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Morphogenesis of *Dictyostelium discoideum* Treated with Lindane

R. Gayatri, S. Chatterjee

School of Life Sciences, Jawahwarlal Nehru University, Delhi-110067, India

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In our earlier report we have shown that lindane, a gamma isomer of benzenehexachloride, affected the growth of *Dictyostelium* vegetative cells (Gayatri and Chatterjee 1992). Further, our other reports indicated that organochlorine pesticides profoundly affect a number of cellular events in slime mold cells (Gayatri and Chatterjee 1993 a,b). In the present investigation the effects of lindane has been studied on the developmental stages of *D.discoideum*. Further we show that the cellular slime molds could be used as a model system for an easy and quick assay for pesticide induced developmental toxicity.

MATERIAL AND METHODS

The axenic strain Ax2 of *Dictyostelium discoideum* provided by Dr. Robert Kay (University of Cambridge, U.K.) was used. The axenic strain was grown at 23°C according to the method of Ashworth and Watts (1970). Exponentially growing *Dictyostelium* cells were treated with lindane (obtained from John Baker Inc., Colrado, USA) as described earlier (Gayatri and Chatterjee 1991).

Following the treatment with lindane for 20 min , the cells were thoroughly washed with Sorenson's buffer ($Na_2HPO_4.2H_2O$-11.87 g; $KH_2PO_4.2H_2O$-9.07 g in 1L of distilled water, pH 6.3). The density of control and pesticide treated cells were then adjusted to 5 x 10^6 cells/mL and plated on non - nutrient agar (containing 20 mM KCl, 1 mM $CaCl_2$, 20 mM NaCl) at 23 °C.

cAMP-chemotaxsis assay was performed by plating the differentiating cells on $1x10^{-6}$M cAMP agar (Ohmori and Maeda 1987). Formation of EDTA-stable cell contact was monitored by counting the number of loose cells at 4, 6 and 8 hr following starvation. (Ohmori and Maeda 1987).

Correspondence to: S. Chatterjee

The supernatant of developing cells were assayed for ePDE according to the method described by Riedel and Gerisch (1971). Morphogenesis of control Ax2 cells and slugs were also studied by plating the cells on lindane agar (5, 10 and 20 μg/ml).

RESULTS AND DISCUSSION

Control *Dictyostelium* cells showed normal morphogenesis which was completed within 24 hr, while the morphogenesis was delayed and abnormal in cells treated with 60 μg/ml lindane (Table 1). Compared to the control, the cell steamings were scanty in the pesticide treated cells. Further, lindane treatment causes the formation of fewer and smaller aggregates, slugs and fruiting bodies (Fig. 1). Morphogenesis was totally blocked in cells treated with 100 μg/ml lindane.

Control cells showed cAMP chemotaxis within 6 hr of plating by which time the cells start to move out of the droplet and by the 12^{th} hr, the droplet spreads out completely. *Dictyostelium* cells treated with a lower dose (60 μg/ml) of lindane showed a delay in outward cell movement by ca. 15 hrs, while there occured a total inhibition of cAMP - chemotaxis when cells were exposed to 100 μg/ml of lindane (Fig. 2).

Dictyostelium cells treated with lindane showed delayed aggregation (Fig. 3). Further the aggregates were fewer and smaller in size. A dose dependent inhibition in the activity of ePDE has been seen in the pesticide treated cells (Fig. 4).

Morphogenesis was totally inhibited when plated on lindane agar with doses above 10 μg/ml (Fig. 5). Cells plated on 10 μg/ml lindane agar showed abnormal developmental stages (smaller and fewer slugs and fruiting bodies) and delay in morphogenesis by 72 hr. Total lysis of slugs occurred when they were plated on 10 μg/ml lindane agar (Fig. 6).

