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Effect of Dichloroglyoxime and Dimethylglyoxime on the Acellular Slime Mold *Physarum polycephalum*

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ABSTRACT. The effect of different concentrations of dichloroglyoxime (DCG) and dimethylglyoxime (DMG) on the cytoplasmic shuttle streaming (*in vivo*) and on the contraction-relaxation activity of excised plasmodial strands (*in situ*) were investigated. In the concentration range between 0.5-2 μg/ml, DCG caused disturbances and subsequent stop of cytoplasmic streaming. Higher concentrations induced a direct and rapid stop of cytoplasmic streaming followed by contraction of the whole plasmodium, blebbing and desintegration of plasma membrane. These effects were completely irreversible. Concentrations of DCG ≤ 0.5 μg/ml induced contraction of excised plasmodial strands measured tensiometrically under isometric conditions. A concentration dependent decrease in frequency of the contraction-relaxation cycle, but an increase in force amplitude were observed. A concentrations of DCG ≥ 2.0 μg/ml caused a single, very strong contraction followed by an irreversible relaxation. DMG did not induce obvious effects on migrating plasmodium or cytoplasmic streaming. When applied to isometrically oscillating excised strands of *Physarum*, DMG showed a concentration-dependent decrease in oscillation frequency. These results showed that some glyoxime derivatives (even in micromolar concentrations) have cytotoxic effects. Further investigations should be done before recommending such derivatives as commercial disinfectants.

It has been found that oximes have many toxic effects (Davis and Willey 1959, Riemschneider and Hecker 1979). The use of glyoxime derivatives in medicinal, analytical and cytological chemistry is well known (Vogel 1968, Rubanyi and Balogh 1982, Khalili and Mahasneh 1986). Khalili and Mahasneh (1986) found that dichloroglyoxime had an antimicrobial activity similar to most commercial disinfectants. Abdalla and Khalili (personal communication) found that dichloroglyoxime caused concentration dependent relaxation of the epinephrine-contracted aortic and pulmonary rings and of the tracheal tone in guinea-pig.

To study toxic effects of chemical compounds as antimicrobial or antifungal substances, the migrating plasmodial stage of *Physarum polycephalum* is a very suitable system. This organism shows a regular shuttle streaming and an oscillatory contraction activity. This communication describes the effect of dichloroglyoxime and dimethylglyoxime on the plasmodia of the acellular slime mold *Physarum polycephalum.*

Materials and Methods

Organism: Plasmodial strands of *Physarum polycephalum* (ATCC 44912) obtained according to the method described previously (Korohoda *et al.* 1983) were used for this study.

Measuring techniques: Live observations of small plasmodia (phaneroplasmodia) migrating on the surface of 1.5% nonnutrient agar were performed using a phase contrast microscope (Zeiss). The specimens were submerged in test solutions for determination of the effect of different concentrations of the two substances on shuttle streaming periodicity and macroscopic responses of plasmodia. Tensiometric measurements of the longitudinal contraction activity of excised plasmodial strands were carried out with the aid of a tension transducer (Wohlfarth-Botterman 1975). During tensiometric investigations the plasmodial strands were submerged in a physiological salt solution containing 6 mM NaCl, 3 mM KCl, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mM $NaHCO_3$; pH 7.0.

Chemicals: Dimethylglyoxime (DMG) was obtained from Fluka, A.G. (West Germany), dichloroglyoxime (DCG) was prepared as mentioned previously (Khalili and Mahasneh 1986). Stock solutions were prepared in absolute ethanol and dilution thereof were made in the physiological salt solution adjusted to pH 7.0.

Results

In studying the effect of DCG and DMG on *Physarum* plasmodia, three criteria were taken into consideration:

1. Macroscopic responses of the whole plasmodium *(in vivo)*.
2. Effects on shuttle streaming periodicity.
3. Effects on longitudinal contraction activity of excised plasmodial strands.

Effect of DCG on macroplasmodia:

The effects of different concentrations of DCG on whole macroplasmodia migrating on agar surface are summarized in Table 1. The table shows the sequence of events that happened after treatment of plasmodia with DCG. The test solution was applied after submersion of plasmodia in the salt solution for about 20 minutes.

Effect of DCG on protoplasmic streaming (in vivo)

Table 2 summarizes the effects of DCG on the shuttle streaming periodicity. Test solutions were applied after submersion of plasmodia for 20 minutes in salt solution and observation was followed for 20-30 minutes.

