**References and Notes**

1. J. J. Loftus-Hills, M. J. Littlejohn, K. G. Hill, *Nat. New Biol.* **233**, 184 (1971).
2. Experiments with earlier sensitive methods based on laser interferometry [S. M. Khanna, J. Tonndorf, W. W. Walcott, *J. Acoust. Soc. Am.* **44**, 1555 (1968)] and the Mössbauer effect [P. Gilad, S. Shtrikman, P. Hillman, M. Rubenstein, A. Eviator, *ibid.* **41**, 1232 (1967); B. M. Johnstone and A. J. Boyle, *Science* **158**, 389 (1967); B. M. Johnstone, K. J. Taylor, A. J. Boyle, *J. Acoust. Soc. Am.* **47**, 504 (1970); W. S. Rhode, *ibid.* **49**, 1218 (1971)] have proved to be remarkably fruitful. Both suffer from the need to fasten a foreign object to the organ. The Mössbauer technique requires attachment of a $\gamma$-ray source, accumulates data slowly, and works in a narrow dynamic range with frequency dependent sensitivity. The interferometer technique requires attachment and maintenance of alignment of a mirror and is extremely sensitive to small animal movements.
3. For an introduction to this technique, see M. J. French, J. C. Angus, A. G. Walton, *Science* **163**, 345 (1969); and P. Rabinowitz, S. Jacobs, R. Targ, G. Gould, *Proc. I.R.E. (Inst. Radio Engrs.)* **50**, 2365 (1962).
4. H. Z. Cummins and H. L. Swinney, in *Progress in Optics*, E. Wolf, Ed. (North-Holland, Amsterdam, 1970), vol. 8, p. 133.
5. The exact expression is

$$i = i_s + i_r + 2\epsilon(i_s i_r)^{1/2}\{\cos\Omega[J_0(4\pi a/\lambda) + 2\sum_{n=1}^{\infty} J_{2n}(4\pi a/\lambda)\sin(n\omega_s t)] - \sin\Omega[2\sum_{n=0}^{\infty} J_{2n+1}(4\pi a/\lambda)\sin(n\omega_s t)]\}$$

where $J_n$ is the $n$th order Bessell function. See H. A. Deferrari, R. A. Darby, F. A. Andrews, *J. Acoust. Soc. Am.* **42**, 982 (1967).
6. $\Omega = 4\pi\Delta l/\lambda$, where $\Delta l$ is the difference in length between the two interferometer arms.
7. The exact expression for the terms discussed in the text is:

$$i = i_s + i_r + 2\epsilon(i_s i_r)^{1/2}[J_0(\alpha)J_0(\beta)J_0(4\pi a/\lambda)\cos\Omega - 2J_1(4\pi a/\lambda)J_0(\alpha)J_0(\beta)\sin\Omega \sin\omega_s t - 2J_1(\alpha)J_0(4\pi a/\lambda)J_0(\beta)\sin\Omega \sin\omega_\alpha t - 4J_1(\alpha)J_1(\beta)J_0(4\pi a/\lambda)\cos\Omega \sin\omega_\alpha t \sin\omega_\beta t + \ldots]$$

where $\alpha$ and $\beta$ are the phase modulation amplitudes at frequencies $\omega_\alpha$ and $\omega_\beta$, respectively. The zeros of the Bessel functions provide basis for absolute calibration.
8. Our lock-in amplifier at $\omega_s$ is a P.A.R. model HR-8, the $\omega_\beta$ lock-in is a P.A.R. model 120, and the $\omega_\alpha$ lock-in is an Ithaco model 353. For an introduction to lock-in techniques, see R. D. Moore, *Electronics* **35** (No. 23), 40 (1962); O. C. Chaykowski and R. D. Moore, *Princeton Applied Research Technical Note T-196* (1968) or *Res./Develop.* (April 1968), p. 32.
9. H. Nocke, *J. Comp. Physiol.* **80**, 141 (1972).
10. B. M. Johnstone, J. C. Saunders, J. R. Johnstone, *Nature (Lond.)* **227**, 625 (1970).
11. A detailed publication is in preparation.
12. The scattering properties of these structures should be adequate. We have succeeded in observing the vibration of a translucent internal partition in the cricket trachea. Furthermore, Kohllöffel was able to observe speckle patterns due to scattered lased light from the basilar membrane in the guinea pig cochlea [L. U. E. Kohllöffel, *Acustica* **27**, 49 (1972)].
13. Considerably higher drift velocities can be canceled out by the feedback system alone, but 1 $\mu$m/sec has proved to be entirely adequate for our present applications.


