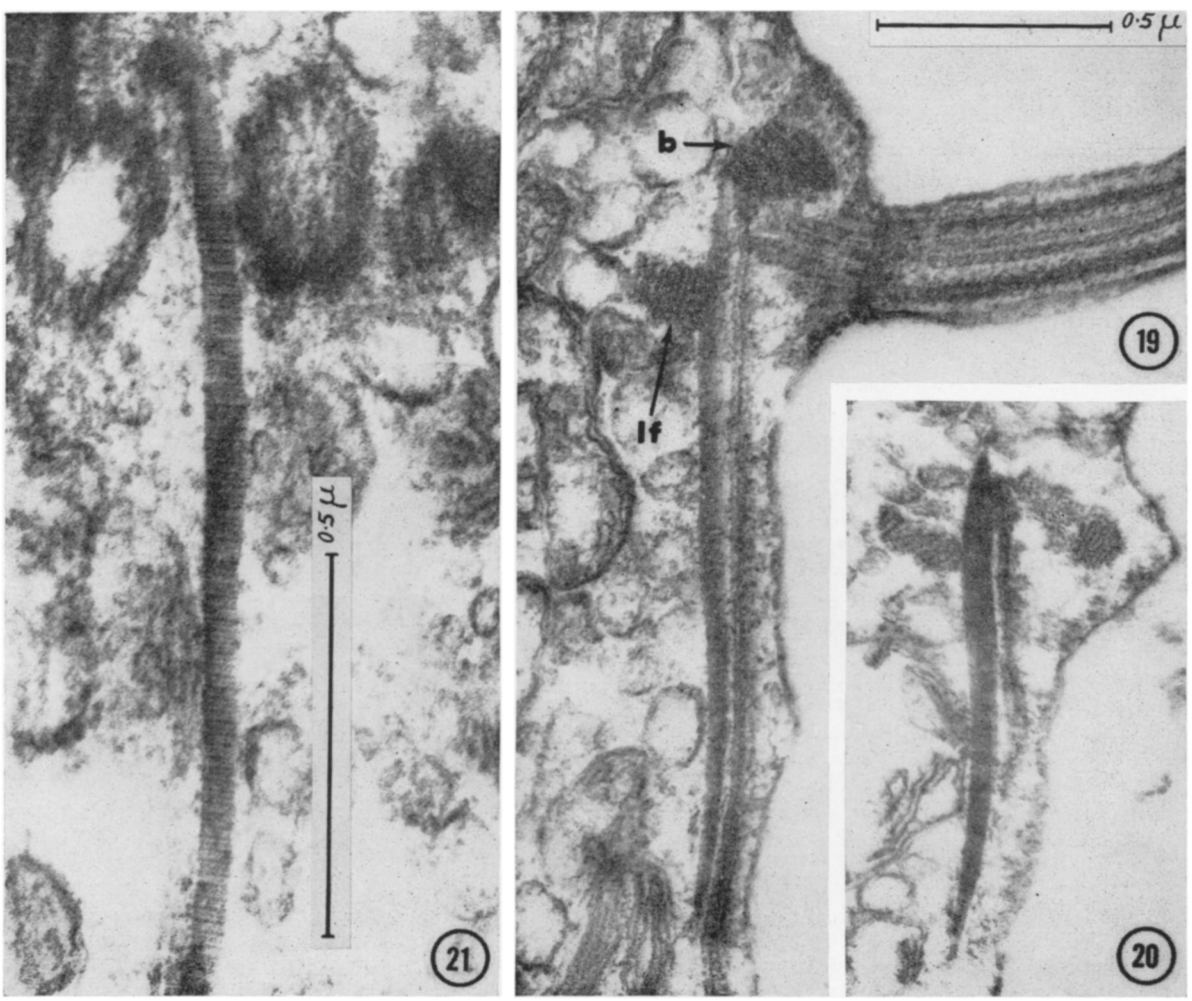


Fig. 19–21. *Oedogonium cardiacum* spermatozoid; further details of the fine structure of "roots" from longitudinal sections.—Fig. 19. Radial longitudinal section showing the 2 components of a "root" in relation to an adjacent flagellum, the fibrous ring (b) and the lower fibers (lf); micrograph B7648. ×60,000.—Fig. 20. Radial longitudinal section midway between flagella (which do not therefore enter the section) showing the shape of the inner component of a "root" more clearly; micrograph B6925. ×60,000.—Fig. 21. Tangential longitudinal section showing the cross-banding on the inner component of a "root" seen between 2 flagellar bases at its anterior end; micrograph B7700. ×100,000.

