in declination and were extrapolated to an antenna temperature of 0°K by assuming a Gaussian shape for the convolution of the beam pattern with the source in this dimension.
9. R. J. Allen, A. H. Barrett, P. P. Crowther, *Astrophys. J.* **151**, 43 (1968).
10. P. A. G. Scheuer and P. J. S. Williams, *Annu. Rev. Astron. Astrophys.* **9**, 321 (1968); J. Schraml and P. Metzger, in preparation.
11. D. Hogg, paper presented at the 127th meeting of the American Astronomical Society, Victoria, British Columbia, 1968.
12. T. P. McCullough and J. W. Boland, *Astron. J.* **69**, 68 (1964).
13. F. T. Barath, A. H. Barrett, J. Copeland, D. E. Jones, A. E. Lilley, *ibid.*, p. 49.
14. J. B. Pollack and A. T. Wood, Jr., *Science* **161**, 1125 (1968).
15. D. O. Muhleman, in preparation.
16. J. R. Dickel, W. J. Medd, W. W. Warnock, *Nature* **220**, 1183 (1968).
17. I thank C. Sagan and J. B. Pollack for suggesting this investigation and for their encouragement and assistance. I am grateful to D. Heeschen and the staff of the National Radio Astronomy Observatory for the use of their facilities, and especially to K. I. Kellermann, I. Pauliny-Toth, and J. Schraml for assistance with observing and data reduction. I thank J. R. Dickel for communicating his results in advance of publication and A. E. Lilley and Y. Terzian for several useful discussions. Supported by NASA grants NGR 09-015-023 and NGR 33-010-082. The National Radio Astronomy Observatory is operated by Associated Universities, Inc. under contract with the National Science Foundation.
* Present address: Center for Radiophysics and Space Research, Cornell University, Ithaca, New York 14850.

15 November 1968

Critical Point Drying for Scanning Electron Microscopic Study of Ciliary Motion

Abstract. *Scanning electron microscopic study of the pattern of ciliary coordination and the form of ciliary beat is now possible. Rapid fixation stops the ciliary activity instantaneously, and critical point drying avoids distortion of the cilia by surface tension forces. Such studies have been made on the ciliate* Opalina *with this new technique.*

The scanning electron microscope provides a powerful new technique for studying the surface structure of biological material. However, the necessity of drying the specimen in air is a major problem in the preparation of material that is distorted by surface tension forces (*1*). In order to use the scanning electron microscope to study ciliary activity, we have devised a new combination of methods that overcomes this difficulty. In brief, the technique is to stop ciliary motion instantaneously by rapid fixation and then prepare the specimens for scanning electron microscopy by critical point drying.

The organism used in this study was the ciliated protozoan *Opalina ranarum*, from the rectum of the common frog *Rana temporaria*. Adjacent cilia beat with regular phase differences, so that waves of activity move over the organism in the same direction as the effective stroke of the ciliary beat (symplectic metachronal coordination). Successive stages in the cycle of one complete beat of a cilium are thus encountered sequentially in one metachronal wave. It is possible to preserve the movement of metachronal waves by instantaneous fixation, and thus analyze the form of the ciliary beat (*2, 3*). The following procedure was used.

Osmium tetroxide (2 percent solution in water) was pipetted rapidly on several hundred *Opalina* swimming in a few drops of suitable Ringer (*4*) in a watch glass, which was then maintained at 0°C for 10 to 15 minutes. The organisms were washed briefly and dehydrated in a graded ethanol series.

Organisms were dried by the critical point method of Anderson (*5*), thus avoiding distortion of the cilia by surface tension forces. The problem of handling during this step was overcome in the following way. *Opalina* in absolute ethanol were pipetted into a tiny bag of fine nylon bolting cloth (60 mesh per centimeter). The alcohol was replaced by clean amyl acetate, and the bag was quickly placed in the bomb of the critical point apparatus. The bomb was flushed out at room temperature with liquid carbon dioxide to replace the amyl acetate.

When it was completely filled with liquid carbon dioxide, the bomb was sealed off and the temperature raised to about 50°C. During this heating the liquid carbon dioxide was taken past its critical point and changed into a gas without the formation of a phase boundary. The valve was then opened slowly to allow the gaseous carbon dioxide to escape.

