Arachidonic Acid and Related Molecules Affect the Behaviour of *Dictyostelium discoideum* Slugs

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Addition of micromolar concentrations of di-2-ethylhexyl phthalic acid ester (DEHP) and arachidonic acid esters to the substratum caused disorientation of phototaxis of *Dictyostelium discoideum* slugs similar to that observed with the as yet unidentified Slug Turning Factor (STF). The decrease in accuracy of phototaxis was dependent on the concentrations of these molecules. Several other structurally related lipid-like molecules did not affect slug phototaxis. Like STF, which affects phototaxis whether or not activated charcoal is present in the agar, arachidonic acid esters retained their activity on charcoal agar, whereas DEHP did not. Both compounds shifted the transition temperatures in slug thermotaxis away from the growth temperature. Unlike STF, they did not reduce the accuracy of positive slug thermotaxis. The data suggest that although none of these molecules can be regarded as a STF analogue, arachidonate and/or some of its metabolites may have a role in the sensory transduction in *D. discoideum* slugs.

INTRODUCTION

The multicellular migratory stage (slug) is a transient step in the morphogenesis of *Dictyostelium discoideum* following the aggregation of starving amoebae via release of and chemotaxis towards cAMP (see, for example Raper, 1939; Gerisch, 1982). Slugs are covered by a cellulose-containing proteinaceous slime sheath which is continuously synthesized and left behind as a slime trail (Hohl & Jehli, 1973; Freeze & Loomis, 1977; Smith & Williams, 1979). Slugs orientate (turn) in response to light (phototaxis; Raper, 1940), temperature (thermotaxis; Bonner *et al.*, 1950), and spontaneous internal stimuli (Fisher *et al.*, 1983). Slug behaviour shows transitions that result in bidirectional phototaxis (slugs 'aim' at points either side of the light source; Fisher & Williams, 1981*a*) and in temperature-dependent orientation up or down heat gradients (Poff & Whitaker, 1979; Fisher & Williams, 1982; Dohrmann *et al.*, 1984). An endogenously produced diffusible factor (Slug Turning Factor, STF) has been proposed to be involved in slug phototaxis and thermotaxis. According to this hypothesis, both light and temperature locally affect production and/or release of STF, thus generating within the slugs lateral STF gradients in response to which they turn (Fisher *et al.*, 1981).

Several aspects of STF action have been established (Fisher *et al.*, 1981; for review see Fisher *et al.*, 1984). STF is secreted into the substratum (more in the light than in the dark), and slugs exhibit negative chemotaxis from crude STF exudates. When STF preparations are added to the agar at high uniform concentrations, the accuracy of both phototaxis and positive thermotaxis is decreased. These interference assays, in conjunction with mutant (Fisher & Williams, 1982) and

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Abbreviations: STF, slug turning factor; DEHP, di-2-ethylhexyl phthalic acid ester.

physiological studies (Dohrmann *et al.*, 1984), demonstrate that phototransduction and thermotransduction pathways converge.

Initial results showed that STF had a molecular weight < 500 . The finding that activated charcoal in water agar did not diminish STF activity (Fisher & Williams, 1981*b*) and the failure of STF from exudates to be strongly adsorbed to activated charcoal indicated that it was unlikely to be a flat non-polar molecule (Fisher, 1981). Further isolation and the identification of STF from *D. discoideum* had proved difficult because of the extremely low quantities in slug exudates. However, during the attempts to extract biological STF, we discovered that concentrated organic solvents already contain high STF-like activity as revealed by their interference with slug phototaxis. These interference assays (Fisher *et al.*, 1981) were highly specific because aggregation of amoebae and formation of slugs are prerequisites for slug behaviour so that agents causing non-specific impairment of amoebal movement are excluded.

We report here the identification of a contaminant present in organic solvents which can mimic STF activity in phototaxis experiments. We show further that a biologically more relevant substance – arachidonic acid – being structurally similar to this contaminant also exhibits STF-like activity. The latter finding is of special interest with respect to recent reports on arachidonic acid as an active chemotactic substance in neutrophils (Turner *et al.*, 1975; Sha'afi & Naccache, 1981).

