important, from heterozygotes for familial metabolic disorders, should prove to be generally useful. For example, similar studies in another lysosomal enzyme disorder, acid phosphatase deficiency, have produced equally informative results (*11*). In the heterozygotes for this disorder, acid α-glucosidase from lymphocytes shows a normal response to stimulation with PHA, while comparison of increase of acid phosphatase to increase of acid glucosidase shows a relative lack of stimulation of acid phosphatase activity. This, as expected, is the opposite result of our findings in patients with Pompe's disease, where the relative lack of stimulation in the heterozygote is that of acid α-glucosidase activity.

This method of analysis may prove useful in the detection of heterozygotes, especially in those diseases in which the defect is not easily detected in uncultured blood specimens. Even in those diseases where the carrier state can be detected in cultured fibroblasts, this approach appears to be preferable in terms of time and expense of materials and labor. These considerations would also make it practical as a screening procedure.

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Ciliary Orientation: Controlled by Cell Membrane or by Intracellular Fibrils?

Abstract. *The cirri of the ciliate* Euplotes *all assume the "reversed" orientation whenever the cell is depolarized and the "forward" orientation whenever the cell is hyperpolarized. Potential changes arise spontaneously or are induced by electrical or mechanical stimuli. The orientation responses of the cirri are apparently independent of intracellular "neuromotor" fibrils previously assigned a coordinating function, as they persist after the fibrils are transected.*

Taylor (*1*) reported that coordination of orientation between the widely separated cirri and membranelles of the hypotrich *Euplotes* (*2*) was lost as a result of microsurgical transection of "neuromotor" fibrils which run through the cytoplasm from a "motorium" to the bases of the organelles. This report, widely quoted, was largely responsible for speculation, still prevalent, that intracellular systems of fibrils, performing a nervelike function, are involved in the coordination of ciliary activity. This speculation has received support from a report that "crawling movements and the characteristic avoiding reaction [in *Euplotes*] . . . are not performed normally after the large fibres, connecting anal cirri with membranelles, have been cut" (*3*). Taylor's experiments were also repeated with the aid of cinematography (*4*) with the opposite conclusion—namely, that sectioning of the fibrils does not destroy coordination between widely separated organelles.

We have reexamined this problem considering both conventional electrophysiological concepts and recent findings on the control of ciliary orientation in *Paramecium* (*5*, *6*). Our results verify the proposal (*4*) that orientation of the motile organelles of *Euplotes* is coordinated by changes in membrane potential which spread over the cell, and is independent of intracellular fibrils. Potential changes which control the orientation of cilia occur both spontaneously and in response to stimuli.

Experimental methods were similar

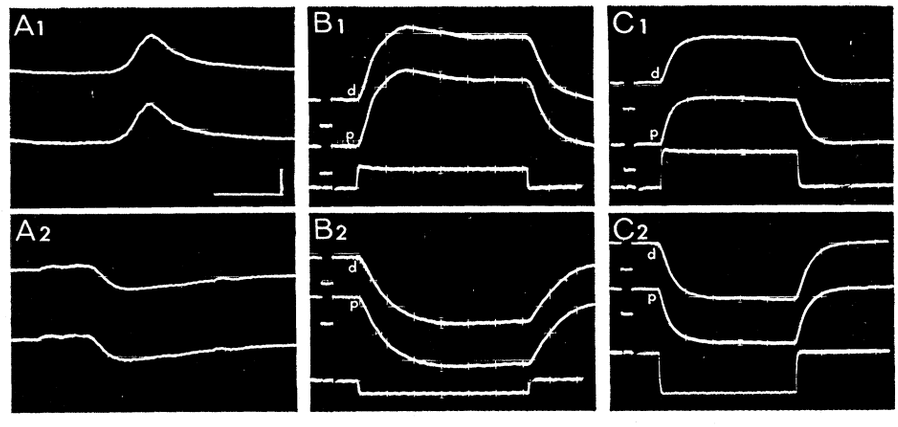


Fig. 1. Membrane potentials recorded with two intracellular electrodes inserted near opposite ends of a specimen. Separation of electrodes was 60 to 80 percent of cell length. (A_1) Spontaneous depolarizing wave; (A_2) spontaneous hyperpolarizing wave in same specimen; (B_1) outward current (bottom trace) injected with polarizing electrode inserted near proximal (*p*) recording electrode; (B_2) same specimen showing response to hyperpolarizing current; (C_1) similar to B_1 but with "neuromotor" fibrils visibly transected by a massive incision halfway between the proximal (*p*) and distal (*d*) recording electrodes; (C_2) same specimen as C_1, showing membrane potential recorded from both sides of incision in response to hyperpolarizing current injected near proximal electrode. Calibrations are 10 mvolt, 200 msec in A; 10 mvolt, 20 msec in B and C.