The present data indicate that organochlorine pesticide at concentrations of 60 and 100 μg/ml severely affects the morphogenesis of *Dictyostelium* cells. Inhibition of cellular streaming and cAMP - chemotaxsis indicates a pesticide induced suppresion of cell motility. Organochlorine pesticides are knwon to interact with the lipid bilayer of liposomes and human erythrocytes thereby altering its structure and functions (Jones and Lee 1985; Madeira and Madeira 1989; Verma and Singhal 1991). Similar membrane alteration might occur in the pesticide treated *Dictyostelium* cells. Inactivation of membrane bound cAMP receptors or alteration of ligand - receptor binding affinity due to pesticide - membrane interactions might lead to inhibition of chemotactic movements (Verma and Singhal 1991). In the developing slime mold cells, binding of cAMP receptors promotes assembly of cytoskeletal proteins which are required for pesudopod formation leading to cellular orientation and directed migration (Devreotes 1982; Fukui 1990). Our unpublished observations show a reduction in both actin and myosin content of the lindane treated (60 and 100 μg/ml) *Dictyostelium* cells. Since cytoskeletal proteins are important for pesudopod formation any alteration in the actin and myosin content would prevent the cell motility.

Expression of cell surface glycoproteins and discoidins during specific stages of development helps in intercellular adhesion in developing cells (Siu 1990). As lindane is lipophilic in nature it is reasonable to assume that pesticide - membrane interactions might lead to conformational changes of membrane proteins thereby preventing the formation of stable cell contact. Further inhibition of cell motility in the pesticide treated cells would also prevent the cells from coming in contact with each other. Our earlier reports on chlorpromazine also shows that any alterations in membrane related activity would affect

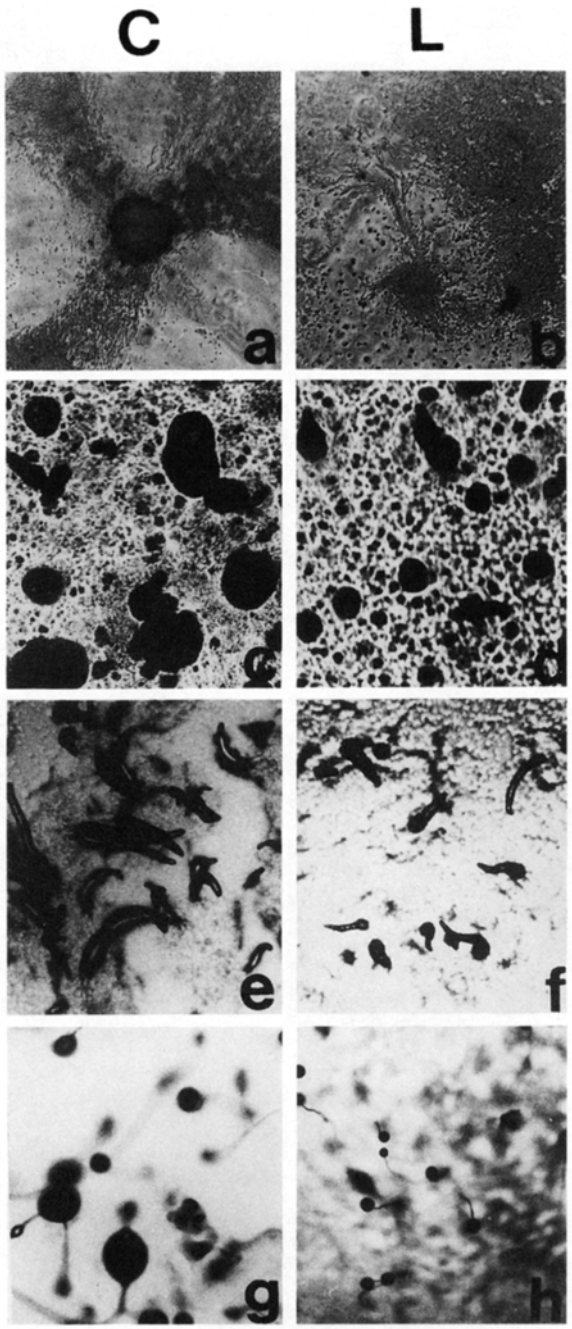


Figure 1. Effects of lindane on the morphogenesis of *Dictyostelium discoideum* cells. Note the well developed streams (a), aggregates (c), slugs (e) and fruiting bodies (g) in control (C) cells and scanty streams (b), aggregates (d), smaller and fewer slugs (f) and fruiting bodies (h) in the lindane treated (L) treated cells. Magnifications a-d (63x); e-h (25x).

