

high level of triterpene, tetrahymanol and 2-amino-ethyl-phosphonolipid is less fluid than pellicle and microsome membranes. Microsomes are most fluid and have the lowest content of these 2 lipids, together with the highest degree of unsaturation in fatty acyl chains.

Alterations in the microviscosities of pellicle and microsome membranes isolated from supplemented-cells with ergosterol or chimyl alcohol are clearly depicted in figure 2. Ergosterol-replaced pellicle and microsome membranes become more rigid, whereas chimyl alcohol-fed membranes are more fluid, when compared with the control native membranes. It is of interest to note that, in pellicles, rigid-making effect of ergosterol-supplementation and fluidizing effect of chimyl alcohol-feeding are pronounced above and below about at 22–25 °C, respectively. Microsome membranes from ergosterol-replaced cells are markedly less fluid above 22 °C. However, at the present time we have no exact explanation for such phenomena on the basis of the lipid composition alterations of the manipulated membranes. Similar trends were also observed with dispersions of lipids extracted from the manipulated pellicles and microsomes (data not shown).

The *in vivo* manipulated *Tetrahymena* membranes with altered fluidities would provide a potential clue towards understanding relationships between fluidities and functions of biological membranes. For example, in our recent work on adenylate cyclase in the pellicle membrane, we have obtained data indicating effects of ergosterol-replacement upon the transition of the activation energy of the enzyme, and the detailed results will be reported elsewhere.

- 1 Acknowledgments. We wish to express thanks to Central Scientific Commerce, Inc., Tokyo for permission to use an Elscint Microviscosimeter MV-1a. This work was in part supported by grant from the Ministry of Education.
- 2 S.J. Singer, *A. Rev. Biochem.* **43**, 805 (1974).
- 3 D. Chapman and P.J. Quinn, *Chem. Phys. Lipids* **17**, 363 (1976).
- 4 Y. Nozawa and G.A. Thompson, *J. Cell Biol.* **49**, 722 (1971).
- 5 H. Iida, T. Maeda, K. Ohki, Y. Nozawa and S. Ohnishi, *Biochim. biophys. Acta*, in press.
- 6 H. Shimonaka and Y. Nozawa, *Cell Structure and Function* **2**, 81 (1977).
- 7 C.E. Martin, K. Hiramitsu, Y. Kitajima, Y. Nozawa, L. Skriver and G.A. Thompson, *Biochemistry* **15**, 5218 (1977).
- 8 R. Kasai, Y. Kitajima, C.E. Martin, Y. Nozawa, L. Skriver and G.A. Thompson, *Biochemistry* **15**, 5228 (1977).
- 9 G.A. Thompson and Y. Nozawa, *Biochim. biophys. Acta* **472**, 55 (1977).
- 10 Y. Nozawa, H. Fukushima and H. Iida, *Biochim. biophys. Acta* **406**, 248 (1975).
- 11 H. Fukushima, T. Watanabe and Y. Nozawa, *Biochim. biophys. Acta* **436**, 249 (1976).
- 12 G.A. Thompson and Y. Nozawa, *J. Cell Biol.* **49**, 712 (1971).
- 13 Y. Nozawa, in: *Methods in Cell Biology*, vol. 10, p. 105. Ed. D.M. Prescott. Academic Press, New York 1975.
- 14 M. Shinitzky and M. Inbar, *J. molec. Biol.* **85**, 603 (1974).
- 15 M. Shinitzky and Y. Barenholz, *J. biol. Chem.* **249**, 2652 (1974).
- 16 Y. Nozawa, H. Iida, H. Fukushima, K. Ohki and S. Ohnishi, *Biochim. biophys. Acta* **367**, 134 (1974).
- 17 H. Fukushima, C.E. Martin, H. Iida, Y. Kitajima, G.A. Thompson and Y. Nozawa, *Biochim. biophys. Acta* **431**, 165 (1976).