Table 1. Effect of different concentrations of DCG on *Physarum* macroplasmodia

Concentration (μg/ml)	Effect
1000	Direct fixation of plasmodium.
50-100	Direct condensation of the whole plasmodium, release of pigments to the outside and consequent decoloration of plasmodium.
25	Disruption of thin veins followed by contraction of the whole plasmodium and loss of pigments.
5	Contraction of plasmodium into protoplasmic clumbs, pigment loss and high vacuolization of endoplasm.
0.5-2	Contraction of the front region started after about 3 minutes, followed by contraction of larger strands, development of large vaculoes in the endoplasmic canal and blebbing of plasma membrane 20-30 minutes after the treatment.

Table 2. Effect of DCG on protoplasmic streaming in *Physarum* plasmodia

Concentration (μg/ml)	Effect
1000	Direct irreversible stop of streaming.
50-100	Direct irreversible stop of streaming.
25	Irreversible stop of streaming in few seconds.
1-2	Streaming went slower, irregular and finally stopped irreversibly in 20-30 minutes.
0.5	Increase in period duration about 65% and streaming was irregular.

Effect of DMG on macroplasmodia and shuttle streaming

Table 3 summarizes the effect of increasing concentration of DMG on phaneroplasmodia. Before treatment, plasmodia were kept for 30 minutes in salt solution containing a concentration of ethanol similar to that found in individual test solutions. The observation was followed 1-2 hours after the treatment. When left overnight in 400 µg/ml DMG, the plasmodium turned into cytoplasmic clumbs and aggregates. After washing with salt solution, the latter could migrate on agar surface and gave rise to new phaneroplasmodia.

Table 3. Effect of DMG on macroplasmodia and shuttle streaming periodicity

Concentration (µg/ml)	Effect
20-100	No obvious effect.
200	Reversible contraction of plasmodium, vacuolization of the flowing endoplasm and a slight increase in the duration of the streaming period.
400	Slow down in streaming velocity, contraction of the whole plasmodium and vacuolization of the streaming endoplasm.

Effect of DCG on the contractile activity of plasmodial strands

Three different concentrations of DCG were tested for their effect on the longitudinal contractile activity of excised plasmodial strands measurd tensiometrically under isometric conditions. We present the main results of our experiments at the end of legends to figures 1, 2 and 3. DCG affected both the period duration and the amplitude of the force output. Table 4 presents statistical analysis of the effect of DCG on these parameters. It becomes apparent that DCG induced a concentration-dependent prolongation of the oscillation period and an increase in the force amplitude.

Table 4. Effect of DCG on the longitudinal contractile activity of excised plasmodial strands

Solution	$\bar{t}$(min)±S.D.	$\bar{t}$(%)	$\bar{A}$(mp)±S.D.	$\bar{A}$(%)	N	n
Salt solution (Control)	2.8 ± 0.3	100	17.0 ± 4.2	100	8	75
DCG (0.5 µg/ml)	3.2 ± 0.5	114	22.1 ± 7.1	130	6	63
DCG (1 µg/ml)	3.5 ± 0.6	125	24.6 ± 7.6	145	6	46

Data are presented as arithmetic means ± S.D. (Standard deviation). $\bar{t}$ = arithmetic mean of period duration (in minutes), $\bar{A}$ = arithmetic mean of force amplitude (in millipounds), N = number of plasmodial strands investigated, n = number of periods analyzed.