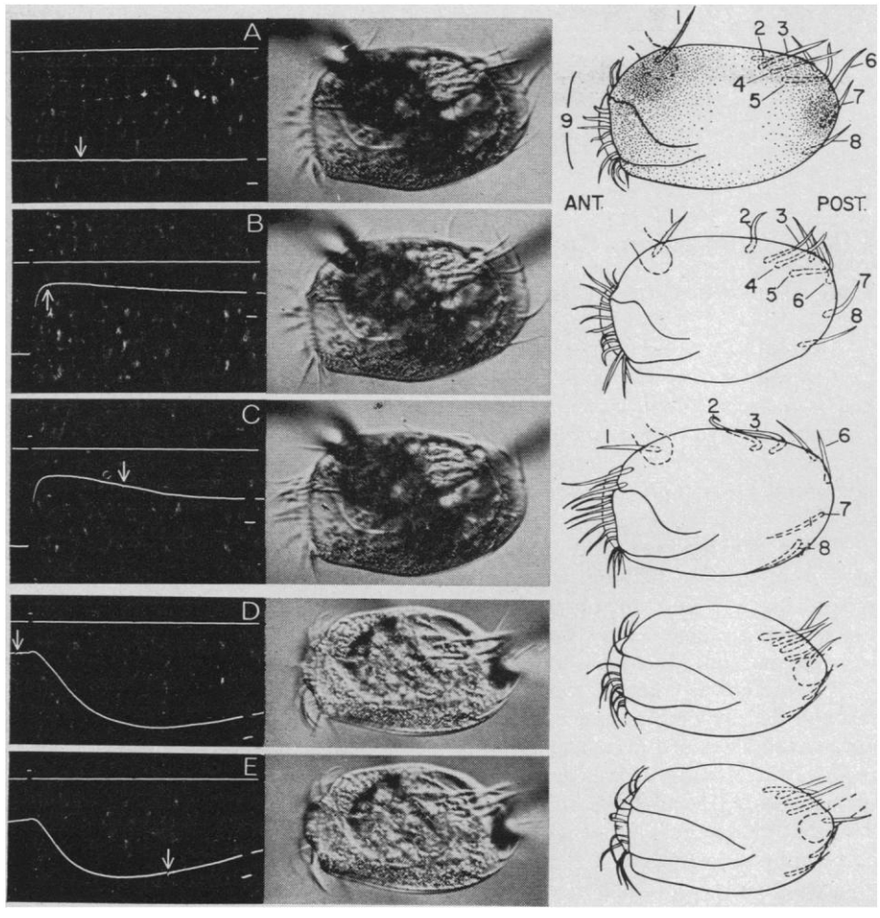
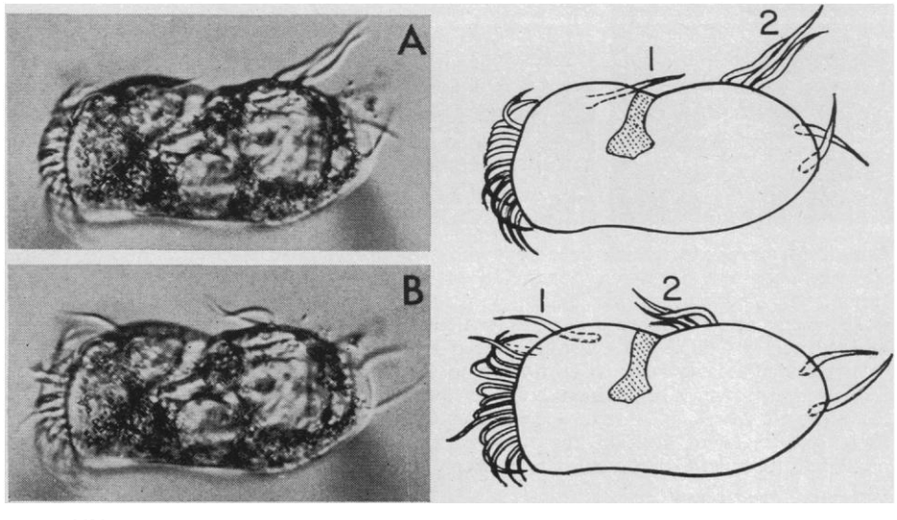