METHODS

Materials. Technical grade petrol (40–65 °C) was from Graen, Munich, FRG. Pure fatty acid and fatty acid esters, analytical grade L- α -phosphatidylcholine, L- α -phosphatidyl-DL-glycerol, L- α -phosphatidyl-L-serine, prostaglandin E₁, and activated charcoal (acid-washed with sulphuric and phosphoric acids) were from Sigma. Phthalic acid esters (Schuchardt, Munich, FRG), 2-ethyl-1-hexanol (Fluka, Buchs, Switzerland), nanograde *n*-hexane and nanograde *n*-pentane (Mallinckrodt Inc., St Louis, Mo., USA), Serva Blau R (Serva Feinbiochemica, Heidelberg, FRG), Noble agar (Difco) and alumina Woelm N-Super 1 (Woelm Pharma, Eschwege, FRG) were obtained as noted. Leukotriene B₄ was a gift from Professor E. J. Corey, Chemistry Dept, Harvard University, USA. Its activity as a leucocyte chemoattractant was confirmed by Dr H. Mensing, University of Hamburg, FRG.

Extraction and purification of STF-like activity from petrol distillate. Normally a 10 l batch of a 40–65 °C fractional distillate of petrol was concentrated to 5–10 ml in a vacuum evaporator at 35 °C. Approximately 1/50 of the concentrated petrol distillate was chromatographed on 1 ml aluminium oxide in a Pasteur pipette. The aluminium oxide was washed with *n*-pentane before application of the sample. Elution was carried out stepwise with 3 $\times$ 2 ml of *n*-pentane, *n*-pentane + 30% (v/v) ethyl acetate, *n*-pentane + 50% ethyl acetate, and ethyl acetate. Each fraction was evaporated to dryness with N₂, and after resuspension in 2 ml *n*-hexane, samples were tested for biological activity. On separate samples gas chromatographic analysis was carried out using a Fractovap chromatograph (Carlo Erba Strumentazione, Milan, Italy). The instrument was equipped with a 26.7 m column of CP Sil 5 (Chrompack WCOT) and linearly programmed from 150 °C to 300 °C at a rate of 10 °C min⁻¹. The carrier gas was helium at a flow rate of approximately 20 cm s⁻¹. The injection and detection temperatures were 275 °C. Gas chromatography-mass spectrometry was performed using a Varian CH7A instrument (70 eV; Bremen, FRG).

Culture of *D. discoideum* strains. Standard strain X22 (Stenhouse & Williams, 1977) was used for most experiments. Two strains, HU1282 (D.L. Welker, unpublished) and NP84 (North & Williams, 1978), which differ in their phototactic behaviour, were used in some experiments. In each case, nutrient SM agar plates were inoculated with about 10⁵ amoebae and *Klebsiella aerogenes* as a food source. The plates were incubated for 48 h in the dark at 21 $\pm$ 1 °C (Williams & Newell, 1976). Amoebae were harvested from the plates, freed of bacteria by washing them several times in Bonner's salt solution (250 g centrifugation), and then resuspended in Bonner's salt solution at 10⁶ ml⁻¹ (Williams & Newell, 1976).

Phototaxis and thermotaxis experiments. Amoebal suspension (normally 20 μ l, i.e. 2 $\times$ 10⁶ cells) was inoculated on a 1 cm² origin at the centre of Petri dishes containing 30 ml water Noble agar or water Noble agar to which 0.5% (w/v) activated charcoal (Fisher & Williams, 1981*b*) had been added. In a few experiments, behaviour of slugs formed from amoebae plated at high cell densities was assayed by inoculating a larger volume of amoebal suspension. After the excess liquid had evaporated, the agar plates were placed into slightly larger opaque PVC dishes and treated as described previously for phototaxis and thermotaxis experiments (Dohrmann *et al.*, 1984). If 2 $\times$ 10⁶ amoebae were inoculated, approximately 20–40 slugs formed and migrated almost to the edge of the plate after 48 h at 21 $\pm$ 1 °C (phototaxis) or 72 h (thermotaxis), the temperature gradient being 0.2 °C cm⁻¹. A permanent record of the migration pattern was obtained by transferring the slugs and the slime trails to clear PVC discs and staining them with Coomassie Blue (3 g Serva Blau R, 500 ml methanol, 400 ml H₂O, 100 ml acetic acid).