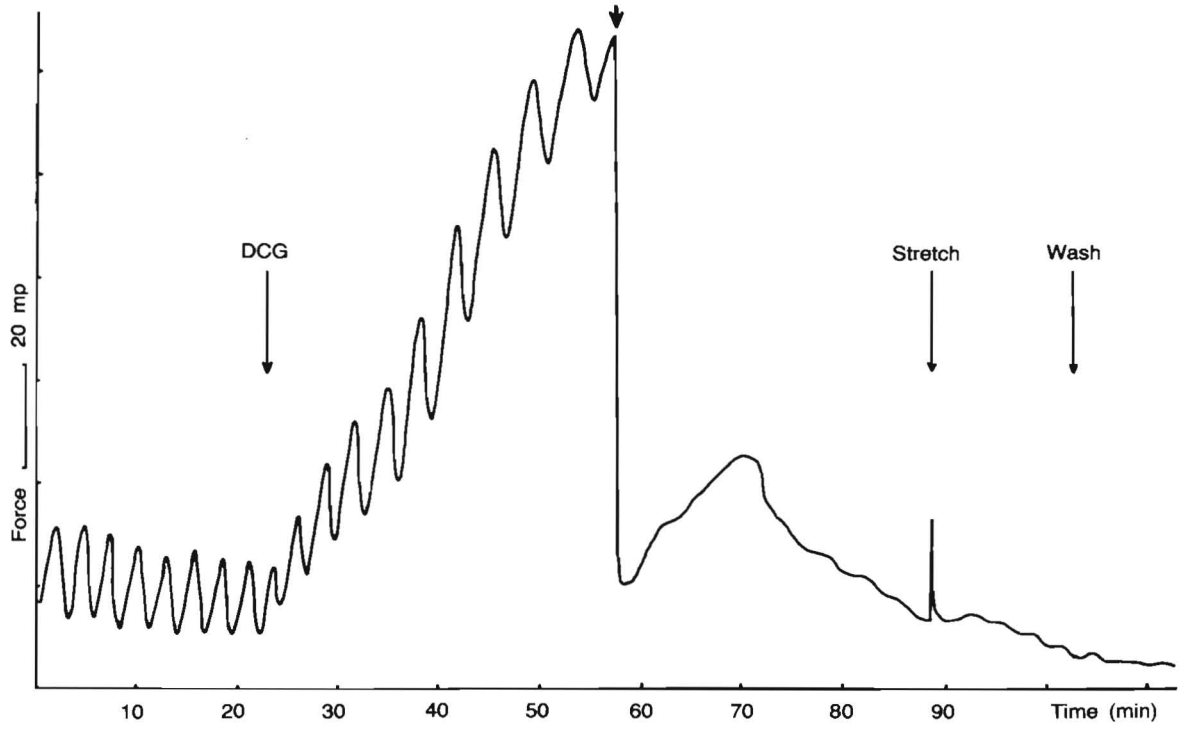

Fig. 1. Time course of longitudinal contractile activity of a plasmodial strand submerged in salt solution followed by application of 1 µg/ml DCG. In this and the following figures (Figs. 1-5) the ordinate represents force output in millipounds (1 mp = 9.81×10^{-6} Newton) and the abscissa shows the time course of the experiment in minutes.
Results: 1 µg/ml DCG induced a very strong contraction, increase in force amplitude and a consequential rupture of the plasmodial strand (arrow). When the strand was directly rejoined to the tensiometer head it did not recover the oscillatory contractile activity. Stretching or washing with salt solution did not reverse the effect of DCG. Note also the prolongation of the periods after DCG treatment.

