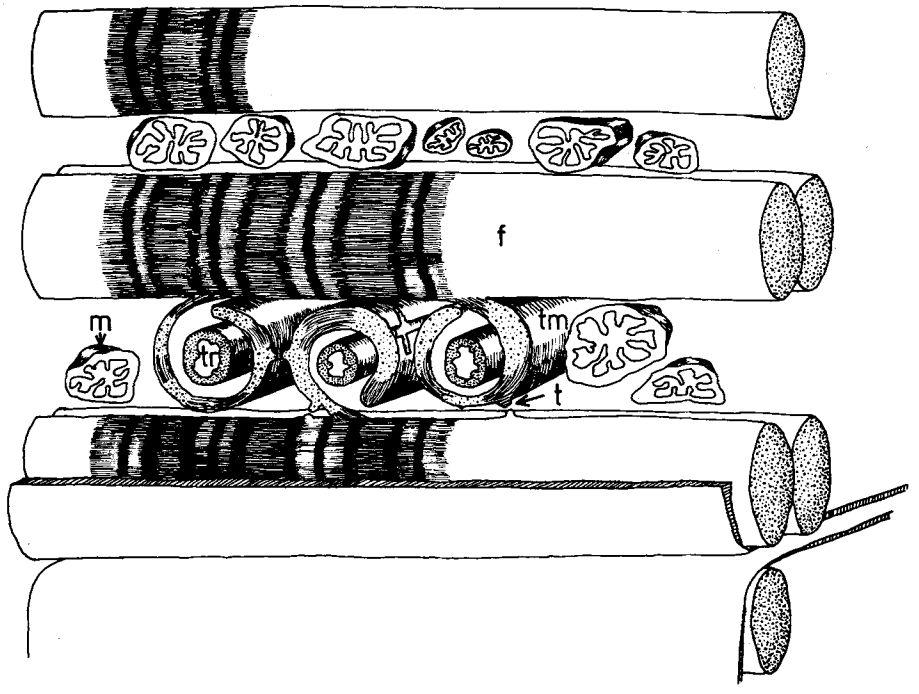

Fig. 3. A diagram to show the arrangement of the tracheal muscles (tm) which arise as extensions of the flight muscle myofibrils (f) and spiral around the tracheoles (tr). Large mitochondria (m) are present between the myofibrils. Dimensions are exaggerated slightly for clarity.

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 whining[6] which is audible to man and the 2nd type of sound is a complex pattern in the ultrasonic frequencies[7]. 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 vaseline[6]. 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 frequencies[8-10]. 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.
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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 hightened interest in the effects of weak, extremely low frequency electromagnetic fields (EMF)[2] on biological systems[3-6]. Observed biological effects range from the microscopic level, such as the modification of Ca^{++} efflux from brain tissue[7], 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

and streaming period return to control levels after 3 to 4 weeks.

In this communication we report that the rate of respiration in EMF-exposed plasmodia is depressed by approximately 15–20% and that the delay in onset of mitosis observed in EMF-exposed *Physarum* persists at a reduced level in heterokaryotic cultures formed by mixing with unexposed *Physarum*. The reduction in delay appears to depend upon the amount of each culture mixed, but not in a simple, linear fashion.

Materials and methods. Maintenance of cultures: A detailed description of our apparatus and culture techniques has been published elsewhere[12,13]. Briefly, submerged microplasmodia are grown in rectangular flasks; electrodes built into the sides of the flasks are connected to circuitry that controls electric field conditions. Magnetic field coils surround a reciprocating platform; both shaker and coils are enclosed in an incubator maintained at 25.5±0.3 °C. Control and experimental incubators are similar in all respects expect that coils and electrodes are not energized in the control incubator.

Oxygen consumption: Control and EMF-exposed microplasmodia were collected in the logarithmic phase of their growth cycle by centrifugation at 250 × g (4 °C). The supernatant was decanted and the pellet resuspended in an equal volume of fresh growth medium. Duplicate 0.5 ml plasmodial samples from each set were placed in sterile Warburg reaction vessels that were calibrated according to Umbreit et al.[14]. Growth medium (2.5 ml) was added and the flasks equilibrated at 25.00±0.015 °C. The CO_2 evolved was trapped in the center well using filter papers saturated with hyamine hydroxide[15]. Protein content was determined by removing plasmodia from the reaction vessel, extracting the pigment in several washes of trichloroacetic acid-acetone-water (50 g TCA; 500 ml acetone; and water to 1 l) and dissolving residual pellet in 0.4 N NaOH. Colorimetric determination of proteins utilized the procedures described by Lowry et al.[16] with bovine serum albumin as a standard.

