the 1st lamellated layer, which is completed 11.5 h later. The growth of the non-lamellated layers takes about 22 h, that of the other lamellated layers almost 2 h. Neither arrests nor phases with enhanced growth could be observed (fig. 2).

Whereas in a synchronized stock moulting occurs during the dark cold phases, the 2nd and the following lamellated layers are deposited during the light warm phases. This fact is a consequence of the prolonged duration of the growth of the 1st lamellated layer (fig. 3).

Discussion. In *Blaberus fuscus,* synchronization of cuticle growth is achieved indirectly by the concentration of moults into a distinct time-span of the day. This gating of moult synchronizes the cuticle rhythm with the environment as well as with internal events and rhythms. Because the free-running period of the growth rhythms differs only very slightly from 24 h[3], a stable phase relationship of the cuticle growth rhythm to the environment is guaranteed, at least during the 1st days after gated moulting.

According to Neville[1] the deposition of lamellated chitin consumes much less energy than the deposition of non-lamellated chitin. In *Blaberus fuscus,* the lamellated layers are deposited during the light warm phases. Since this cockroach is a dark-active species, the less energy-consuming process takes place during the diurnal resting time. The same is true in the light-active locusts: *Schistocerca gregaria* deposits lamellated chitin during the dark phases[5]. Thus, phase controlled cuticle growth could be an internal adaptation to the circadian availability of metabolic and/or anabolic molecules in the hemolymph.

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Effect of trifluoperazine and calcium ions on gregarine gliding

C.A. King and K. Lee[1]

Department of Zoology, University College, Gower Street, London WC1 6BT (England), 2 December 1981

Summary. The drug trifluoperazine (TFP) inhibits the gliding motility of gregarine protozoans. This suggests that the Ca^{++} binding protein, calmodulin, plays a role in the motility process. However the presence of extracellular Ca^{++} was not required for gliding to occur.

The gliding movement of gregarine protozoans has fascinated biologists for many years, and various theories to account for their movement[2,3] have been proposed. We have recently suggested that the motility process involves mechanochemical force transduction occurring at or near the cell surface[4,5]. It was previously suggested[4] that the gliding process might not involve actin, however in more recent experiments we have clearly shown that the gliding motility is inhibited in a quantitative manner by the presence of cytochalasin B[6]. The importance of Ca^{++} ions in the regulation of many motility events, especially muscular contraction, has been clear for some time[7]. In striated vertebrate muscle troponin C plays the major role in Ca^{++} activation of the actin-myosin system. However, in many calcium activated intracellular systems another calcium binding protein-calmodulin-plays a pivotal role[8,9]. In the context of cell motility particular interest has focussed on the activation of myosin kinase by Ca^{++}-calmodulin.

Although external (extracellular) Ca^{++} is required for some motile processes e.g. heart muscle contraction, locomotion of *Amoeba proteus,* in other cases external Ca^{++} does not appear to be required e.g. locomotion of *Naegleria gruberi* amoebae[10], contraction of vertebrate striated muscle[11]. The intracellular level of Ca^{++} within cells would be expected to lie between 10^{-6} and 10^{-8} M[7], and alteration in this level has been shown to have profound effects, e.g. cilia reversal in ciliates[7]. The anti-psychotic drug trifluoperazine (stelazine) complexes selectively with Ca^{++}-calmodulin, thereby inhibiting Ca^{++}-calmodulin dependent enzyme reaction. It was decided to see if TFP would act as an inhibitor of gregarine locomotion and also study the effect of extracellular Ca^{++} on gregarine gliding.

The gregarines were obtained from the gut of meal worm larvae *(Tenebrio molitor)* and washed with Hedon-Fleig medium. (7.0 g NaCl, 0.3 g KCl, 0.1 g $CaCl_2$, 0.5 g $NaHCO_3$, 0.3 g $MgSO_4$ per l)[4]. Calcium free Hedon-Fleig solution was prepared by ommitting the $CaCl_2$ from the basic recipe given above. Measurements on gregarine motility were then carried out as descibed previously[4]. It was found that no significant difference inlocomotion rates was observed when Ca^{++} free or complete media were used. The speed of movement of gregarines in Ca^{++} free medium was found to lie in the range $2.2 \rightarrow 5.3$ µm/sec (7 moving individuals studied) i.e. about the same value as that found using the complete medium. To preclude the possibility that the water used contained trace amounts of Ca^{++} ion (e.g. at µM levels), experiments were also carried out using Ca^{++} free medium in the presence of the Ca^{++} chelating compound ethylene glycolbis (β-amino ethyl ether) N, N′-tetra-acetate (EGTA) at a final concentration of 10 mM. No alteration in motility was noted. Thus it

Inhibition of gregarine motility with TFP

TFP solution (final concentration)	Motility index* 0 min	20 min	40 min	60 min	80 min
0	100	107	105	100	107
10 µM	100	104	100	68	77
50 µM	100	73	41	23	9
100 µM	100	27	11	11	0

* Motility index $= \frac{\text{No. of motile gregarines at time t}}{\text{No. of motile gregarines at time 0}} \times 100.$

appears (at least in the short term experiments described here) that extracellular Ca^{++} is not required for gregarine motility.