Table 1. Development (in hr) of *D. discoideum* on non-nutrient agar

Dose (µg/ml)	cAMP-Chemotaxis (hr)	Cell-Streaming (hr)	Cellular Aggregates (hr)	Slugs (hr)	Fruiting Bodies (hr)
	+	+	+	+	+
0	6	8-10	12-16	18-20	24-28
	±	±	±	±	±
60	15-20	48-52	54-58	62-68	72-78
100	-	-	-	-	-

+ present, - absent; ± delayed; hr hours in development

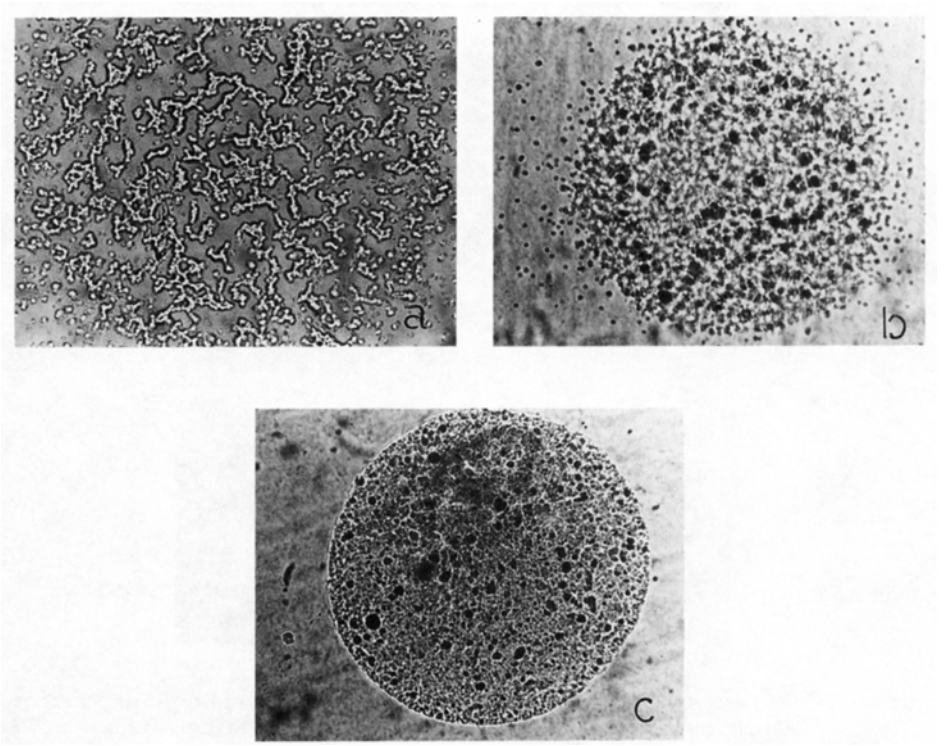
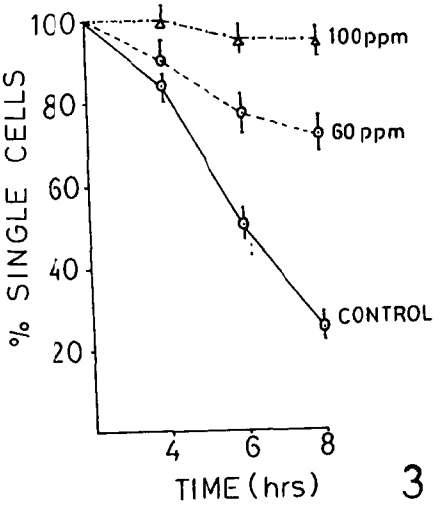
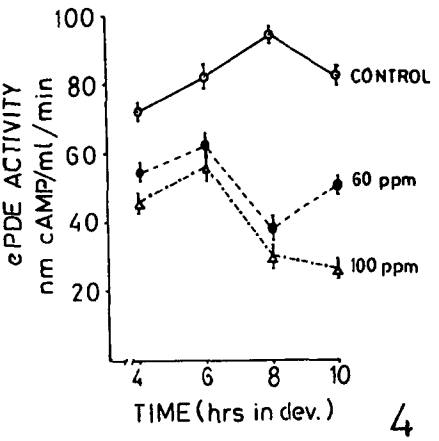
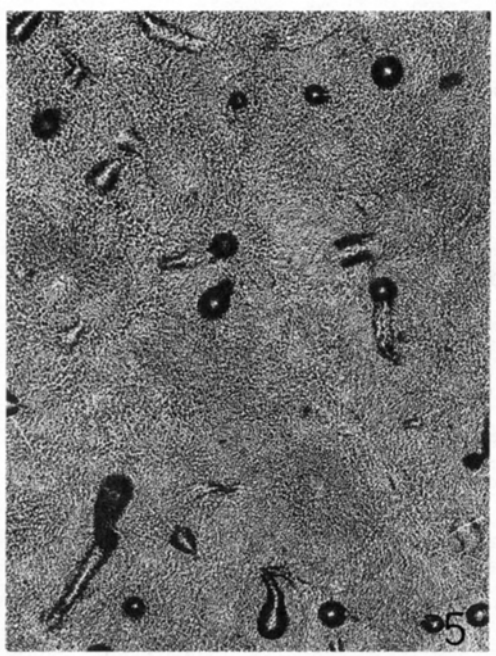