Fig. 2. Orientation of ciliary organelles of *Euplotes* in response to mechanical stimulation. Membrane potential is shown in the lower trace of each recording. Deflection of the upper trace indicates pulse applied to mechanical stimulator. Specimens paralyzed with $NiCl_2$ to permit still photography of cirri. (Arrows) Electrical artifacts coincident with electronic flash discharged for corresponding photomicrographs in center column. (A) No stimulus, membrane potential steady at −25 mvolt, cirri in "resting" orientation. (B) Light tap at anterior (*ANT*) end evoked a depolarization. Organelles began to assume a more anterior orientation. (C) Flash discharged later during similar depolarization. (D) Different specimen, light tap at posterior (*POST*) end of cell evoked a hyperpolarization. Flash discharged prior to the hyperpolarization to show "resting" orientation of organelles. (E) Flash discharged after peak of hyperpolarization to show slight accentuation of "resting" (forward swimming) orientation. Stippling in tracing A indicates regions of highest mechanical sensitivity. 1, Frontal cirrus; 2–5, anal cirri; 6–8, caudal cirri; 9, adoral zone of membranelles. Where an organelle was oriented in a position outside optical section it is unclear or undiscernible in the photomicrograph. Our numbering is arbitrary and for purposes of reference only. Calibration, 10 mvolt and 10 msec.



in all essentials to those reported earlier (*5, 7*). Specimens of *Euplotes* sp. were secured to the lower surface of a cover glass with microneedles, and were bathed in a standard solution of 1.0 m*M* KCl, 0.5 m*M* NaCl, 0.5 m*M* $CaCl_2$, and 1.0 m*M* tris(hydroxymethyl)aminomethane at *p*H 7.2. When we wished to prevent beating of the cilia, $NiCl_2$ was added to a final concentration of 5 mmole/liter until beating ceased (*8*). The organism was then washed free of nickel with the standard solution. Cilia treated with Ni reverse their direction of orientation in response to the same stimuli which elicit the reversal of actively beating cilia (*9*). Photomicrographs, by stroboscopic illumination and Zeiss interference contrast, were made to document ciliary orientation. Interference contrast also enabled us to visualize Taylor's neuromotor fibrils during experiments. Intracellular recording and polarizing electrodes, of the conventional glass capillary type, were below 0.5 μm in tip diameter, and were filled with 0.1*M* KCl (*7, 10*).

Identical membrane potentials were recorded simultaneously from opposite ends of the cell. Resting potentials ranged from −18 to −26 mvolt, and exhibited spontaneous depolarizing and hyperpolarizing deviations (Fig. 1A). The potential changes shown in Fig. 1B were in response to currents applied through a polarizing electrode inserted at one end of the cell. Figure 1C is similar, except that a transverse incision was made halfway through the specimen between the two recording electrodes. This incision transected the neuromotor fibrils of Taylor (*1*). The potentials recorded from different ends of the cell were essentially identical in spite of the deep incision, and in spite of a drop in the input resistance of the cell. The formation over the exposed cytoplasm of a membrane continuous with the plasma membrane presumably accounts for the electrical integrity of the cell after the incision.

Fig. 3. Specimen with transected "neuromotor" fibrils. Massive incision was made with fine glass needle halfway through the organism on its right side. This is equivalent to the operation illustrated by Taylor's plate 29 and figure 1 (*1*). The incision visibly severed the neuromotor fibrils of Taylor and also resulted in some loss of cytoplasm before new membrane formed over exposed cytoplasm. (A) Photomicrograph made in absence of applied current; (B) reorientation of cirri in response to a depolarization induced by outward current injected near one end of cell (Fig. 1, C_1).

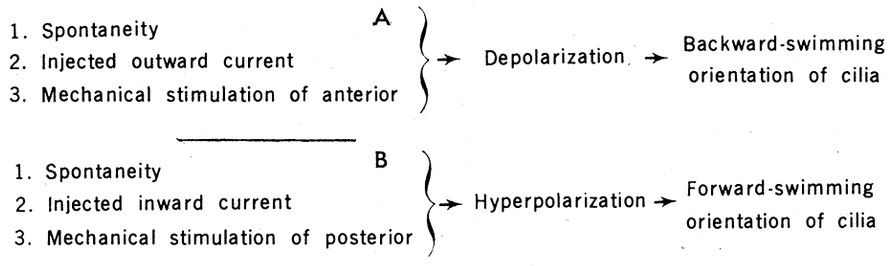


Fig. 4. Summary of bioelectric events which influence the locomotor behavior of *Euplotes*. Events A1 and B1 are illustrated in Fig. 1, A_1 and A_2. Events A2 and B2 are illustrated in Fig. 1, B_1 and B_2. Event A3 is illustrated in Fig. 2, B and C, while event B3 is illustrated in Fig. 2, D and E.