$^{14}CO_2$ Evolution: Microplasmodia were prepared as described above, with a 0.5 ml suspension placed in sterile Wheaton $^{14}CO_2$ collection flasks (Wheaton Glass Co., Millville, N.J.)[17]. To this suspension 3.5 ml of glucose-free nutrient medium containing 3.5 μCi (^{14}C) D-glucose (specific activity 240 μCi/μM) and 0.14 ml of 1.0 M glucose was added. Scintillation vials containing filter paper strips saturated with hyamine hydroxide were attached to each flask. Vials were removed after a 30 min incubation period, and radioactivity was determined by counting the trapped $^{14}CO_2$ in Bray's solution using a Nuclear Chicago Scintillation counter[17]. Data were analyzed using the Nuclear Chicago CURVFIT program; protein content was determined as outlined above.

Mixing experiments: Measurements of mitotic cycle timing in *Physarum* are made by placing a sample of microplasmodia on a filter paper supported on surgical sponges. The microplasmodia are allowed to coalesce, forming 1 large macroplasmodium containing about 10^8 individual nuclei. Following coalescence approximately 25 ml of nutritive media is added to the culture. The coenocytic plasmodium can be thought of as a single multinucleate cell where all of the nuclei undergo simultaneous division at predictable intervals making observation of the timing a relatively simple matter.

Statistical analysis: Average differences were computed by treating data for each day as paired observations and pooling the variance[18].

Results and discussion. Exposing *Physarum polycephalum* to an extremely low frequency electromagnetic field of 75 Hz, 2.0 G, and 0.7 V/m resulted in a depression in respiration rate of the cultures. The rate of O_2 uptake for control and exposed cultures was measured for 65 separate cultures, on 16 days over a period of 4 months. The QO_2 (μl O_2/mg protein/min) for control cultures was 0.55 whereas exposed cultures had a QO_2 of 0.46; this corresponds to a 16% decrease in respiration for exposed cultures. The average difference in QO_2 is 0.086 with a SD of 0.0087; the t-statistic for this difference is 9.9 with 33 degrees of freedom ($p < 0.001$).

These findings were corroborated by measuring the rate of $^{14}CO_2$ production. Average values of 478 and 419 DPM/mg protein were obtained for control and exposed cultures, respectively, for 30 min collection periods (5 days over 4 months and 20 experiments). This corresponds to a decrease in CO_2 evolution of 12% for exposed cultures which matches the decrease observed in oxygen uptake to within experimental error. The average difference in ^{14}C activity is 59 DPM/mg protein with a SD of 12; the t-statistic for the difference is 5.1 with 10 degrees of freedom ($p < 0.001$). It is noteworthy that the degree to which respiration has been depressed in EMF-treated cultures is the same as that observed in the mitotic cell cycle[10,11].

The results of mixing microplasmodia from both control and exposed cultures in equal volumes are shown in the table. After mixing, 1 single macroplasmodia is formed which undergoes synchronous mitosis at a time between those of the 2 parent cultures. This averaged behavior continues even when the mixture is predominantly non-exposed plasmodia, 3:1 by volume, or vice versa. In mixed (M) cultures made up 1 part control (C) to 3 parts exposed (E) culture, the average differences observed were 0.75 h (M–C) and 0.30 h (E–M). Comparable experiments with 3 parts control and 1 part exposed culture gave average differences of 0.27 h (M–C) and 0.79 h (E–M). These averages are computed from experiments involving 192 separate cultures and were conducted on 12 different days over a period of 1 year. The variation in these data is similar to that seen in the table for 1:1 mixtures. The length of the mitotic cycle in the mixture appears to depend on the

Time to second post-fusion mitosis for control, exposed, and mixed cultures

Control	Δ	Mixed	Δ	Exposed
14.79 (0.09)	0.33	15.11 (0.09)	0.46	15.57 (0.07)
15.67 (0.10)	0.24	15.92 (0.14)	0.66	16.58 (0.06)
16.53 (0.12)	0.25	16.78 (0.12)	0.49	17.27 (0.10)
16.11 (0.11)	0.24	16.35 (0.10)	0.67	17.02 (0.06)
15.24 (0.08)	0.18	15.42 (0.15)	0.48	15.90 (0.09)
15.37 (0.02)	0.02	15.39 (0.05)	1.22	16.61 (0.08)
15.39 (0.08)	0.17	15.56 (0.04)	0.33	15.89 (0.03)
15.96 (0.06)	0.13	16.09 (0.07)	0.71	16.80 (0.06)
15.08 (0.05)	0.50	15.58 (0.05)	0.16	15.74 (0.03)
15.40 (0.11)	0.68	16.08 (0.08)	0.23	16.31 (0.04)
15.76 (0.04)	0.15	15.91 (0.07)	0.88	16.79 (0.03)
15.26 (0.07)	0.34	15.60 (0.06)	0.24	15.84 (0.03)
16.07 (0.03)	0.40	16.47 (0.03)	0.16	16.63 (0.08)
15.27 (0.06)	0.11	15.38 (0.09)	1.25	16.63 (0.10)
15.32 (0.07)	0.35	15.67 (0.05)	0.91	16.58 (0.05)
15.45 (0.14)	0.21	15.66 (0.11)	0.58	16.24 (0.04)
14.82 (0.04)	0.17	14.99 (0.04)	0.42	15.41 (0.06)
15.38 (0.08)	0.17	15.55 (0.04)	0.88	16.43 (0.07)
	0.26		0.60	