Spirally arranged muscles associated with tracheoles in tsetse fly flight muscles; their possible involvement in sound production

M. Anderson¹

Department of Zoology and Comparative Physiology, University of Birmingham, P.O. Box 363, Birmingham B15 2TT (England), 17 October 1977

Summary. A set of muscle fibres have been found associated with certain tracheoles in the flight muscles of the tsetse fly. It is proposed that those muscles may be involved in sound production in the fly.

The production of sounds in insects can be divided into 2 main categories: 1. sounds produced by frictional methods and 2. sounds produced by movement of air². If, as is suggested, within the 2nd category the sounds are produced by air being pulsed out of the spiracles, a mechanism for controlling the air flow would be necessary. Control of spiracular opening is well documented³ and this operation plays an important part in sound production, however, I propose in addition to the spiracular control that muscle fibres which I have discovered in close association with tracheoles in the indirect flight muscles in the tsetse fly could perform some additional function in sound production.

Ultrathin sections were cut of the indirect flight muscles from the tsetse fly thorax. Fixation, embedding, sectioning and staining for electron microscopical investigation were all carried out according to conventional methods⁴.

In the indirect flight muscles of the tsetse fly tracheation is similar to that described in other flight muscles⁵, with the tracheoles situated within deep clefts created by invaginations of the sarcolemma. Within these intuckings in certain of the indirect flight muscles an elaborate muscular ar-

rangement has been found closely associated with the tracheoles (figures 1–3). I shall refer to these muscles as tracheal muscles to distinguish them from the flight muscles.

The tracheal muscles clearly arise as extensions of the flight muscle myofibrils (figure 1), and are arranged spirally around the tracheoles (figure 2). The spiral arrangement can be clearly appreciated by the transverse, longitudinal and oblique profiles which are observed in these muscle fibres when either longitudinal or transverse sections of the muscle are examined. Surrounding these tracheal muscles are areas of extracellular space (figure 1), and the membranes of the spiralling muscles make tight junctions with the membrane of the flight muscle fibres (figures 1 and 2).

I propose the following model as to how these tracheal muscles may function. The fibres are spirally arranged around the tracheoles, therefore when the muscle fibres contract they will constrict the tracheoles and when the muscles relax the tracheoles will regain their original dimensions. The extracellular space surrounding the tracheal muscles will allow for any size changes which might take place in the diameter of the tracheoles during such