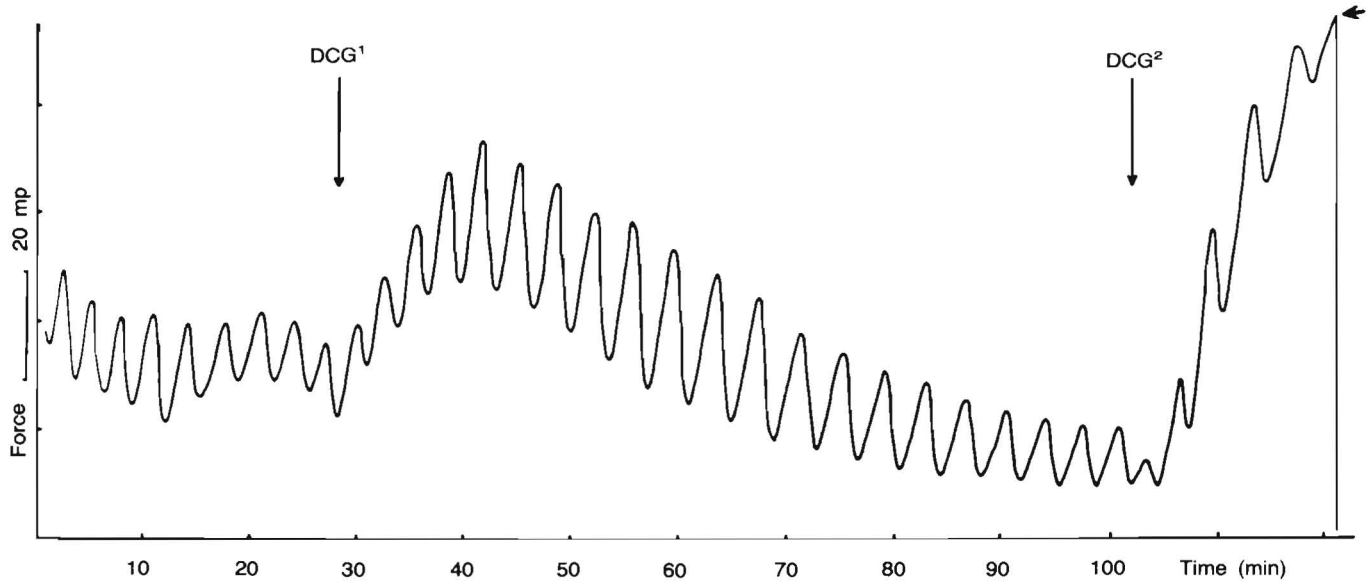

Fig. 2. Contraction behaviour of a plasmodial strand submerged in salt solution as a response to subsequent application of 0.5 µg/ml DCG (DCG^1) and 1 µg/ml DCG (DCG^2).
Results: Note the increase in the period duration and the increase in force amplitude. 1 µg/ml DCG induced a very strong contraction, increase in force output and a consequential rupture of the plasmodial strand (arrow).

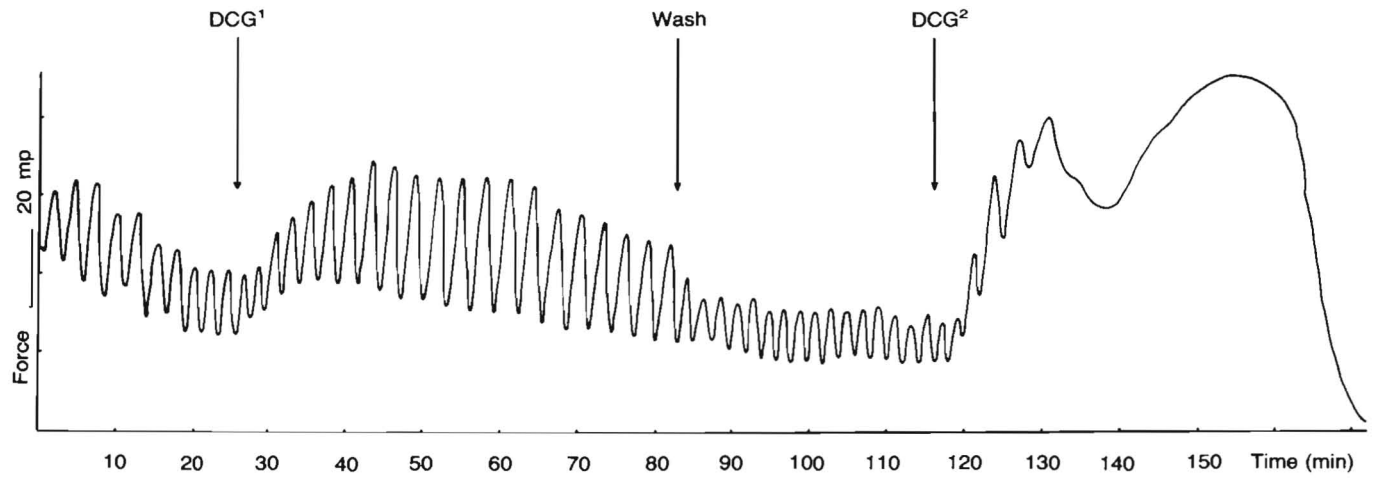

Fig. 3. Frequency response of oscillatory contraction activity of a plasmodial strand submerged in salt solution as a result of sequential application of 0.5 µg/ml DCG (DCG^1), washing with salt solution and application of 2 µg/ml DCG (DCG^2).
Results: 0.5 µg/ml DCG induced a prolongation of periods and increase in force amplitude. Washing recovered the normal oscillation frequency but not the level of force amplitude. 2 µg/ml DCG induced a very strong contraction followed by an irreversible relaxation.

Test of Vitality

It was of interest to know whether the plasmodia and the strands are still vital after treatment with the different concentrations of DCG. For this purpose, the treated specimens were washed thoroughly with the salt solution and deposited on nonnutrient agar plates. Only specimens treated with concentrations of 0.5 μg/ml DCG or less grew to small plasmodia within 24 hours, but specimens treated with higher concentrations of DCG failed to do so. The latter plasmodia and strands were depigmented and did not grow to locomotor macroplasmodia.