14. Research supported by NSF and NIH. We thank J. J. Loftus-Hills for help in the initial phase of this research.

19 March 1974


## Woodruffia metabolica: An Exception to the Rule of Desmodexy


Abstract. Woodruffia metabolica *(Protozoa, Ciliophora) has a compound fiber on the left side of its ciliary rows composed of parallel, overlapping ribbons of kinetosome-based microtubules. No similar structure is found connecting the kinetosomes on the right side of the ciliary rows. This arrangement is in opposition to the rule of desmodexy.*


In the ciliated protozoans, one finds some of the most structurally complex cells in existence. Much of this complexity lies in the intricate arrangement of fibrillar systems. Varieties and arrangements of these seem endless but a few common elements have been documented (*1*). One of these, the kinetodesmos, has been known for more than 35 years. As defined by Chatton and Lwoff (*2*), the kinetodesmos is a fiber which connects the kinetosomes (basal bodies) of a ciliary row (kinety); this fiber is always seen accompanying the kinety on its right. It is conventional to refer to the right of a row as if the observer were inside the cell looking outward, with the cell's anterior end uppermost. This constant position of the kinetodesmos led Chatton and Lwoff to propose this orientation as a general feature of many ciliated protozoa, and they termed this generalization desmodexy (*2*). In the subsequent three decades, no exceptions to this generalization were reported; thus, it became known as the rule of desmodexy and has been helpful to electron microscopists for orientation purposes.

The rule of desmodexy has received little challenge in subsequent ultrastructural studies. In 1961 Dumont (*3*) reported an unusual fibrillar arrangement in the proboscis of *Dileptus anser* which, he felt, violated the rule of desmodexy. This apparent exception to the rule cannot be considered valid for two reasons: first, the kinetosomes which give rise to the fiber in question are part of the feeding apparatus (the rule of desmodexy pertains only to body kinetosomes); and, second, the fiber stops short of entering a compound fiber (*4*) and therefore does not connect the kinetosomes in a row. A possible exception to the rule does exist in the ciliate *Colpoda cucullus* (*5*). Here, there is a fiber on the left side of the kineties, but published micrographs do not present a clear picture of the extent of this fiber or whether it connects the kinetosomes in a row. To my knowledge, the arrangement of the newly described fiber in *Woodruffia metabolica* is the first fully demonstrated valid exception to the rule of desmodexy.

Organisms for the study (strain WR1) were fixed for 1 hour in glutaraldehyde in phosphate buffer (2 percent by volume) at *p*H 6.8, postfixed in aqueous osmium tetroxide (2 percent, weight to volume) for 90 minutes, and embedded in an Araldite-Epon mixture (*6*). Flat embedding was employed in order to achieve precise specimen orientation. Thin sections were cut with glass knives on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-11E electron microscope operated at 75 kv.

*Woodruffia metabolica* is a large ciliate, frequently measuring 400 $\mu$m or more in length (Fig. 1A), flattened ventrally and somewhat flattened dorsally. Viewed from the ventral aspect, its shape varies from an elongate ovoid to somewhat pear-like. The mouth (buccal area) appears as a crescent-shaped slit in the anterior third of the ventral surface. The body has a uniform ciliation arranged in nearly meridional rows originating at the border of the buccal area from which they course posteriad. They are visible in Fig. 1A as dark stripes.

The cell surface shows a series of longitudinal ridges with the cilia arising between them. Kinetosomes of these cilia are always found in pairs; sometimes both kinetosomes bear cilia but often the anterior of the two kinetosomes is barren.

Each kinetosome pair has many of the established fibrillar and microtubular accessories. Figure 2 shows the relationships between the kinetosomes and the fibrillar structures. The orientation of the figure is with anterior to the observer's left and posterior to the right. In Fig. 2, A to D, the views are from inside the cell looking outward and the kinety's left is toward the bottom of the figure; the series shows kinetosome pairs cut at successive levels, with Fig. 2A the most proximal (deepest in the cytoplasm). Figure 2E shows the kinetosome pair in oblique section and Fig. 2F in almost exactly longitudinal section; in both the view is from the left. A postciliary ribbon of microtubules is