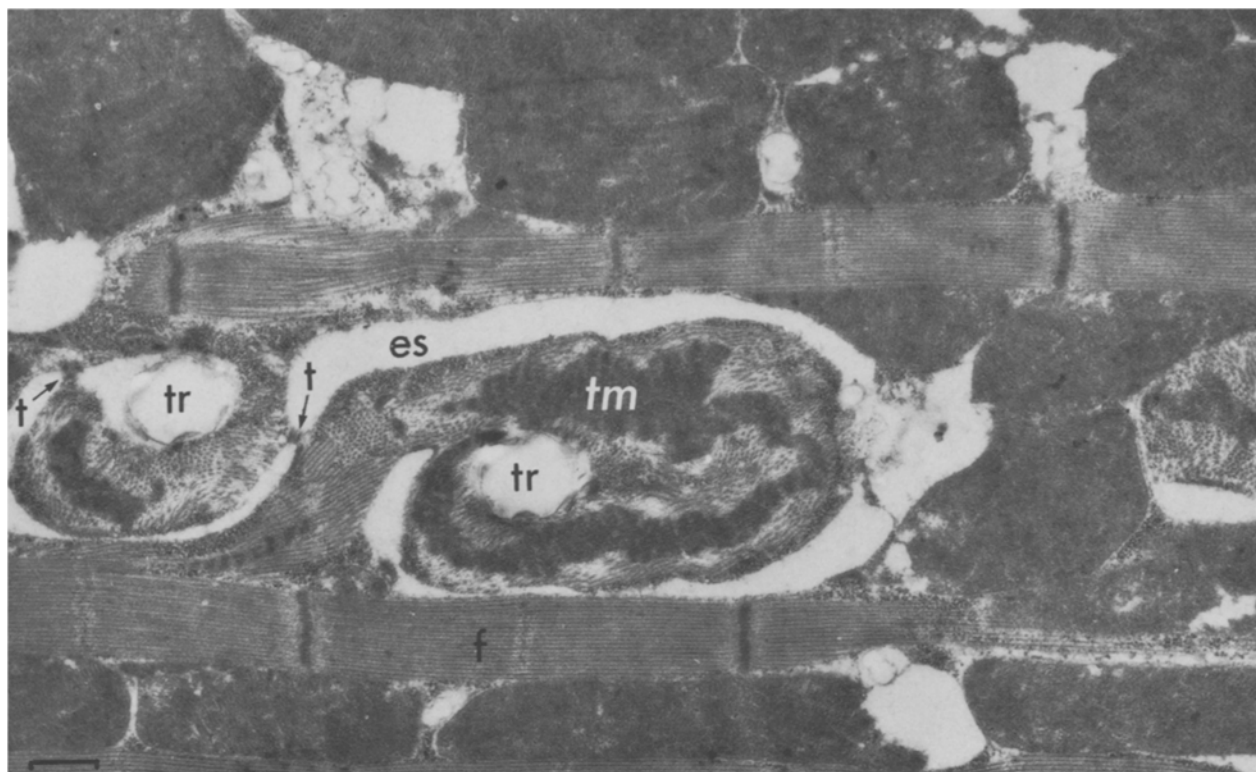


Fig. 1. An electron micrograph of a longitudinal section through a flight muscle of a tsetse fly. Note the tracheal muscles (tm) arising from the myofibrils (f) of the flight muscle and spiralling around the tracheoles (tr). These tracheal muscles make occasional tight junctions (t) with the flight muscle membrane and are surrounded by extensive extracellular space (es). Scale bar 1 μ m.



Fig. 2. An electron micrograph of a transverse section through a flight muscle (f) of a tsetse fly. Within the tracheal muscle (tm) longitudinal, oblique and transverse sections through the contractile material are apparent indicating the spiralling of this muscle around the tracheole (tr). Tight junctions (t) between the membranes of the tracheal muscle and the flight muscles are present. Scale bar 1 μ m.

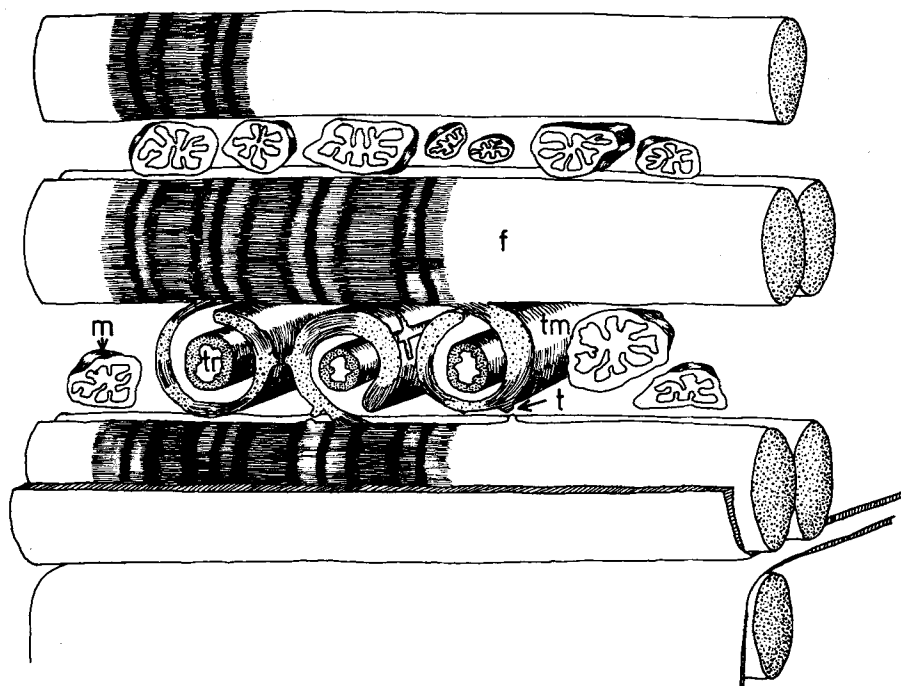


Fig.3. A diagram to show the arrangement of the tracheal muscles (tm) which arise as extensions of the flight muscle myofibrils (f) and spiral around the tracheoles (tr). Large mitochondria (m) are present between the myofibrils. Dimensions are exaggerated slightly for clarity.