A stock solution of 1 mM TFP (Smith, Klinde & French, Welwyn Garden City, Herts.) in Hedon-Fleig medium was prepared and appropriate dilutions made. The potential inhibitory effect of TFP was tested using the experimental protocol described by King and Lee[6], i.e. 0.1 ml of gregarine preparation (20 → 60 individuals) was placed in a single compartment (depth ~ 1 cm, diameter = 0.6 cm) of a flat bottomed plastic tissue culture tray, and the number of motile gregarines present was recorded; 0.1 ml of TFP solution was added and the number of motile gregarines present at 20-min intervals was recorded. The original number of motile cells counted just prior to the addition of test solution was normalized to a value of 100%. Suitable controls were carried out using Hedon-Fleig solution in place of the TFP solutions under test. The table shows that TFP markedly inhibited the motility of gregarines in a dose dependent manner and within the range of concentration ($10^{-5} \rightarrow 10^{-4}$ M) previously found to be effective in other systems[12,13].

At the light microscope level no change in gregarine shape was observed in the presence of TFP in contrast to the marked change in shape (discoidal → spherical) of human blood platelets when transfered to solutions containing TFP[12]. External Ca^{++} is not required for gregarine motility but the inhibition of motility by TFP indicates that Ca^{++}-calmodulin plays an important role. One would assume that sequestered Ca^{++} stores occur within the gregarine having a role similar to the sacroplasmic reticulum of muscle[11] and the Ca^{++} storage sites in the dense tubular system of platelets[12]. One presumes that under conditions suitable for motility to occur, Ca^{++} is released from these stores, complexes with calmodulin leading to the activation of enzyme system(s) required for motility. One prime candidate would be myosin kinase, however there are a large number of Ca^{++}-calmodulin activated enzyme systems[8,9]. Gregarine motility would seem to be worthy of further study particularly with regard to Ca^{++} stores and possible role of calmodulin. Since gregarines are large cells (volume $10^4 \rightarrow 10^6$ μm^3) they can be conveniently microinjected[14] and might offer an interesting model system for the study of calcium intracellular reactions.

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Induction of cell contact sites by Ca^{2+}-EDTA pulses in *Dictyostelium discoideum*

S. Miyamoto[1]

Department of Biology, Tsukuba University, Sakura-mura, Ibaraki (Japan), 31 October 1980

Summary. The effect of extracellular Ca^{2+} on the differentiation of the cellular slime mold *D. discoideum* was examined. In the case of sustained application of calcium, cells required at least 60 min for the presence of calcium to show the induction of EDTA resistant cell contact sites (csA). However, the application of 12 pulses of calcium, followed after 30 sec by EDTA, giving a total time of Ca^{2+}-exposure of 6 min, could induce csA on the cell surface.

Cellular slime mold *Dictyostelium discoideum* ameobae enter a phase of aggregation when they are starved. Cells aggregate by chemotaxis towards cAMP producing cells. Cyclic AMP pulses, either spontaneously generated or artificially applied, were reported to induce some proteins specific for the development phase[2]. Expression of contact sites (csA)[3], that mediate tight intercellular cohesion, is also developmentally regulated and is induced by cAMP pulses. The mechanism of the induction of csA by cAMP pulses was not defined. But calcium ions are believed to play some role in the inducing mechanism because calcium influx was observed within 10 sec after stimulation of cells with cAMP[4]. It was reported that cells could not aggregate in the calcium free medium[5]. Most work concerning the effects of calcium was performed using a sustained application of calcium[6,7]. But in this report, it was added in a pulsatile manner. Calcium pulses instead of cAMP pulses also induced csA.

Materials and methods. Dictyostelium discoideum NC-4 cells were cultivated on nutrient agar in association with *Enterobacter aerogenes* at 22 °C. Growth phase cells were collected, washed twice with 17 mM phospate buffer and resuspended in the same buffer. Aliquots of 6 ml of the cell suspension in 20 ml flasks were incubated in the shaker (T_0). After 2 h of incubation (T_2), 30 µl of $CaCl_2$ and/or EDTA solution was added manually with a micropipette. Protocols for pulsatile application and sustained application are shown in figure 1. In the case of Ca^{2+}-EDTA pulses, EDTA was added equimolar to the $CaCl_2$ solution 30 sec after the $CaCl_2$ addition. This alternate addition, which represented 1 pulse, was repeated at 5-min intervals. During the 5-min interval, calcium ions existed in the suspension for the 1st 30 sec, then for at least 4.5 min, the calcium was absorbed by EDTA. In all cases, either the pulse or sustained application, EDTA solution that was equivalent to the Ca^{2+} was added after the $CaCl_2$ solution was applied in order to avoid the effect of Ca^{2+} remaining in the suspension. Because, at the end of incubation, the cell contact sites A was measured as the amount of cell aggregates that were resistant to EDTA.