For preparing the figures, the discs were photographed and the positives were re-photographed to enhance the visibility of the migration patterns; it should be noted that the slime trails are not as clearly seen on the original discs as they are in the figures.

Bioassay for STF-like activity. The interference of slug phototaxis and thermotaxis by fractions obtained from petrol and by lipid-like substances was monitored after their addition to molten agar. An appropriate amount of stock solution of the desired compound was added to the agar at about 70 °C immediately before pouring the plates which contained 30 ml agar. The solvents were ethyl acetate for fatty acids and fatty acid esters, and *n*-hexane for phthalates and chloroform for triglycerides. Control experiments were done with the highest amount of solvent used. Phototaxis and thermotaxis experiments were as outlined above.

RESULTS

Detection and identification of STF-like activity in petrol.

During the initial experiments aimed at the isolation of STF a high amount of STF-like activity was detected in technical grade petrol (R. Mahurin, P. R. Fisher & K. L. Williams, unpublished). The amount of activity found in 10 l technical grade petrol was equivalent to that from at least 10^{11} cells (10^6 phototaxis interference units, PIU; Fisher *et al.*, 1981). Assuming that the 'petrol molecule' (being derived from organic matter) and STF might at least be structurally related, we undertook its purification and identification. After concentration by distillation, the activity present in petrol was recovered by aluminium oxide column chromatography in a fraction eluted with pentane containing 30% ethyl acetate. Other fractions obtained from the aluminium oxide column were inactive in the bioassay. Because gas chromatographic analysis of the active fraction indicated a high degree of purification (Fig. 1) mass spectroscopy could be applied. The two major peaks from the mass spectrum were identified as dibutyl phthalic acid ester and DEHP, which are the most common plasticizers found as contaminants not only in organic solvents but in the overall environment (e.g. see Ishida *et al.*, 1980; for biochemical effects see e.g. Bell, 1983). They chromatographed with retention times of 538 s and 838 s under the conditions used, and at a ratio of 1:3.5, respectively. The commercially obtained pure compounds chromatographed in these positions.

When the two phthalic acid esters were examined for disorientation of *D. discoideum* slug phototaxis, DEHP decreased the accuracy of phototaxis by slugs of the wild-type strain X22 at 1 μ M (Fig. 2, Table 1). It was calculated that 1 l petrol contained approximately 2 mg DEHP, and this amount was sufficient to account for almost all of the phototaxis interference activity occurring in petrol. Dibutyl phthalate did not disorientate slugs, but at 5 μ M slugs migrated only half as far as the control slugs and at 50 μ M no slugs were formed from the aggregates (data not shown).

X22 slug phototaxis in the presence of DEHP and related molecules

DEHP had a pronounced effect on the phototaxis of strain X22. Fig. 2 shows slug migration patterns on water agar in the presence of DEHP (1–100 μ M; Fig. 2*b, c, d, f*) or dimethyl phthalate (100 μ M; Fig. 2*g*). The phototaxis-impairing activity of DEHP was observable in slugs formed at low cell density (Fig. 2*b–d*) but was more pronounced in slugs formed at high density (Fig. 2*f*), i.e. at high STF concentrations (Fisher *et al.*, 1981). Hence its effect seemed to be additive with that of STF already present due to high cell density (Fig. 2*e*). In order to eliminate interference by STF, experiments were preferentially performed using 'low density' slugs. Whereas DEHP affected phototaxis at concentrations as low as 1 μ M, the dimethyl ester (Fig. 2*g*) had little effect, even at concentrations as high as 400 μ M. In Table 1, minimal effective concentrations of DEHP and some analogues are listed. The minimum concentration of STF reliably detected by the bioassay is about 50 PIU ml⁻¹ (Fisher, 1981; Fisher *et al.*, 1981).