movements. When the tracheal muscles contract they will bring about movements of air within the tracheoles, and I propose that 1 possible result of this air movement would be the production of sound at the spiracles.

Much debate surrounds the production of sound by tsetse flies, however, it seems generally accepted that the flies are capable of producing sounds of 2 types. The 1st type of sound is a chirping and/or whining⁶ which is audible to man and the 2nd type of sound is a complex pattern in the ultrasonic frequencies⁷. The methods by which these sounds are produced are far from clear. Some evidence is available to show that the low frequency sounds are produced by distortion of the thorax, in tsetse these sounds continue to be produced even when the wings and halteres are removed and the spiracles are blocked with vaseline⁶. In other dipterans there is evidence that some of the sound is caused by air being pulsed out of the spiracles and causing some membranous foldings to vibrate at high frequencies⁸⁻¹⁰. It is my belief that some of the ultrasonic component in the tsetse fly sound could be produced in a

similar fashion, with some structure in the region of the spiracular openings capable of vibrating at an extremely high frequency and thus produce high frequency sounds.

- 1 I am grateful to the Ministry of Overseas Development for a grant in support of this work and to Professor L.H. Finlayson for his advice and criticism.
- 2 P.T. Haskell, *Insect sounds*. Witherby, London 1961.
- 3 G. Hoyle, *J. Insect Physiol.* 7, 305 (1961).
- 4 M. Anderson and L.H. Finlayson, *J. Insect Physiol.* 19, 1989 (1973).
- 5 O.W. Tiegs, *Phil. Trans. R. Soc. B* 238, 221 (1955).
- 6 F.F. Kolbe, *Zoologica africana* 8, 241 (1973).
- 7 E.C. Erickson and A.R. Møller, *J. acoust. Soc. Am.* 57, 984 (1975).
- 8 W.H. Harris, *J. Quekett microsc. Club* 8, 513 (1903).
- 9 B.T. Lowne, *The Anatomy, Physiology, Morphology and Development of the Blowfly (Calliphora erythrocephala)*. R.H. Porter, London 1890.
- 10 H. Landois, *Z. wiss. Zool.* 17, 105 (1867).

Effects of weak electromagnetic fields on *Physarum polycephalum*: Mitotic delay in heterokaryons and decreased respiration

M. T. Marron, E. M. Goodman and B. Greenebaum

University of Wisconsin-Parkside, Kenosha (Wisconsin 53141, USA), 10 October 1977

Summary. Continuous exposure of *Physarum polycephalum* to a 75 Hz, 2.0 G, and 0.7 V/m electromagnetic field results in a depressed respiration rate and a lengthening of the mitotic cell cycle. If unexposed *Physarum* are mixed with exposed *Physarum* the onset of synchronous mitosis in the mixed culture is delayed, occurring at a time between those of the 2 parent cultures.

In recent years there has been a heightened interest in the effects of weak, extremely low frequency electromagnetic fields (EMF)² on biological systems³⁻⁶. Observed biological effects range from the microscopic level, such as the modification of Ca^{++} efflux from brain tissue⁷, to macroscopic observations that weak low frequency EMF can

affect migrating birds^{8,9}. We have previously shown that continuous exposure of the acellular slime mold *Physarum polycephalum* to EMF (45, 60, 75 Hz at 2.0 G and 0.7 V/m) results in a 10-15% lengthening of the mitotic cell cycle and retardation of reversible protoplasmic streaming^{10,11}. When plasmodia are removed from the fields, both the cell cycle