We examined electrical responses to mechanical stimulation (*5*). The depolarizations were evoked by a light tap applied to the anterior end of the specimen with a fine glass stylus driven by a ceramic phono cartridge (Fig. 2, B and C). Hyperpolarizations were evoked by a similar stimulus applied to the posterior end (Fig. 2, D and E). As in *Paramecium* (*5*), mechanical stimulation at the anterior end invariably results in a depolarization, and stimulation at the posterior end always elicits a hyperpolarization.

The ciliary organelles of *Euplotes* invariably assume an anterior-pointing (reversed) orientation in response to depolarization (Fig. 2, B and C) and an augmented posterior-pointing (forward locomotion) orientation in response to hyperpolarization (Fig. 2, D and E). This correlation between membrane potential and the orientation of cirri and membranelles was exhibited by beating cilia and by paralyzed cilia regardless of whether the potential changes were spontaneous, evoked by mechanical stimulation, or evoked by the injected current.

In specimens with transected neuromotor fibrils, depolarizing current injected at one end of the cell evoked reversal of the organelles on both sides of the incision (Fig. 3). The reasons for Taylor's (*1*) conclusion that fibrillar transection abolishes coordination are not clear. However, we did find that massive incisions cause some deterioration and depress spontaneous fluctuations in membrane potential. By supplying a depolarizing current we have shown that the organelles still reverse in unison to depolarization after transection of the fibrils.

The evidence reported here and elsewhere (*5*, *11*) indicates that the coordination of ciliary orientation results primarily from electrotonic spread of spontaneous and evoked membrane potentials (Figs. 1A and 2) which control the positions of the individual cilia, cirri, and membranelles (Fig. 4). The view that systems of fibrils coordinate the orientation of cilia (*12*) is no longer credible.

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Codon Recognition by Enzymatically Mischarged Valine Transfer Ribonucleic Acid

Abstract. *Direct evidence for the adaptor hypothesis has been obtained by examining the codon recognition of a purified* Escherichia coli *valine transfer ribonucleic acid which was enzymatically mischarged with phenylalanine labeled with carbon-14 by reaction with purified phenylalanyl-transfer ribonucleic acid synthetase from* Neurospora crassa. *The mischarged transfer ribonucleic acid recognized the valine codons but failed to recognize the phenylalanine codon when tested in trinucleotide-directed ribosomal binding assay.*

The adaptor hypothesis of Crick (*1*) states that the amino acid sequence of a protein is determined, during the course of the translation and protein synthesis, by the alignment of aminoacyl transfer ribonucleic acids (tRNA) at corresponding nucleotide triplets (codons) in messenger RNA (mRNA). The specificity of the translation mechanisms depends on base pairing between a nucleotide region of tRNA, the anticodon, and nucleotides in the codon. The hypothesis predicts that the amino acid bound to tRNA does not participate in this recognition. The first evidence for the passive role of the amino acid was obtained by Chapeville *et al.* (*2*); they showed that [^{14}C]cysteine, while attached to its tRNA (tRNA^{Cys}), (*3*), was converted by catalytic reduction with Raney nickel to [^{14}C]alanine. When the resulting complex, [^{14}C]-alanyl-tRNA^{Cys}, was tested in a protein synthesizing system, radioactivity was incorporated under the direction of poly UG, a polymer which normally stimulates the incorporation of cysteine but not alanine (*2*). Similarly, von Ehrenstein *et al.* (*4*) found that the chemically altered aminoacyl-tRNA delivered [^{14}C]alanine, in place of cysteine, in a peptide of hemoglobin.

We used a different system to confirm the results of Chapeville *et al.* (*2*). Our approach was made possible by the discovery (*5*) that a phenylalanyl-tRNA synthetase of *Neurospora crassa* can mischarge phenylalanine onto two *Escherichia coli* tRNA's which have been identified as tRNA^{Val} and tRNA^{Ala} (*6*). Earlier studies had indicated that the phenylalanyl-tRNA^{Ala} responded to poly UCG in the same manner as alanyl-tRNA^{Ala} of *N.* crassa and *E. coli* (*6*, *7*). We have asked whether va-