None of the compounds structurally related to DEHP decreased the accuracy of phototaxis at concentrations < 100 μ M. This means that changes in the length and/or branching of the alkyl group in DEHP resulted in loss of activity. The importance of the carbon number of the alkyl groups of phthalates was demonstrated previously with studies on human plasma lecithin/cholesterol acyltransferase: among various phthalates tested, DEHP reduced the enzyme activity

Fig. 1. Gas chromatographic analysis of the pentane/30% ethyl acetate fraction of petrol obtained from an aluminium oxide column. The fraction contained STF-like activity. Conditions for distillation of 10 l petrol, and column chromatography were as described in Methods. The first 2 ml eluate with pentane/30% ethyl acetate was evaporated with liquid nitrogen and resuspended in 2 ml *n*-hexane, 1 μ l was injected into the gas chromatograph. Gas chromatography of pure dibutyl phthalate and pure DEHP confirmed the identity of the two main peaks which were determined by mass spectroscopy.

most effectively (Lagente *et al.*, 1979). Note that in contrast to most of the phthalates, *N*-(*p*-aminobenzoyl)-L-glutamic acid, a constituent of the amoebal attractant folic acid (Pan *et al.*, 1972), caused not only disoriented but pronounced bimodal phototaxis at 200 μ M (see Fig. 6e).

DEHP at concentrations above 10 μ M had other effects on slug behaviour. Substantially more slugs were present in the 10 μ M and 100 μ M treatments (Fig. 2c, d), partly because many slugs split longitudinally from the tip to form two slugs under these conditions. Furthermore, the aggregation territory size decreased so that more numerous, smaller aggregates were formed. The folate derivative did not cause either effect even at 200 μ M when it impaired phototaxis (see Fig. 5e).

Arachidonic acid and arachidonic acid esters alter X22 slug behaviour but related compounds do not

Because of some similarity of the structural formula of arachidonic acid to that of DEHP (compare Table 1 with Table 2), we tested slug phototaxis in the presence of this fatty acid and some of its esters. Disorientation of slug phototaxis was most pronounced when esters of arachidonic acid were present in the substratum, the minimal effective concentration being 10 μ M (Table 1). The simplest explanation for the esters being more active than free arachidonic acid itself is that they can enter the cell membrane more easily because of their greater hydrophobicity.

In contrast to phthalic acid esters, the hydrophobic chain length of the arachidonate esters was clearly not critical for their activity. Slug phototaxis in the presence of methyl arachidonate is illustrated in Fig. 3 for slugs formed at low densities. From comparison of Fig. 3(c) with Fig. 2(c) it can be seen that 50 μ M-methyl arachidonate affected slug phototaxis to a similar extent as did 10 μ M-DEHP. As in the case of DEHP, the effect of the arachidonate was superimposed upon the effect of STF at high cell density (data not shown).

Unlike DEHP, the arachidonate acid esters did not cause slugs to divide, nor was there a substantial effect on aggregation territory size, even at high concentrations (see Fig. 3c). Examination of the slime trails under the microscope revealed that unlike DEHP, the

Table 1. Phototaxis of strain X22 in the presence of phthalic acid derivatives and related molecules

The listed compounds were added to the molten agar at the concentrations indicated before pouring the plates. The plates were inoculated with 2×10^6 starving amoebae and phototaxis experiments were carried out as described in Methods.

Compound	Structure	Minimum effective concn*
DEHP		1 μM
Dibutyl phthalate		5 μM †
Diethyl phthalate		> 150 μM
Dimethyl phthalate		> 400 μM
2-ethyl-1-hexanol		> 100 μM
<i>N</i> -(<i>p</i> -aminobenzoyl)- <i>L</i> -glutamic acid		> 100 μM

* Lowest concentration at which significant impairment of phototaxis was observable. The '>' sign means that higher concentrations could not be or were not tested.

† As stated in Results, dibutyl phthalate did not impair phototaxis at this concentration, but had other effects.

arachidonate esters caused many cells to be left behind in the trail as slugs migrated. The dropping of cells was especially pronounced at concentrations > 100 μM , where a continuous stream of highly vacuolated cells was observed.