Dried *Opalina* were sprinkled on metal specimen stubs made sticky with a thin layer of a chloroform solution of Cellotape. The organisms were coated with approximately 30 nm of gold-palladium by rotating the stubs at an angle of 45° to the evaporation source. They were viewed in a "Stereoscan" (*6*) scanning electron microscope at a beam accelerating voltage of 20 kv with the stub tilted at an angle of 45° to the electron beam. Rotation of the

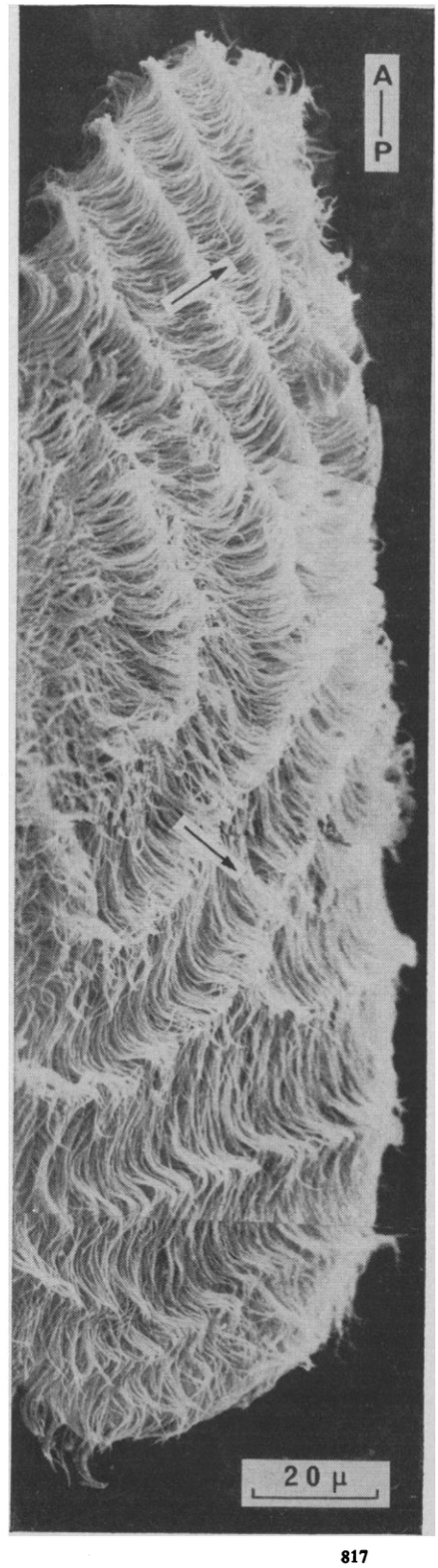

Fig. 1. Scanning electron micrograph of an *Opalina* fixed with metachronal waves traveling simultaneously in different directions. Arrows indicate direction of wave transmission and the effective stroke of cilairy beat in different regions. *A–P*, anterior-posterior axis.

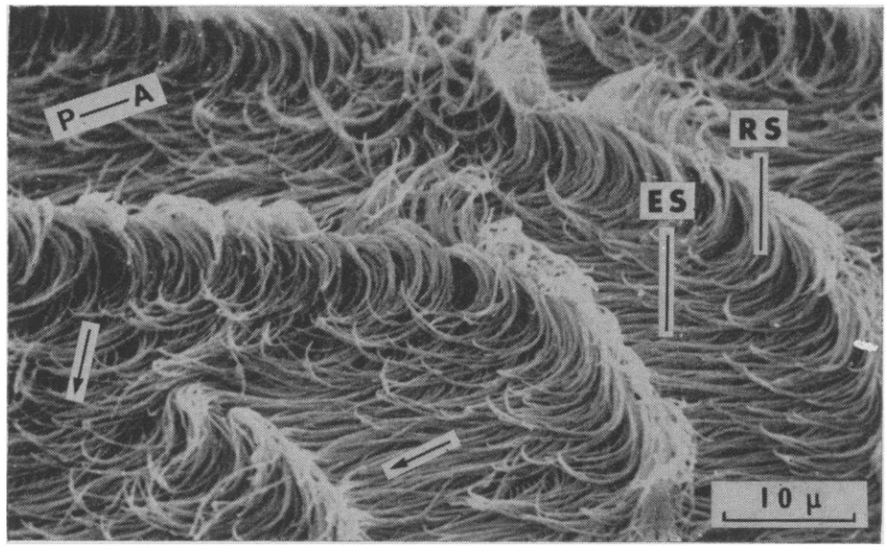

Fig. 2. Higher magnification of metachronal waves on a different animal, showing posterior side of waves. Arrows have the same meaning as in Fig. 1. *RS*, cilia in recovery stroke; *ES*, cilia in effective stroke; *P–A*, posterior-anterior axis.

stub allowed the organism to be viewed at 45° from different sides.

Opalina can change its direction of swimming by changing the direction of the effective stroke of ciliary beating, with corresponding changes in the direction of transmission of metachronal waves (*7*). This situation is illustrated in Fig. 1 in which the organism is fixed with waves traveling simultaneously in different directions. The wave pattern is similar to that in a living animal photographed while turning to the left by Sleigh (*8*). We find that the form of the waves is the same for waves moving in different directions.