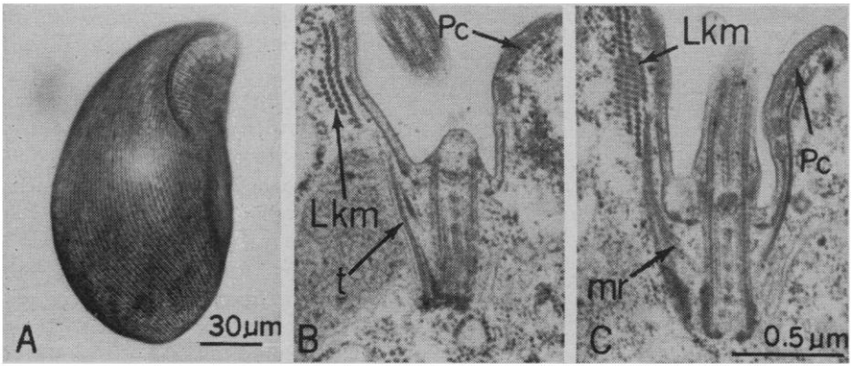


Fig. 1. (A) Light micrograph of *Woodruffia metabolica* showing the pattern of kineties (dark stripes) (× 270). (B) Transverse section through the organism showing an anterior member of a kinetosome pair in longitudinal section. The position of the transverse ribbon of microtubules (*t*), the left Km fiber (*Lkm*), and the postciliary ribbon of microtubules (*Pc*) can be seen (× 30,000). (C) Posterior kinetosome and its cilium in longitudinal section showing the newly described microtubular ribbon (*mr*) turning laterally and upward into the left pellicular ridge to join the Lkm fiber (× 30,000). Both (B) and (C) are oriented so that the sections are viewed looking toward the anterior with the left toward the viewer's left.

adjacent to triplet 9 of the posterior kinetosome [De Puytorac convention (*7*)] and courses upward into the right pellicular ridge and posteriad for a distance of one or two kinetosomal pairs (*Pc* in Figs. 1, B and C, and 2A). A transverse or tangential ribbon of microtubules arises to the left of the anterior kinetosome (*t* in Figs. 1B and 2, A to E) and courses upward into the left pellicular ridge; also present is some dense fibrous material connecting the kinetosomes proximally and at about one-third of the way distally (Fig. 2, A, B, and F). The structures described thus far occur commonly in ciliates and show no significant variation from the norm.

Also present in each kinetosomal doublet is a previously undescribed fiber, a ribbon of microtubules (*mr* in Figs. 1C and 2, A to F), which arises between the two kinetosomes of a kinetosomal pair, angles upward to the left, and then turns posteriad. The origin of the ribbon is in the area of triplets 4 and 5 of the posterior kinetosome. Favorable sections indicate that it arises at about a 45° angle with only one edge near or in contact with a kinetosomal triplet (Fig. 2, E and F). Posteriorly the ribbon aligns itself adjacent to other ribbons which have arisen from other kinetosomes located more anteriorly. Thus, in each pellicular ridge a compound fiber is formed composed of several kinetosome-based microtubular ribbons (*Lkm* in Figs. 1, A to C, and 2D), each entering the fiber at its right. The ribbons terminate at the left margin of the fiber approximately three to five kinetosomal pairs posteriad. The fiber corresponds to a kinetodesmos inasmuch as it connects the kinetosomes in a row, but it does not conform to the kinetodesma of other ciliates in its position.

Introduction of a new term for this fiber has been avoided for two reasons. First, a good, simple, descriptive term does not fall readily to hand, and, second, it may be a disservice to create new terminology in an area yet to be further standardized. An attempt has recently been made to standardize terminology (*7*) but is slow in gaining universal acceptance. At present, the term kinetodesmos is restricted to the right-anterior, striated fiber bundle found in some ciliates, since that is the fiber Chatton and Lwoff were looking at when they coined the term. Some confusion has arisen because many ciliates in the order Heterotrichida

