axons from brain and glandular lobes whose intrinsic cells produce adipokinetic hormones[19-21]. The corpus cardiacum plays a vital role as a neurohemal organ from which trophic hormones like allatotropin originating in the neurosecretory cells of the brain are released.

The role of allatotropin in activating juvenile hormone synthesis by corpora allata[22], and the gonadotropic activity of juvenile hormone[23, 24] are well documented. Though these trophic hormones have not been chemically identified, there is ample proof that the neurosecretory proteins (carrier/precursor proteins) are rich in cysteine[25, 26]. The study on the turnover of L-[^{35}S]cysteine labeled secretory proteins demonstrates a remarkable difference between the maturing control females and the experimental females, whose ovarian development was inhibited by a single dose of azadirachtin A. In the experimental group, poor turnover is attributed not only to reduced transport from neurosecretory cells but also to its marginal release. Difference in uptake of cysteine could also contribute to poor turnover. However, such a difference in uptake of cysteine into the cerebral neurosecretory cells alone cannot be measured, owing to the impossibility of removing the surrounding non-neurosecretory tissue[26].

Synthesis and release of neurosecretory material are at an equilibrium controlled by feedback regulation operating under normal conditions of development[27]. In addition to recovery of a larger quantity of [22,23-$^{3}H_2$]dihydroazadirachtin A from the corpus cardiacum, histological studies[28] indicate that while this drug does not penetrate the blood-brain barrier efficiently, it covers the entire corpus cardiacum and concentrates more in the neurosecretory axons located in the storage lobes. The poor turnover of the neurosecretory proteins could hence be a result of the interference of azadirachtin A with the mechanism of their release from the corpus cardiacum.

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Regular oscillations in suspensions of a putatively chaotic mutant of *Dictyostelium discoideum*

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Summary. We have tested the light-scattering properties of suspensions of the *Dictyostelium discoideum* mutant *HH201* derived from the mutant *Fr17*. Previous studies indicated that *HH201* and *Fr17* possess highly irregular rhythmic properties which might represent aperiodic oscillations, i.e. chaos. We report that the former mutant can display regular oscillatory behavior. Possible explanations for this result are discussed, including that of a transition from chaotic to periodic behavior resulting from some parameter change or from strong intercellular coupling in cell suspensions.
Key words. Dictyostelium discoideum; cAMP oscillations; biological rhythms; chaos.

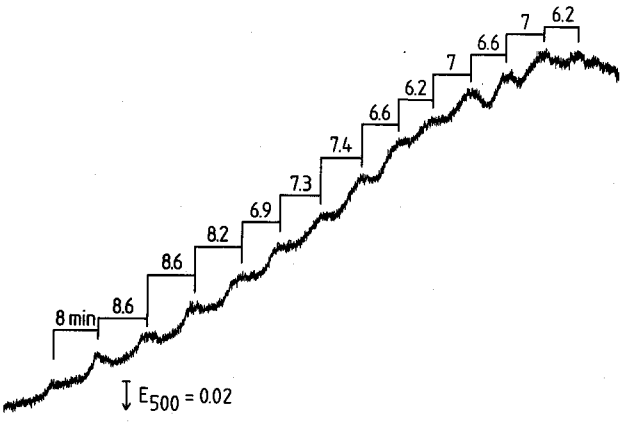
After starvation, the cellular slime mold *Dictyostelium discoideum* aggregates in a wavelike manner by a chemotactic response to cyclic AMP (cAMP) signals emitted by cells which behave as aggregation centers [1, 2]. In the wild type, aggregation is periodic as cAMP signals are released by the centers with a frequency close to one pulse every 8 min. These cAMP oscillations have also been observed in cell suspensions [3] and represent one of the best-known examples of periodic signalling in intercellular communication. In the mutant *Fr17* which develops rapidly as a result of some alteration in cAMP metabolism [4], time intervals between successive waves of aggregation can, however, be highly irregular as they may extend from 4 to 20 min. This observation led Durston to suggest [5] that *Fr17* is characterized by the property of aperiodic signalling. A study of the temperature-sensitive mutant *HH201* derived from *Fr17*, based on a chemical assay, later provided preliminary evidence for 'erratic' oscillations of cAMP in cell suspensions [6].

Martiel and Goldbeter recently proposed a model for cAMP signalling in *Dictyostelium* which accounts for the periodic synthesis of cAMP pulses [7] but also indicates [8] that the signalling system is capable of producing irregular cAMP oscillations, with time intervals between successive peaks varying by a factor of up to 3 in an apparently random manner. Such behavior, known as 'chaos', has been encountered in a number of chemical and biological systems [9, 10]. The main characteristic of chaotic systems is the unpredictable nature of their time evolution, as they are capable of generating oscillations with an element of randomness in the absence of external noise. Thus, the theoretical analysis of the cAMP signalling system raised the possibility that the irregular oscillatory behavior of *Fr17* and *HH201* might represent a first example of chaos at the cellular level [8, 11].

Oscillations in intercellular communication in *Dictyostelium* amoebae can be conveniently monitored by recording light-scattering changes in cell suspensions [12, 13]. In order to test for the occurrence of chaotic behavior, we have investigated the light-scattering properties of the mutant *HH201*. The search for aperiodic oscillations has not been conclusive so far, but we observed, somewhat unexpectedly, rather regular oscillations. In four out of eight experiments, suspensions of this mutant displayed sustained oscillations in light-scattering shortly after the beginning of starvation. The period of these oscillations was relatively stable as it varied between 8.6 and 6.2 min over 13 successive cycles in the longest record (fig.); this range, as well as the number of cycles, are close to those observed in the wild type [3, 12, 13]. Most of the variation in cycle length (fig.) can be interpreted as being due to the slight acceleration of a periodic oscillator in the course of time, owing to developmental changes in the signalling system. A similar drift to shorter periods is observed in the wild type [13].

