blood lymphocytes we used as controls proved to be totally negative.

TEM, at small magnifications, showed cells with a large irregular nucleus containing a regular nucleolus. The cells were connected by processes forming a three-dimensional network (fig. 2). A few lymphoid cells and other degenerating cells were observed inside the meshes of this network during the previous stages of culture. At a later stage, flocculent material and dead cell debris appeared among the meshes (fig. 2). Certain cytological features were observed at greater magnifications. In 3-day cultures, free ribosomes were prominent, and vacuoles were present, together with filaments presumably belonging to the class of keratin-made tonofilaments (fig. 3). Desmosomes connecting the cellular processes to one another were frequently observed (fig. 4).

Discussion

The aim of the present work was to isolate REp cells from the medulla of chicken bursal follicles, and to characterize them by means of immunofluorescence with an anticytokeratin monoclonal antibody and TEM. Our results show that the method we used makes it possible to isolate REp cells. This is demonstrated by the positivity towards the anticytokeratin monoclonal antibody and by the ultrastructural features of the cultured cells. Indeed, the immunofluorescence technique shows an intense positivity in all the cells, while electron microscopy shows the typical morphological pattern of the REp cells; desmosomes, connecting the cellular processes to one another, and tonofilaments were the main features. REp cells do not seem to modify their pattern during the period of culture, even if growth becomes slower after a log phase during the first week of culture. In the past, a similar method was used by others to isolate the cells of the bursal follicle medullae. Boyd et al. used virtually the same technique of dissociation, followed either by irradiation [11], or enzymatic treatment [9], to improve the isolation of the REp cells and to eliminate lymphocytes. In the present work, we show that successive changes of the medium make it possible to eliminate lymphocytes. The adhering non-epithelial cells are discarded because the REp cells stick on lightly, and gentle shaking resuspends them, so that it is then possible to seed supernatants that contain REp cells. The possibility of isolating REp cells, and regenerating in vitro the three-dimensional network that they normally form in vivo, may be an interesting starting-point for further morphological and biochemical research on interactions between bursal lymphocytes and epithelial cells during lymphocyte differentiation in the primary lymphoid organs.

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Novel oscillations in cell suspensions of *Dictyostelium discoideum*

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Summary. With a light-scattering technique, two novel rhythms were discovered in cell suspensions of *Dictyostelium discoideum*. One is a damped oscillation with a period of 2 to 2.5 min (at 23 °C) induced by folate in EDTA-dissociated undifferentiated cells. The other is a sinusoidal oscillation with a period of about 12 min occasionally observed with late differentiated cells. Obviously, the repertoire of rhythms of this simple eukaryotic organism is larger than previously assumed.
Key words. Dictyostelium; cell communication; biological rhythms; oscillations; cAMP; folate; calcium.

Periodic processes are widespread in biological systems and they control important life functions including heartbeat, fertility, and leaf movement. Periodic activities also regulate cell aggregation and differentiation in the cellular slime mold *Dictyostelium discoideum*. This organism feeds and divides as solitary amebae. Upon depletion of the food source, growth phase cells differentiate to aggregative ones which assemble to form multicellular structures[1]. Growth phase cells respond chemotactically to folate and pterin[2]. This ability is assumed to reflect a food-seeking device of cells which naturally prey on folate- and pterin-releasing bacteria in the soil[2]. Aggregative cells respond sensitively to cAMP[3,4]. During the course of differentiation, cells acquire the capacity to synthesize and release cAMP in a periodic manner[5,6]. In a field of cells those that first emit cAMP signals are likely to become aggregation centers. Cells in the vicinity move towards the source of cAMP[3] and they amplify and relay the cAMP signals[7–10]. The occurrence of cAMP concentration waves in fields of aggregating cells has been demonstrated[6].

Not only on a solid substratum but also in an agitated suspension *D. discoideum* cells differentiate and aggregate. The terms undifferentiated, early differentiated, and late differentiated refer to cells starving in phosphate buffer for 0 to 1 h, 3 to 7 h, and 7 to 10 h, respectively. Undifferentiated cells form agglomerates which can be dissociated by means of EDTA while differentiated cells produce EDTA-stable aggregates[11]. After 3–4 h of differentiation in suspension cells acquire the capacity of periodic signalling. With an optical technique two types of periodic phenomena in the light-scattering properties of cell suspensions, spike-shaped and sinusoidal, have been observed[12] (fig. 2A). Light-scattering changes reflect structural changes such as alterations in cell shape or aggregate size. Spike-shaped oscillations are mainly due to changes in cell shape while sinusoidal oscillations primarily involve alterations in aggregate size[13]. During differentiation, spike-shaped oscillations occur prior to sinusoidal ones. At 23 °C the periods of spikes and sinusoids are 8 ± 1 min and 6 ± 1 min, respectively. Spike-shaped oscillations are accompanied by the periodic synthesis and release of cAMP[5], and exogenous cAMP causes phase-shifts[12]. These experimental results, and theoretical work[14], suggest that cAMP functions as a synchronizer of cells during spike-shaped oscillations and also is a constituent of the reaction system underlying these oscillations. Spikes are obviously related to the periodic cAMP emission of aggregation centers on a solid substratum. In contrast to spikes, sinusoids occur in the absence of measurable oscillations in the cAMP concentration[15]. The reactions controlling sinusoidal oscillations are obscure, although there are indications that calcium[16,17] and cell adhesiveness[13] may participate in this process. Sinusoidal oscillations may reflect the organization of cell types in the aggregates[13]. In our studies on the molecular basis of spike-shaped and sinusoidal oscillations two novel periodic phenomena were observed which are described here.