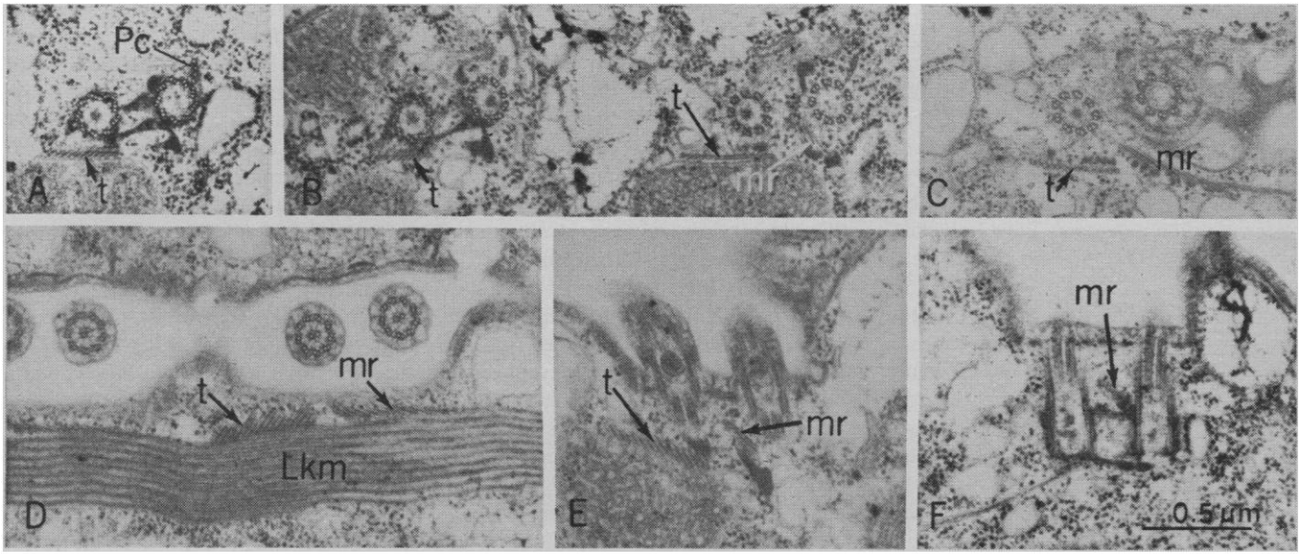


Fig. 2. (A to D) Cross sections through kinetosomes at successive levels from the proximal portion (A) to a point just above the emergence of the cilia (D). Various components of the infraciliature are shown: postciliary ribbon of microtubules (*Pc*), transverse ribbon of microtubules (*t*), newly described microtubular ribbon (*mr*), and the left Km fiber (*Lkm*). Sections are viewed from the inside of the cell looking outward; anterior is toward the viewer's left and posterior toward the viewer's right. The left of each kinety is toward the bottom of the figure. (E) Oblique section through a kinetosome pair showing the microtubular ribbon (*mr*) near its origin and the transverse ribbon (*t*). (F) A nearly longitudinal section through a kinetosome pair showing the origin of the microtubular ribbon (*mr*). Magnification (A through F), × 30,000.

possess a fiber along the right of their kineties which is structurally quite different from the kinetodesmos. This fiber, found originally in *Stentor* (*8*) and termed the Km fiber, is composed of stacks of parallel, overlapping, kinetosome-based microtubular ribbons which course to the right of the kineties and posteriad. Both these fibers satisfy the rule of desmodexy but are considered nonhomologous; as a result, the term Km fiber has been retained by most authors to distinguish the structure found in heterotrichs from the kinetodesmos. Therefore, for *Woodruffia metabolica* at least, the letters Km are retained and L is added to indicate left; hence the term Lkm fiber because of its superficial resemblance to the Km fiber of many heterotrichs. It is emphasized that the use of the term Lkm for the structure described in this report is merely to indicate similarity in appearance to the heterotrich fiber. I do not believe that there is any homology in these structures because the ribbons of microtubules comprising the true Km fiber are postciliary microtubules and course to the right of a kinety, while ribbons of the Lkm fiber differ in their origin and position. *Woodruffia metabolica* also has a postciliary ribbon of microtubules associated with each kinetosomal pair, but it is poorly developed, only extending for one or two kinetosomal pairs posteriad. These postciliary microtubules do run parallel to the kinety and they do overlap the postciliary ribbons from the next one or two kinetosomal pairs posteriad. However, they do so in a linear fashion and thus form a single ribbon of microtubules. The true Km fiber, on the other hand, is composed of stacks of parallel, overlapping ribbons of microtubules. The conspicuous difference in construction between the postciliary ribbons in *Woodruffia* and the Km fiber of many heterotrichs makes it difficult to consider the *Woodruffia* structure a true Km fiber. However, since the single ribbon in *Woodruffia* is composed of microtubules from more than one kinetosome, it may well be that this type of configuration represents a Km prototype or perhaps an attenuated Km fiber. At any rate, the postciliary microtubules in *Woodruffia* seem almost certainly homologous to Km fibers.