In addition to arachidonic acid and its esters, we tested whether other fatty acids or their derivatives exhibited similar effects on slug phototaxis. As seen in Table 2, the esters of oleic,

Fig. 2. Phototaxis of *D. discoideum* X22 slugs in the absence and presence of DEHP and dimethyl phthalate. Washed amoebae were placed on a 1 cm² origin in the centre of water agar plates at low cell density (2×10^6 ; a-d, g) and at high cell density (2×10^7 ; e, f). The agar contained no phthalates (a, e); 1 μ M-DEHP (b, f); 10 μ M-DEHP (c); 100 μ M-DEHP (d); or 100 μ M-dimethyl phthalate (g). The lateral light source is arrowed. Here, as in all subsequent figures, the rectangle from which the slugs migrate is 1 cm².

linoleic and γ -linolenic acid decreased the accuracy of slug phototaxis but only at concentrations higher than those of the arachidonic acid esters. Ethyl acetate, which was used as a solvent for the fatty acid molecules, impaired slug phototaxis only at concentrations above 50 mM. Such a high concentration of ethyl acetate was never present when fatty acid esters were to be assayed. However, even in the presence of such high concentrations of ethyl acetate or the highest concentrations of fatty acid esters which were tested (i.e. 200 μ M), amoebae aggregated and slugs migrated.

A number of other substances such as triglycerides were assayed for interference with X22 slug phototaxis but none of these were active at the highest concentration that could be tested (Table 2). Because metabolites of arachidonic acid such as prostaglandin E₁ and leukotriene B₄ (Borgeat & Samuelsson, 1979; Samuelsson *et al.*, 1979) have been shown to be endogenous effector molecules in other systems, we investigated their possible interference with slug behaviour. Prostaglandin and leukotriene could only be applied at relatively low concentrations (10 μ M for prostaglandin and 1 μ M for leukotriene B₄). Neither compound altered *D. discoideum* slug phototaxis at the concentrations tested, which were in the concentration range where DEHP was proved to be active.

Table 2. Disorientation of slug phototaxis of strain X22 by fatty acids, fatty acid esters and lipids

The experiments were performed as described for Table 1.

Compound	Structure	Minimum effective concn
Arachidonic acid		20 μM
Methyl arachidonate Ethyl arachidonate Propyl arachidonate	$\left. \begin{array}{l} \text{R} = \text{CH}_3 \\ \text{R} = \text{C}_2\text{H}_5 \\ \text{R} = \text{C}_3\text{H}_7 \end{array} \right\}$	10 μM 10 μM 10 μM
Methyl oleate		100 μM
Methyl linoleate		100 μM
Methyl γ -linolenate		200 μM
L- α -Phosphatidylcholine	$\left. \begin{array}{l} \text{R}_3 = \text{CH}_2\text{---CH}_2\text{---}\overset{\oplus}{\text{N}}\text{---}(\text{CH}_2)_3 \\ \text{R}_3 = \text{CH}_2\text{---}\overset{\text{H}}{\underset{\text{OH}}{\text{C}}}\text{---CH}_2\text{---OH} \\ \text{R}_3 = \text{CH}_2\text{---}\overset{\text{H}}{\underset{\text{COO}^\ominus}{\text{C}}}\text{---}\overset{\oplus}{\text{N}}\text{H}_3 \end{array} \right\}$	> 100 μM
L- α -Phosphatidylglycerol		> 100 μM
L- α -Phosphatidyl-L-serine		> 10 μM
Ethyl acetate		50 mM

X22 slug thermotaxis in the presence of DEHP and methyl arachidonate

When slugs developed from amoebae plated at low cell density (Fig. 4*a-c*) were assayed for thermotaxis, both DEHP and methyl arachidonate shifted the temperature of transition to negative thermotaxis away from the temperature at which the amoebae had been grown (Fig. 4*b, c*). The transition temperature was also lowered when 'high density' slugs were assayed on agar without additions (Fig. 4*d*), although the effect was not as pronounced as in Fig. 4*(b, c)*. Unlike high cell density and STF itself (Fisher *et al.*, 1981), methyl arachidonate and DEHP did not decrease the accuracy of positive thermotaxis near the growth temperature. In fact methyl

Fig. 3. Effect of arachidonate on the accuracy of phototaxis by *D. discoideum* X22 slugs. Inoculation of amoebae was as in Fig. 2. Slugs developed from cells at low density (2×10^6). The experiments were performed on water agar without addition (a) or on water agar containing $20 \mu\text{M}$ (c), and $50 \mu\text{M}$ (b) methyl arachidonate.

arachidonate enhanced it slightly (data not shown). These results indicate that neither of these molecules is STF.