Suspended *D. discoideum* cells respond to their chemoattractants with rapid and transient decreases in light-scattering[12,13]. The pattern of the light-scattering changes is due both to changes in cell shape and to alterations in the size of cell agglomerates[13]. When agglomerates of undifferentiated cells were dissociated by means of 1 mM EDTA[11] the light-scattering response to a folate pulse (now largely due to changes in cell shape) appeared as a damped oscillation (fig. 1). At 23 °C the oscillation period is 2 to 2.5 min (fig. 1). Similar oscillations were induced by folate concentrations of 0.01, 0.1, and 1 µM. Also, a damped light-scattering oscillation was elicited in such cell suspensions by exogenous cAMP (not shown). Previous experiments indicated in response to folate a damped oscillation in the extracellular Ca^{++} concentration[18]. In addition, small successive changes in extracellular Ca^{++} were elicited by cAMP in these undifferentiated cells[18]. The period of the Ca^{++} oscillation of about 2 min is similar to the period of the light-scattering oscillation, suggesting that the two phenomena are interrelated.

The other novel rhythm was observed with differentiated cells after sinusoidal oscillations with periods of 6 ± 1 min had ceased. This oscillation also has a sinusoidal shape (fig. 2B) but both the period and the amplitude are about twice as big as those of the earlier sinusoids. We examined the sensitivity to exogenous cAMP of cells displaying big sinusoidal oscillations. Cyclic AMP at 0.3 nM did not induce a significant change in light-scattering whereas 3 nM cAMP elicited a rapid response and also caused an increase of the period length (fig. 2B). The latter result contrasts with that obtained with early sinusoids, which were not affected in period length by exogenous cAMP[15]. Big sinusoidal os-

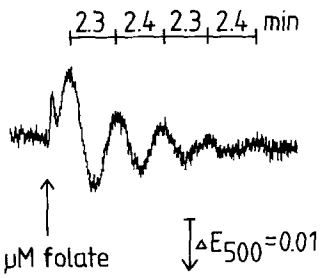

Figure 1. Folate-induced damped oscillations in the light-scattering properties of EDTA-dissociated *D. discoideum* cells. Strain Ax2 was grown in axenic medium supplemented with 1.8% maltose[23]. Cells were harvested at densities of $3–8 \times 10^6$ cells/ml, washed twice with cold (4–8 °C) 17 mM Sorensen phosphate buffer pH 6.0, resuspended in this buffer at 2×10^7 cells/ml, and shaken at 150 revs/min on an orbital shaker. Growth of cells and subsequent steps were performed at 23 °C. In the experiment shown, a 2-ml sample of the shaken cell suspension was transferred into a cuvette after 30 min and agitated by bubbling water-saturated oxygen through the suspension[11]. Cell agglomerates were largely dissociated by 1 mM EDTA (added as 20 µl of a 100 mM solution). About 20 min later folate was added as 2 µl of a 0.1 mM solution. The optical density at 500 nm was monitored with a Zeiss PM6 spectrophotometer. The experiment was repeated three times with similar results.

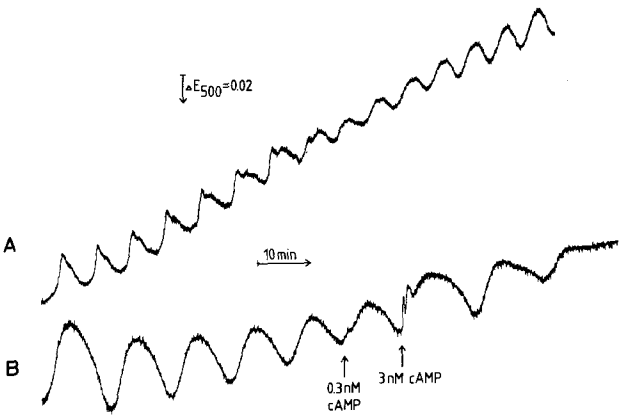

Figure 2. Autonomous oscillations in the light-scattering properties of cell suspensions of *D. discoideum*. *A* Spike-shaped and early sinusoidal oscillations are shown for comparison. Records were obtained from a 2-ml sample shaken for 4 h in phosphate buffer and then transferred into the cuvette. *B* Late, big sinusoidal oscillations. In this experiment a 2-ml sample was transferred into the cuvette after shaking in phosphate buffer for 8 h. Cyclic AMP was added as 2 µl of 0.3 µM and 3 µM solutions, respectively.

cillations were observed only in five out of twenty experiments. These figures do not argue against the significance of the observed rhythm. Theoretical studies demonstrate that systems capable of periodic behavior show stationary behavior in a large domain in parameter space, and small changes in conditions suffice to shift the system from the periodic to the non-periodic mode of operation [19].

Obviously, the repertoire of rhythms of *D. discoideum* cells is larger than previously noted. Although the light-scattering technique has been very helpful for the discovery of periodic phenomena in cell suspensions of *D. discoideum*, it cannot yield molecular information. A common component of different light-scattering oscillations is Ca^{++}. Periodic changes in the extracellular Ca^{++} concentration accompany spike-shaped and early sinusoidal oscillations [17] and presumably also attractant-induced damped oscillations [18]. Ca^{++} concentrations during late sinusoidal oscillations remain to be investigated. Periodic Ca^{++} changes relate oscillations in *D. discoideum* to Ca^{++} oscillations in other biological systems [20, 21].

Why should periodic processes occur? It has been pointed out that oscillatory changes would be more appropriate than constant signals if adaptation occurs in the signal transduction pathway [17] which, in fact, is the case in *Dictyostelium* [9, 22]. Oscillatory variations of a component can be considered as a frequency-encoded signal. In contrast to an amplitude-encoded signal, which contains only information on the concentration of the component, a frequency-encoded signal comprises information on concentration, frequency, and wave shape. The additional information may allow a more precise regulation of cellular responses.

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