The desmodexy problem is quite interesting: Why should there be so consistently a fiber to the right of a kinety in so many different types of ciliates, and why should this precise arrangement of parallel, overlapping fibrils be retained in nonhomologous structures? There is good evidence that sliding of the microtubules in the Km fiber plays a role in cell extension in the contractile ciliate *Stentor* (*9*) but there is almost no direct evidence as to the function in noncontractile ciliates. The puzzle becomes even more complex with the present demonstration that the usual dextral orientation is not necessary; the same kind of composite bundle can be formed by sinistrally oriented fibrils.

THOMAS K. GOLDER
*Department of Zoology, University of California, Davis 95616*

**References and Notes**

1. D. R. Pitelka, in *Research in Protozoology*, T. T. Chen, Ed. (Pergamon, New York, 1969), vol. 3, p. 280; J. Grain, *Ann. Biol.* **8**, 54 (1969).
2. E. Chatton and A. Lwoff, *C. R. Seances Soc. Biol. Fil.* **118**, 1068 (1935).
3. J. N. Dumont, *J. Protozool.* **8**, 392 (1961).
4. J. Grain and K. Golinska, *Protostologica* **5**, 269 (1969).
5. P. Didier and M. G. Chessa, *ibid.* **6**, 301 (1970).
6. J. N. Grim, *J. Protozool.* **14**, 625 (1967).
7. P. De Puytorac, *ibid.* **17**, 358 (1970).
8. J. T. Randall and S. F. Jackson, *J. Biophys. Biochem. Cytol.* **4**, 807 (1958).
9. B. Huang and D. R. Pitelka, *J. Cell Biol.* **57**, 704 (1973).
10. I thank D. R. Pitelka, L. E. Rosenberg, and S. L. Wolfe for their valuable assistance in the preparation of the manuscript. Supported by research funds from the Department of Zoology, University of California, Davis.

18 September 1973; revised 17 April 1974

## Photochemical Activity of Single Chloroplasts Recorded by the Use of Nuclear Track Emulsion

Abstract. *The photochemistry done by single chloroplasts can be measured when the chloroplasts are embedded in nuclear track emulsion. It has been known for more than 50 years that certain chemicals will blacken photographic plates (chemical fogging). Although this effect has been little used to measure chemical reactions, it may be particularly useful in photochemistry and electrochemistry, since as little as $10^{-18}$ mole can be measured.*

In the study of the process of photosynthesis in green plants, one of the most informative and widely used procedures is the Hill reaction (*1*). A suspension of chloroplasts provided with an electron acceptor will, when illuminated, oxidize water to oxygen and reduce the electron acceptor, thus separating the photochemical reaction completely from the complicated series of enzyme reactions that make up the Calvin cycle.

Our purpose in this report is to describe a modification of the Hill reaction that is so sensitive that one can easily measure the photochemistry done by a single chloroplast. Chloroplasts are embedded in nuclear track emulsion and then, while wet, are exposed to red light. The red light by itself does not affect the emulsion. The illumination of the chloroplasts reduces some of the silver ions in the nearby grains to form a "latent image." It is thought that in the photographic process the latent image is made by the reduction of some small number (three to ten) of silver ions in a single grain (*2*). This small amount of metallic silver located at a "sensitive speck" makes it possible for the photographic developer to reduce all of the silver in that grain. In this emulsion the grains are 0.2 to 0.3 $\mu$m in diameter and thus contain from $1 \times 10^8$ to $3 \times 10^8$ silver ions. Therefore, the small amount of silver reduced by the chloroplast is amplified by the photographic process some millions of times to make a black grain that can be seen with the light microscope.

This procedure is somewhat reminiscent of the Molisch reaction—that is, the reduction of silver nitrite by chloroplasts (*3*)—but differs from it in two important respects. First, in the Molisch reaction there is considerable reduction of silver in the dark, whereas we find no grains above the background at all in the dark. Second, the Molisch reaction requires bright illumination for some minutes, whereas this new procedure can be done with small amounts of light.

"Broken" chloroplasts were isolated from greenhouse-grown Good-King-Henry (*Chenopodium bonus-henricus*) by a variation of the method of Walker (*4*). Leaves were kept in running water for about 1 hour, after which 50 g was ground in a Waring blender by means of three high-speed blasts of 5 seconds each. The blender contained 200 ml of the following ice-cold grinding medium: 0.02*M* tris(hydroxymethyl)aminomethane hydrochloride (*p*H 7.4), 0.45*M* sucrose, 0.001*M* $MgCl_2$, and 0.001*M* NaCl. The leaf