Effect of DMG on the contractile activity of plasmodial strands

DMG at concentrations less than 200 μg/ml did not affect the contractile activity of excised plasmodial strands. Figures 4 and 5 show the response of plasmodial strands to higher concetrations of DMG. Results are presented at the end of each legend. Statistical analysis of the effect of DMG on the longitudinal contraction activity of plasmodial strands are presented in Table 5.

Table 5. Effect of DMG on the longitudinal contractile activity of excised plasmodial strands

Solution	$\bar{t}$(min)±S.D.	$\bar{t}$(%)	$\bar{A}$(mp)±S.D.	$\bar{A}$(%)	N	n
Salt solution + 1% ethanol (Control)	3.4 ± 0.4	100	17.1 ± 2.1	100	5	50
200 μg DMG/ml	4.0 ± 0.3	117	12.3 ± 3.8	72	5	50
Salt solution + 1% ethanol (Control)	3.6 ± 0.5	105	9.2 ± 5.8	54	5	52
Salt solution + 2% ethanol (Control)	3.1 ± 0.4	100	2.3 ± 5.7	100	6	45
400 μg DMG/ml	4.7 ± 0.7	152	23.5 ± 8.9	102	6	52
Salt solution + 2% ethanol (Control)	4.7 ± 0.6	152	11.0 ± 2.2	48	6	51

Data are presented as arithmetic means ± S.D. (Standard deviation). $\bar{t}$ = arithmetic mean of period duration (in minutes), $\bar{A}$ = arithmetic mean of force amplitude (in millipounds), N = number of plasmodial strands investigated, n = number of periods analyzed. Each treatment with DMG was preceeded and followed by a treatment with salt solution containing the appropriate concentration of ethanol as a control solution.

Discussion

The plasmodia of the acellular slime mold *Physarum polycephalum* inhabit the soil and represent a suitable object for studying the effect of toxic substances that are used as antimicrobial or antifungal agents. Excess amounts of these substances are used in the treatment of plant diseases mainly caused by bacteria and fungi. Two glyoxime derivatives, dichloroglyoxime (DCG) and dimethylglyoxime

(DMG) have been tested for their effect on *Physarum* plasmodia. DCG has a drastic effect on *Physarum* plasmodia. As the immediate response of the plasmodia, we registered a 65% increase in the duration of the period of cytoplasmic streaming following the application of 0.5 μg/ml DCG. In the concentration range between 0.5-2 μg/ml DCG caused disturbances and subsequent stop of cytoplasmic streaming as a result of drastic morphological changes in the cellular organization. Higher concentrations induced a direct, rapid and irreversible stop or cytoplasmic streaming and contraction of the whole plasmodium followed by blebbing and desintegration of plasmalemma (see Table 1). Concentrations of DCG above 1 μg/ml brought about a stop of cytoplasmic streaming in a time inversely proportional to the concentration (see Table 2). As shown clearly in figures 1, 2 and 3, DCG induced a concentration-dependent decrease in frequency of the contraction-relaxation cycle, and an increase in the force output of excised plasmodial strands. A concentration above 2 μg/ml caused a very strong contraction followed by an irreversible stop of oscillation and release of pigments.

The ability of DCG to induce vacuolization of endoplasm, blebbing of plasma membrane, stop of oscillations and loss of pigments indicates that this compound affected the membranous system in *Physarum*. These effects were followed by the disruption of the whole structural organization of the cell. This could lead also to a disturbance in the free calcium concentration, which may be responsible for some of the observed effects (vacuolization, contraction of plasmodia and plasmodial strands and the increase in both the force output and the period duration). Similar effects have been observed when the plasmodial strands were treated with chemicals that affect directly or indirectly the calcium homeostasis in *Physarum* plasmodia (Shraideh *et al.* 1983).

On the other hand DMG even at high concentrations did not induce obvious effects on migrating plasmodia or tensiometrically oscillating excised plasmodial strands (Table 3, Fig. 4). It could reversibly affect the morphology of macroplasmodia, the oscillation frequency and the force output of plasmodial strands (Fig. 4).

The finding that DCG affects the contraction of heart and smooth muscles in guinea-pig (Abdalla and Khalili, personal communication) makes the results of this investigation very interesting since *Physarum* plasmodia possess a system of non-muscle actomyosin which seems to act by a mechanism similar to that of smooth muscles (Wohlfarth-Bottermann 1977). The comparison between the effect of chemical agents on smooth muscles on the one hand and *Physarum* actomyopsin on the other hand, may help in elucidating the mechanism by which the actomyosin in *Physarum* works.