Mixed cultures are formed from equal volumes of control and exposed microplasmodia. In each row is given mean times in h for cultures examined on a particular day. The number in parentheses next to the entry is the SD of the mean. Entries in columns headed by a '*Δ*' are differences between the means appearing on either side of the entry; the average difference is given at the bottom of these columns. This table presents data taken for 290 separate cultures collected on 18 different days over a period of 1 year.

amount of each type of culture in the mixture, however the data are too sparse to determine a precise relationship.

Several investigators have previously shown that joining 2 existing macroplasmodia in different phases of the cell cycle can alter the timing of mitosis[19-21]. The mixing experiments described here differ in that 1. contact between the 2 types of plasmodia occurs at the time the macroplasmodia are formed rather than after each individual macroplasmodium has established a synchrony of its own and 2. the 2 partners do not have the same cycle lengths.

These experiments permit several conclusions to be drawn. First and foremost is that exposure of *Physarum* to weak electromagnetic fields produces biological effects. This conclusion is also supported by our previous findings that exposure causes the mitotic cycle and protoplasmic shuttle streaming to slow. We may also conclude that the exposed cultures have been altered in some way that immediately manifests itself when control and experimental cultures are brought together. Similar averaging of the cell cycle has been reported by Haugli et al.[22] in mixtures of normal cultures and cell-cycle mutant cultures that have a lengthened cell cycle. A 3rd important conclusion is that the alteration brought about in exposed cultures is not drastic from the point of view of basic cellular processes. When exposed and unexposed microplasmodia are mixed together and allowed to fuse to form a single large macroplasmodia, the fusion takes place and all nuclei behave normally to the extent that they undergo synchronous mitosis. They behave abnormally in that the mitotic cycle time is different from either parent culture. Since all of the processes we have observed to be influenced by EMF require energy, we suspect that weak low frequency EMF may interfere with either energy generating processes or the transport of essential metabolites in *Physarum*.

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2 Abbreviations: EMF for electromagnetic field; QO_2 for rate of oxygen uptake; C for control cultures, E for exposed cultures, and M for a mixture of E and C cultures.
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Activity of the reticuloendothelial system following exposure to electric stress and thymectomy

A.L. Granata[1], C. Vecchi and A. Corsi[2]

Istituto di Patologia Sperimentale dell'Università, via Grotte di Posatora, I-60100 Ancona (Italy), 13 September 1977

Summary. After electric stress stimulation, granulopectic activity is reduced in otherwise normal rats, whereas it appears to be increased in thymectomized animals. The differences between the 2 groups of animals seem to support the hypothesis that the effects of stress upon the overall phagocytic capacity may be mediated by the products of lymphocyte breakdown.

It has long been known that the phagocytic activity of reticuloendothelial cells is affected by exposure to stress. Some conflicting results in the literature can be explained, at last partially, by the use of different stress procedures, many of which obviously possess specific undesirable components like tissue damage, superimposed infections, blood or plasma loss[3,4]. It is mostly agreed that the phagocytic activity is reduced as a consequence of the rise of plasma corticosteroids acting directly on the macrophage[5-8], although the action mechanism of corticosteroids is still under investigation; recent observations seem to indicate that the role of corticosteroids may be questioned[9].

The effects of thymectomy on the reticuloendothelial system have been reported under several experimental conditions[10-15]: evidence was provided that the phagocytic response to repeated stimulation by colloidal suspensions is much higher in thymectomized rats, whereas it is reduced in normal animals[12].

In the present research, experiments were designed to investigate the interference of thymectomy and stress in the phagocytic activity of the reticuloendothelial system and to clarify to some extent the mechanism by which the phagocytic activity is affected in stressed non-thymectomized animals.

All experiments were carried out with Wistar albino rats at 5-6 weeks of age. The granulopectic activity was estimated by measuring the rate of removal from the blood of colloidal carbon (16 mg/100 g b.wt) by the method previously described[12]. Thymectomy was performed 2 days after birth according to the method of Miller[16]. Stress was applied in grid-floored cages according to the method of Hall et al.[3], by electric shocks of 200 V and 2000 cps over