The form of the ciliary beat and pattern of coordination in *Opalina* has recently been described with the use of photography of living animals (*8*) and also by the instantaneous fixation technique coupled with hematoxylin staining and light microscopy (*2*). We confirm Párducz's statement that ". . . contrary to Sleigh's description, the whole of the beating cycle involves more than a single plane . . . the cilium bends out of the plane of the effective beat after performing it, and during the greater part of the recovery phase it rotates counterclockwise, parallel to the body surface, gradually emerging into the preparatory position for the next stroke" (*2*, p. 107). The counterclockwise bending of cilia in the recovery stroke can be clearly seen in Fig. 2 (*RS* cilia). Apart from this large divergence from one plane, the general form of the beat is as figured by Sleigh (*8*).

In conclusion, the combination of instantaneous fixation, critical point drying, and scanning electron microscopy offers a powerful new method for the study of ciliary motion, not only in Protozoa, but in metazoan tissues as well. The introduction of the critical point drying technique greatly increases the usefulness of the scanning electron microscope for studying any material that suffers distortion by air drying.

G. A. Horridge
S. L. Tamm*
Gatty Marine Laboratory and
Department of Natural History,
University of St. Andrews,
St. Andrews, Scotland

References and Notes

1. V. C. Barber and A. Boyde, *Z. Zellforsch.* **84**, 269 (1968).
2. B. Párducz, *Int. Rev. Cytol.* **21**, 91 (1967).
3. P. Satir, *J. Cell Biol.* **18**, 345 (1963).
4. H. Kinosita, *J. Fac. Sci. Univ. Tokyo Sect. IV Zool.* **7**, 1 (1954).
5. T. F. Anderson, *Trans. N.Y. Acad. Sci. Ser. II* **13**, 130 (1951).
6. Cambridge Instrument Company, England.
7. A. Okajima, *Jap. J. Zool.* **11**, 87 (1953).
8. M. A. Sleigh, *J. Exp. Biol.* **37**, 1 (1960).
9. We thank H. MacGregor and J. Mackie of the Zoology Department at the University of St. Andrews for valuable advice and the use of their critical point drying apparatus. D. Bradley of the Zoology Department at the University of Edinburgh generously provided facilities for coating the specimens. We are very grateful to A. R. Dinnis and J. Goodall of the Electrical Engineering Department at the University of Edinburgh for examining the specimens in their scanning electron microscope, and to J. Stevenson of the Gatty Marine Laboratory for his expert photographic assistance. S.L.T. is a National Science Foundation postdoctoral fellow.

* Present address: Department of Zoology, Indiana University, Bloomington, Indiana.

9 September 1968

Microspikes on the Lymphocyte Uropod

Abstract. *Lymphocytes have anatomic and functional characteristics reminiscent of the amoeba. The capacity to form microspikes on the uropod suggests a high degree of specialization essential to the lymphocyte's function in immunologic reactions.*

The motile lymphocyte in vitro has a characteristic configuration (*1*). Locomotion is essentially amoeboid, but the tail section of the cell is so prominent (*2*) that the cell can readily be distinguished from other motile leukocytes which may, irregularly and briefly, display a "tail."

Cinephotomicrographic studies of human lymphocytes in immunological reactions in vitro indicate that the cytoplasmic process forming the tail is used by the lymphocyte to contact and attach to debris, the surface of the culture vessel, and other cells in the environment (*3*). In many instances, as during interaction with macrophages, this attachment lasts for prolonged periods, even hours, with the tail process forming the connecting stalk. Thus the term "uropod" was applied to describe a process which was both a tail and a connecting stalk (*3*). By means of phase contrast microscopy, threadlike projections could often be seen extending from the uropod to contact neighboring objects. I have studied the uropod with electron microscopy to determine its structure in more detail.

Leukocytes (0.5×10^6) from two unrelated human donors were cultured in medium 199 containing 20 percent autologous plasma derived equally from the cell donors (*4*). On day 3, while the culture was maintained at 37°C, the supernate was decanted quickly, and 2.5 percent gluteraldehyde at 4°C was added gently and rapidly. The cell button was loosened with a rubber policeman, fixed in osmium tetroxide, and embedded in Epon for examination in an RCA EMU-4 electron microscope.

Most lymphocytes appeared round in cross section, suggesting a spherical shape with occasional regularly spaced microvilli projecting from the cell's surface. In some instances, the microvilli were so numerous that the lymphocyte appeared shaggy or burrlike. Occasional lymphocytes were elongated and had the configuration typical of the