Activity of X22 slug orientation impairing molecules in the presence of activated charcoal

It was previously shown that neither STF nor any other phototaxis-impairing metabolites of *D. discoideum* were adsorbed by activated charcoal in the substratum (Fisher & Williams, 1981 *b*). This is confirmed in Fig. 5(g) (vs Fig. 5b) where the presence of activated charcoal did not prevent impairment of phototaxis at high cell density (i.e. high STF levels). Like STF, methyl arachidonate disoriented slug phototaxis on charcoal agar as well as on water agar (Fig. 5h vs 5c). However, we show here that the activity of the aromatic molecules DEHP (Fig. 5d vs 5i) and *N*-(*p*-aminobenzoyl)-L-glutamic acid (Fig. 5e vs 5j) was abolished by activated charcoal in the agar. These findings support the suggestion that STF is not a molecule containing a flat aromatic group like DEHP (Fisher, 1981).

Phototaxis of strains NP84 and HU1282 in the presence of DEHP and methyl arachidonate

We examined whether the effects of DEHP and methyl arachidonate observed in X22 slug behaviour could also be seen in other strains with different genetic backgrounds. Fig. 6 shows the results obtained for two strains: NP84 and HU1282. NP84 was chosen because it only begins to show disorientation of slug phototaxis at cell densities of $> 10^7 \text{ cm}^{-2}$, whereas X22 slugs become disorientated at $3 \times 10^6 \text{ cells cm}^{-2}$ on water agar with or without activated charcoal (Fisher *et al.*, 1981; Fisher & Williams, 1981 *b*). This could be due either to insensitivity to STF or to a lower production of STF in NP84. NP84 was insensitive to DEHP (Fig. 6b) as well as to methyl arachidonate (Fig. 6c), although in the latter case slugs did not migrate so far. It should be noted that slugs of strain NP84 were also insensitive to fluoride (unpublished results) in the concentration range which affected X22 (Dohrmann *et al.*, 1984).

Fig. 4. Migration patterns of *D. discoideum* X22 slugs at low or high cell density in the absence or presence of DEHP or methyl arachidonate in a temperature gradient. About 2×10^6 amoebae (a–c) or 2×10^7 amoebae (d) were placed in a 1 cm^2 origin in the centre of water agar plates which contained no additional compounds (a, d), DEHP (b), or methyl arachidonate (c), both at $20 \mu\text{M}$. The temperature at the origin was 16°C and the temperature gradient was $0.2^\circ\text{C cm}^{-1}$. The warmer side of the plates is indicated by the arrows.

Strain HU1282 was chosen because it exhibited poor slug phototaxis even when slugs were formed at low cell density (Fig. 6d). Unlike NP84 but like X22, HU1282 slugs became more disoriented with both DEHP (Fig. 6e) and methyl arachidonate (Fig. 6f). Note that DEHP did not cause slugs to split into two, nor did it alter the aggregation territory size in this strain.

Fig. 5. Comparison of *D. discoideum* X22 slug phototaxis in the presence of different lipid-like molecules added to water agar or to water agar containing activated charcoal. Amoebae were inoculated, (i) on water agar plates (upper row), (ii) on water agar containing activated charcoal (lower row) at 2×10^6 per 1 cm^2 origin on each plate except for (b) and (g) in which cases inoculation was at 2×10^7 cells. Added to the agar were: 100 μM -methyl arachidonate (c, h); 10 μM -DEHP (d, i); or 200 μM -N-p-aminobenzoyl glutamic acid (e, j). The other plates (a, b, f, g) were without addition.

Fig. 6. Influence of DEHP and methyl arachidonate on the accuracy of phototaxis by slugs of strain NP84 (a-c) and strain HU1282 (d-f). Amoebae were inoculated at 2×10^6 per 1 cm^2 origin on water agar plates. The agar contained no additions (a, d); $20 \mu\text{M}$ -DEHP (b); $50 \mu\text{M}$ DEHP (e); or $50 \mu\text{M}$ -methyl arachidonate (c, f).