Figure 2. Cyclic AMP chemotaxis in control (a) and lindane treated cells (b-60 µg/ml; c - 100 µg/ml).







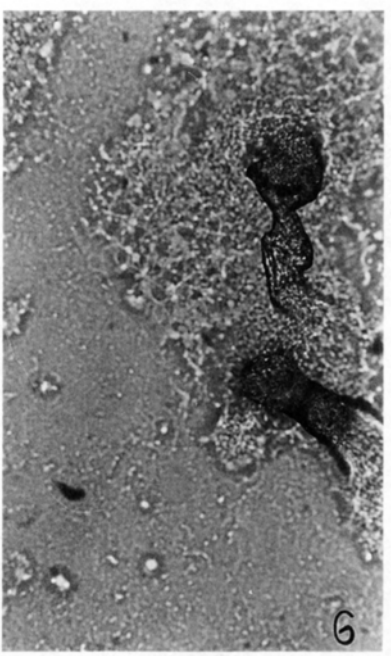


Figure 3. EDTA - stable cell adhesion in control and lindane treated slime mold cells.

Figure 4. Extracellular phosphodiesterase profile in control and lindane treated *Dictyostelium discoideum* cells.

Figure 5. Morphogenesis of *Dictyostelium discoideum* cells on lindane agar. Note the smaller slugs and aggregates. (25x)

Figure 6. Photomicrograph of lysing slug on 20 µg/ml lindane agar (at T_{12}). (200x).

the cellular contact formation in the developing *Dictyostelium* cells (Gayatri and Chatterjee 1991).

The inhibition of ePDE activity in the lindane treated *Dictyostelium* cells would lead to cAMP accumulation in the extracellular medium, which in turn might disturb the cAMP gradient essential for the normal morphogenesis. A reduction in ePDE activity could also be due to alterations in the levels of Ca^{2+} and calmodulin when cells are treated with lindane (Salimath et al. 1988). A similar reduction in calmodulin activated phosphodiesterase (from rat brain) by organochlorine compounds have been reported by Vig et.al. (1990).

During morphogenesis of *Dictyostelium* intercellular communications play an important role in aggregation and sensing of morphogens which ultimately determines the fate of the developing cells (Bloom and Kay 1988). The intercellular commmunication is well pronounced in the slug stage. Inhibition in intercellular communication is reported in chinese hamster cells exposed to organochlorine pesticide DDT (Warngard et. al. 1989). Lysis of slugs when exposed to lindane thus indicates a disruption of signaling mechanisms, affecting the normal course of morphogenesis.

From the present study it is evident that lindane severely affects the developmental events in *Dictyostelium* cells by interfering with the membrane related activities . Undertaking a full range of toxicity tests on hazardous chemicals using conventional animal models is not feasible because of the cost, maintainence and longer gestational period. Therefore there is a need for simpler models for easy and quick toxicity assay. In this regard *Dictyostelium* offers a good model system as the results on the developmental toxicity can be easily and quickly performed as morphogenesis is completed within 24 hr. Further, in *Dictyostelium* many of the morphogenetic events such as cell movement, cell recognition and cell differentiation are similar to embryogenesis of higher organisms. Thus the cellular slime molds can be used for preliminary and rapid screening for effects of various toxic chemicals on higher organisms.

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