More investigations on the cytotoxic effects of DCG have to be done in order to clarify the mechanism of action of this glyoxime and other antimicrobial and antifungal agents.

The results of this investigation show that some glyoxime derivatives are very toxic to soil microorganisms and have cytotoxic effects even at concentrations that are 2000 times less than the effective dose found by Khalili and Mahasneh (1986). Further studies are necessary on the effect of such chemical agents before recommending them for commercial use.

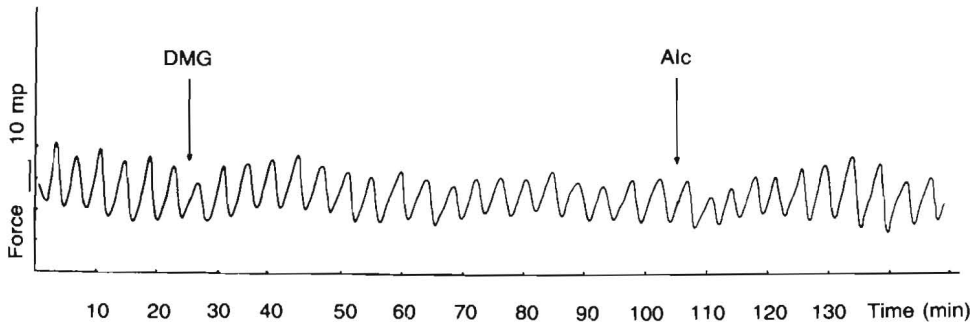

Fig. 4. Contraction behaviour of a plasmodial strand submerged in salt solution containing 1% ethanol and after application of 200 µg/ml DMG.

Results: Treatment with DMG induced a slight reversible prolongation of periods but no obvious effect on force amplitude.

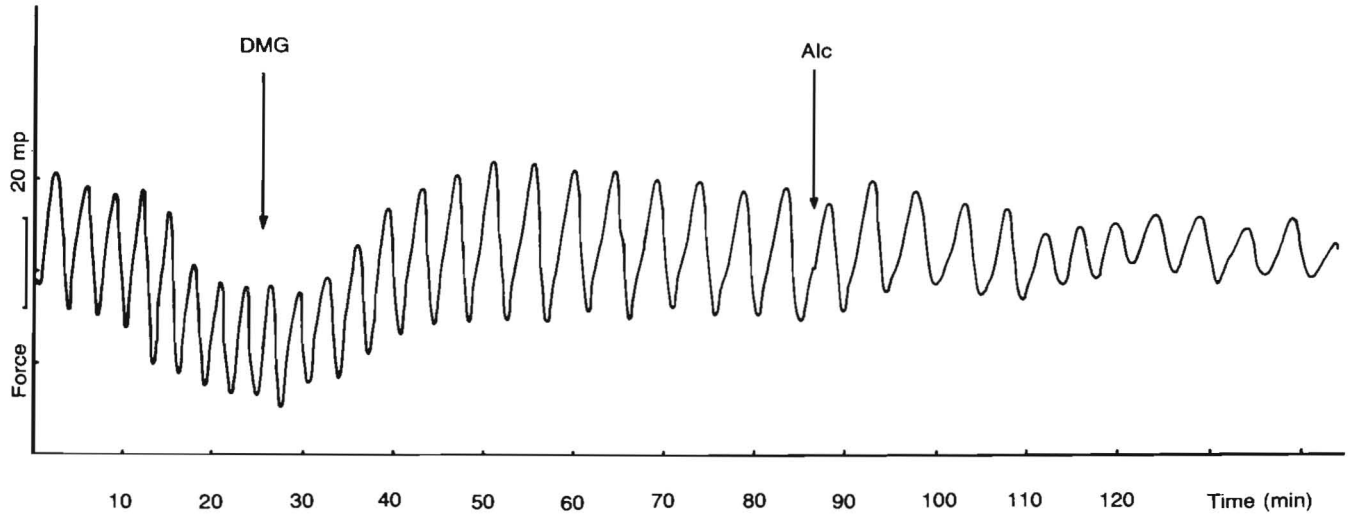

Fig. 5. Time course of longitudinal contractile activity of a plasmodial strand submerged in salt solution containing 2% ethanol and effect of application of 400 µg/ml DMG.
Results: note the irreversible prolongation of periods and the reversible increase in force amplitude.