In the specimen illustrated (Fig. 21), some of the thinner lines are indistinct but measurements of spacing between successive thicker lines, regardless of whether the complete pattern of intervening thin lines is clearly expressed or not, gives an average distance apart of ca. 150 A. This is exactly the distance previously recorded for each repeat of pattern (i.e., involving 1 thick and 1 thinner line) in the zoospore (Hoffman and Manton, 1962).

DISCUSSION—Probably the most important general observation resulting from the present work is the demonstration of the high degree of structural resemblance existing between the zoospore and the spermatozoid of one and the same species of *Oedogonium*, in spite of the very great differences in size and in function between the 2 types of cells. A feature common to both that should be specially noted is the apparent absence of any structural connection between the flagellar apparatus and the nucleus. When this negative character was first encountered in the zoospore it was pointed out as somewhat unusual (Hoffman and Manton, 1962) but it is even more unusual in a motile gamete. In the best-known flagellated gametes from the standpoint of fine structure, namely the spermatozoids of fucoids (Manton and Clarke, 1956), *Dictyota* (Manton, 1959b), and of *Prasiola* (Manton and Friedmann, 1960), the flagellar bases are anchored directly to the nucleus in a manner suggesting functional significance in fertilization. That this is not necessarily the case is indicated by our present findings.

The other characteristics which the gamete shares with the zoospore have already been discussed in a general way in our previous paper to which the reader is referred. The spermatozoid has confirmed the observations already recorded for the zoospore on details such as the orientation of flagella and the structure and arrangement of "roots." Salient comparisons with other organisms include the zoospores of other green algae for which the fine structure is partly known, notably *Draparnaldia* and *Chaetomorpha* (Manton, Clarke and Greenwood, 1955), the spermatozoid of a fern (Manton, 1959a), and certain animal cells (Fawcett and Porter, 1954). These references have previously been discussed with regard to the zoospore of *Oedogonium* (Hoffman and Manton, 1962) and those same comments are applicable to the spermatozoid of *Oedogonium*.

Structural differences between the spermatozoid and the zoospore of *O. cardiacum* include the greatly reduced chloroplast without eyespot in the gamete, the relatively increased vacuole, the quantitative differences in mechanical materials involved in the flagellar ring, the absence of vesicles with dense contents from the forwardly directed end, the absence or apparent absence of dense contents within the mitochondria, and probably other details involving the less conspicuous components of both types of cells when these have been more fully examined. It is to be expected that some of these differences will prove to be merely incidental consequences of over-all differences of cell size which seem likely to be the significant factors in determining features such as the numbers of flagella and "roots" and the relative amounts of supporting mechanical materials. Other characters are likely to bear a closer relation to the functional activity of the cell; but until we have some comparable facts for other stages and perhaps also for other species and types of cells (androspores for example), attempts at more detailed interpretation are better deferred.

LITERATURE CITED

FAWCETT, D. W., AND K. R. PORTER. 1954. A study of the fine structure of ciliated epithelia. Jour. Morph. 94: 221–282.

HOFFMAN, L. R., AND I. MANTON. 1962. Observations on the fine structure of the zoospore of *Oedogonium cardiacum* with special reference to the flagellar apparatus. Jour. Exptl. Bot. 13: 443–449.

MANTON, I. 1959a. Observations on the microanatomy of the spermatozoid of the bracken fern (*Pteridium aquilinum*). Jour. Biophys. Biochem. Cytol. 6: 413–418.

———. 1959b. Observations on the internal structure of the spermatozoid of *Dictyota*. Jour. Exptl. Bot. 10: 448–461.

———, AND B. CLARKE. 1956. Observations with the electron microscope on the internal structure of the spermatozoid of *Fucus*. Jour. Exptl. Bot. 7: 416–432.

———, B. CLARKE, AND A. D. GREENWOOD. 1955. Observations with the electron microscope on biciliate and quadriciliate zoospores in green algae. Jour. Exptl. Bot. 6: 126–128.

———, AND I. FRIEDMANN. 1960. Gametes, fertilization and zygote development in *Prasiola stipitata* Suhr. II. Electron microscopy. Nova Hedwigia 1: 22–42.

STARR, R. C. 1960. The culture collection of algae at Indiana University. Amer. Jour. Bot. 47: 67–86.