Whereas numerous cycles are readily obtained in chemical oscillatory systems such as the Belousov-Zhabotinsky reaction where chaos has been shown to occur [14], the limited number of cycles that can be recorded in a *Dictyostelium* cell suspension makes it difficult to carry an unambiguous identification of the phenomenon. However, the variation of the interval between two successive peaks of light transmission in the figure is definitely much smaller than that reported for the time intervals between successive waves of *Fr17* amoebae aggregating on agar [5]. The oscillatory behavior of figure 1 therefore departs from that described in the available studies on the pacemaker mutants of *Dictyostelium* [5, 6].

While our investigations have led to the observation of regular oscillations but not of chaos, they by no means rule out the occurrence of the latter phenomenon in the mutant *HH201*. Theoretical studies of models show that even in systems capable of chaos, periodic behavior remains a common dynamic phenomenon that generally occurs in a much larger domain in parameter space [15]. The present results should therefore not be viewed as contradicting previous reports on the irregular oscillatory properties of *HH201* and *Fr17*. Indeed, slight changes in conditions suffice to bring a chaotic system into a periodic mode of operation [16]. That such transition might also occur in vivo is suggested by experimental observations [5] which show that the irregular, aperiodic signalling properties of the mutant *Fr17* can spontaneously become regular in the course of aggregation. Besides the above explanation, there exists, however, another possible reason for the observation of rather regular light-scattering oscillations in suspensions of the mutant *HH201*. When the behavior is periodic, we may



Regular oscillations in the light-scattering properties of the *Dictyostelium discoideum* mutant *HH201* in cell suspension. The mutant (a gift from Dr P. Newell, Department of Biochemistry, University of Oxford) was grown with *Escherichia coli* (strain *B2*) on nutrient agar, harvested, and separated from bacteria as described by Wurster and Mohn [13]. Growth of mutant cells and subsequent experiments were performed at 23 °C. The amoebae were adjusted to 2×10^7 cells/ml in 17 mM Sorensen phosphate buffer pH 6.0 and shaken at 150 revs/min on an orbital shaker. A 2-ml sample of the shaken cell suspension was directly transferred into a cuvette and agitated by bubbling water-saturated oxygen through the suspension [12]. The optical density at 500 nm was monitored with a Zeiss PM16 spectrophotometer. Oscillations developed about 4 h after the beginning of starvation.

expect that cells in the suspensions synchronize, owing to their coupling through extracellular cAMP. Much less is known about the dynamic behavior of coupled chaotic systems. If individual cells were evolving on the same strange – i.e., chaotic – attractor, at different phases (owing to different initial conditions), or on different strange attractors, their coupling might well result in the transformation of aperiodic into regular oscillations. Such a possibility is currently under investigation, by means of numerical simulations with the model for periodic and aperiodic cAMP signalling [7, 8, 11]. If it turns out that a population of individually chaotic cells shows global periodic behavior as a result of strong intercellular coupling, one might expect that experiments in suspensions would not yield results similar to those obtained in the experiments in which the amoebae were aggregating on agar [5] and were therefore not subjected to the strong coupling that occurs in cell suspensions.

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Endocytosis and inositol hexakisphosphate levels in *ras* transformants of *Dictyostelium discoideum* amoebae [1]

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Summary. Fluid-phase pinocytosis kinetics and lysosomal enzyme secretion parameters were measured in *Dictyostelium discoideum* amoebae constructed from strain AX3 by transformation with a multicopy plasmid carrying either a normal *ras* gene (*ras*-Gly12), a mutated *ras* gene (*ras*-Thr12) or by the vector carrying the geneticin resistance gene only (pDNEO2). It was found that the pinocytosis rate and extent as well as the lysosomal enzyme secretion were slightly different in the three strains. These changes, however, were related to minor modifications of the cellular volumes. The overall concentration of inositol hexakisphosphate was similar in the three strains.
Key words. Fluid-phase pinocytosis; enzyme secretion; inositol hexakisphosphate; *ras* genes; *Dictyostelium discoideum*; amoebae.

The protein products of the *ras* proto-oncogenes (p21 *ras*) bind GTP and show a restricted homology with signal-transducing guanine nucleotide binding proteins (G-proteins). Point mutations at specific positions in the *ras* genes have been associated with phenotypic transformation leading to increased malignancy [2–4]. In the slime mold *Dictyostelium discoideum*, a *ras* gene (Dd *ras*) is developmentally regulated and seems to be essential for growth [5–7]. The introduction of a missense mutation at the amino acid 12 induced phenotypic changes and perturbations in the formation of aggregation centers during the early chemotactic phase [8, 9]. Recent studies suggest that *Dictyostalium ras* is involved in the inositol-lipids pathway of the signalling system [10].

Microinjection of human Ha-*ras* protein in rat embryo fibroblasts has been shown to enhance membrane ruffling and fluid-phase pinocytosis of fluorescein labelled dextran (FITC-dextran) by a factor of ten [11]. Similarly, measurements of Lucifer Yellow pinocytosis in a *Saccharomyces cerevisiae* strain mutated at the level of its RAS2 gene (analogous to the Ki-*ras*) have revealed a higher level of endocytosis than in the isogenic strain with the normal *ras* gene [12]. Uptake of horse radish peroxidase was found to be increased by a factor of two in NIH/3T3