Acknowledgements

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References

Davis, D.R. and **Willey, G.L.** (1959) Toxicity of the oxime 2-hydroxyiminomethyl N-methyl-pyridinium-methanesulphonate (P25). *Br. J. Pharmacol.* **14:** 186-191.

Khalili, F. and **Mahasneh, A.** (1986) Preparation and antimicrobial activity of glyoximes. *Dirasat,* **13:** 186-188.

Korohoda, W., Shraideh, Z., Baranowski, Z. and **Wohlfarth-Bottermann, K.E.** (1983) Energy metabolic regulation of oscillatory contraction activity in *Physarum polycephalum. Cell and Tissue Res.* **231:** 675-691.

Riemschneider, R. and **Hecker, A.** (1979) Determination of the lethal dose of metaldehyde and evidence of a synergetic effect in snails. *Z. Pflanzenkr. Pflanzenschutz,* **86:** 479-482.

Rubanyi, G. and **Balogh, I.** (1982) Effect of nickel on uterine contraction and ultrastructure in the rat. *Am. A. Obstet. Gynecol.* **142:** 1016-1020.

Shraideh, Z., Baranowski, Z., Korohoda, W. and **Wohlfarth-Bottermann, K.E.** (1983) Consequences of impeding in mitochondrial function in *Physarum polycephalum.* 1. Reversible effects of anoxia, KCN, and influences on the Ca^{+2} ionophore A-23187. *European Journal of Cell Biology,* **31:** 175-186.

Vogel, A.I. (1968) *A textbook of quantitative inorganic analysis,* 3th ed. Longmans, Green & Co. Ltd., London, 479 p.

Wohlfarth-Bottermann, K.E. (1975) Tensiometric demonstration of endogenous, oscillatory contractions in plasmodia of *Physarum polycephalum. Zeitschrift für Pflanzenphysiologie,* **76**(1): 14-27.

Wohlfarth-Bottermann, K.E. (1977) Zellmotilität im Transmissions-Elektronen-Mikroskop: Cytoplasmatische Actomyosine als Ursachen von Zellbewegungen, Beitr. *Elektronemikroskop. Direktabb. Oberfl.* **10:** 97-138.

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تأثيرات ثنائي كلور الجليوكسيم وثنائي ميثايل الجليوكسيم على الفطر الهلامي اللا خلوي «فيزاروم بوليسيفالم»

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لقد درست تأثيرات كل من ثنائي كلور الجليوكسيم(ث.ك.ج)وثنائي ميثايل الجليوكسيم (ث.م.ج) على الانسياب البروتوبلازمي المنعكس الرتيب والنشاط الانقباضي ـ الانبساطي في بلازموديوم وعروق الفطر الهلامي «فيزاروم بوليسيفالم». وقد سبب تركيز ٠,٥ ـ ٢ ميكروغرام / مللتر من (ث.ك.ج) اضطراباً ومن ثم توقفا غير منعكس للانسياب البروتوبلازمي في طور البلازموديوم. أما التراكيز الأعلى فأحدثت توقفاً مفاجئاً غير منعكس للانسياب البروتوبلازمي، تبعه انقباض كلي للبلازموديوم وتبثر وتلف في الغشاء الخلوي. وقد أحدث تركيز ٠,٥ ميكروغرام / مللتر أو أقل من (ث.ك.ح) انقباضاً معتمداً على التركيز في العروق البروتوبلازمية المعزولة من الفطر، وأدى إلى زيادة في السعة ونقصاً في تكرار الانقباضات متساوية الطول. وجد أيضاً أن ٢ ميكروغرام / مللتر أو أكثر م (ث.ك.ج) أحدثت انقباضاً واحداً قوياً عقبه أرتخاء غير منعكس.

لم يكن للمادة (ث.م.ج) تأثير واضح على الانسياب البروتوبلازمي في البلازموديوم. وعند اضافتة إلى العروق البروتوبلازميه المعزولة أحدث إنخفاضاً معتمداً على التركيز في تكرار الانقباضات متساوية الطول. تقترح مجموعة هذه الملاحظات أن بعض مشتقات الجليوكسيم تؤدي إلى تأثيرات سامة في الخلايا الحية، ولابد من دراسات مستفيضة قبل التوصية باستعمالها كمبيدات بكتيرية أو فطرية.