Whereas X22 was more sensitive to DEHP than to methyl arachidonate, in HU1282 a similar sensitivity towards both compounds was observed. However, bimodality in HU1282 was more apparent with slugs that migrated in the presence of arachidonate as they did not switch frequently from one preferred direction to the other.

DISCUSSION

Until recently the only molecule shown to affect *D. discoideum* slug behaviour was STF, which is secreted by slugs and decreases the accuracy of phototaxis and positive thermotaxis (Fisher *et al.*, 1981). Subsequently, it was demonstrated that inorganic ion concentrations (F^- , Ca^{2+}) influenced both accuracy of orientation and transitions in phototaxis and thermotaxis (Dohrmann *et al.*, 1984), but neither of these ions mimics STF.

Over 30 compounds, including amino acids, sugars, plant hormones, neurotransmitters and cAMP were screened for STF-like phototaxis interference activity at concentrations up to $500 \mu\text{M}$ in charcoal agar, but were negative (Fisher, 1981). As demonstrated here for strains X22

and HU1282, the presence in the substratum of organic molecules such as DEHP (not regarded as of biological origin) and a biological molecule such as arachidonic acid or its esters at 1–10 μM resulted in decreased accuracy of slug phototaxis, and the transition temperature from negative to positive thermotaxis of X22 slugs was shifted away from the growth temperature of the amoebae. Because of the effects on slug phototaxis, we concluded initially that DEHP and arachidonate exhibit STF-like activity. However, unlike STF neither molecule impaired the accuracy of positive thermotaxis. Furthermore, whereas phototactic impairment by STF or arachidonate was observed using water agar with or without activated charcoal, the effect of DEHP was abolished by the charcoal. In strain X22, DEHP and arachidonate not only influenced slug behaviour but had a broader spectrum of activities in that DEHP decreased territory sizes of aggregating amoebae and caused splitting of migrating slugs. Arachidonate also enhanced 'dropping' of slug cells into the slime trail.

Nevertheless, these molecules may play an as yet unknown role in slug behaviour. At least two explanations are possible. The fluidity and/or fatty acid composition of the *D. discoideum* membrane may play a role in the transduction of signals. The possible significance of an altered physical structure of membranes due to perturbation by endogenously produced or exogenously applied fatty acids has been pointed out (Anderson & Jaworski, 1977; Hauser *et al.*, 1979; Klausner *et al.*, 1980; Creutz, 1981; Hoover *et al.*, 1981; Elhai & Scandella, 1983). Very little is known about fatty acids and fatty acid metabolism in *D. discoideum*; however, arachidonic acid is only found in traces (Weeks, 1976; Long & Coe, 1977; Hase, 1981). For this reason, exogenous arachidonic acid may alter membrane properties more significantly than shorter chain lipids already well represented. This, or the greater degree of saturation of the other fatty acids tested here, could explain their lower activity.

Alternatively, arachidonate may itself play a specific role in signal processing in slugs analogous to that postulated for leucocyte chemotaxis where it has an important function in surface receptor-response coupling upon stimulation by chemotactic factors (for reviews see Sha'afi & Naccache, 1981; Irvine, 1982; Schiffmann, 1982; Wilkinson, 1982). Substances such as the bacterial *N*-formyl peptides, arachidonic acid (Turner *et al.*, 1975) or arachidonate metabolites such as leukotriene B_4 (Ford-Hutchinson *et al.*, 1980) can induce chemotaxis and aggregation of leucocytes. As well as stimulating chemotactic motility, these factors evoke secretion of arachidonic acid/metabolites (Hirata *et al.*, 1979) to which neighbouring leucocytes might respond, a situation similar to that in aggregating *D. discoideum* amoebae where chemotaxis and relay is via cAMP. If this scheme for sensory transduction in leucocytes also applies to *D. discoideum* slugs, then arachidonic acid or one of its metabolites would be a third candidate as an extracellular chemical messenger in slug behaviour, in addition to STF and cAMP. However, the two metabolites tested here (prostaglandin and leukotriene B_4 , respectively) did not impair slug orientation. Future experiments using, for example, radioactively labelled arachidonic acid and/or its precursors or metabolites, are necessary to clarify possible roles for these molecules in *D. discoideum* behaviour.

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