DDT Uptake and Growth of *Euglena gracilis*[1]

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Although aquatic organisms concentrate DDT from water (1), little is known about the rate of DDT accumulation or the influence of environmental factors on the process.

This paper reports on experiments which examine the effect of DDT on the growth rate of *Euglena gracilis* and the rate of DDT uptake by actively growing Euglena cultures.

Materials and Methods

Euglena gracilis, Indiana Type Culture No. 12716 were grown in a completely synthetic medium (2) at room temperature (24-26°C). The cultures were maintained in Erlenmeyer flasks on a reciprocating shaker (110 excursions/min) in daylight plus 500 foot candles of mixed fluorescent and tungsten light on a 16 hour day basis.

The p,p'-DDT (or ^{14}C-p,p'-DDT) was added to the cultures as a solution in ethanol. In a few cases the DDT was deposited in the Erlenmeyer flasks by evaporation of a benzene solution prior to the addition of the Euglena culture. Control flasks received equal treatment except that DDT was omitted. Both control and sample cultures were replicated 3-6 times for each experiment, with reproducibilities ±5 to ±10 percent.

For cell number determinations, one ml aliquots of cell suspension were appropriately diluted with 0.9% saline solution, then counted in the Coulter Cell Counter (Model B).

The ^{14}C-DDT distribution in the cultures was determined in the following manner. Five ml of culture were mixed with 5 ml of hexane, shaken for 10 sec, then centrifuged for one min at 2000 rpm. The hexane layer was separated and set aside. Of the remaining 5.0 ml of aqueous layer, 4.0 ml were removed and discarded, the Euglena cell pellet was then resuspended in the remainder. This cell suspension was added to 9 ml of

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scintillation fluid ("Aquasol", New England Nuclear Corp). Aliquots of the hexane extract from above were also dispersed in the same scintillator solution and both were counted in the scintillation counter (Beckman LS-200). Color quench correction was made by the internal standard method with ^{14}C-toluene.

Cells were extracted and prepared for DDT analysis by gas chromatography as follows. The Euglena cells were separated from another aliquot of culture by direct centrifugation, washed with distilled water, and suspended in 50 ml of 80% ethanol-water. The cells were broken by sonication for one minute (Branson Sonifier) and the debris was spun down. The debris was re-extracted with 80% ethanol and the combined ethanol extracts were shaken in a separatory funnel with two successive 150 ml portions of hexane. The combined hexane was dried over anhydrous Na_2SO_4 and reduced in volume to 10 ml in an oil-filtered air stream. This hexane concentrate was passed through a 10 cm column packed with Florisil which had been activated at 135°C for 48 hr. DDT was eluted from the column in 100 ml of 15% benzene in hexane. Gas chromatographic analysis of the column eluate was completed on a Pye model 104 fitted with a ^{63}Ni electron capture detector.

Results and Discussion

The curves in Figure 1 compare the increase in cell number in Euglena cultures following the addition of 1.0 ml of ethanol, 0.1 ml of ethanol or 1.0 ml of water. Growth during the exponential phase was similar in the three cases. However, during the first 3 days cultures developing in the presence of 1.0 ml of ethanol were distinctly less green than the others. This is indicative of heterotrophic growth during the early stages.

When 10 µg of DDT was added to cultures in either 1.0 or 0.1 ml of ethanol, the effect was apparently related to the ethanol concentration. The data in Table I illustrate that DDT suppressed growth of Euglena when added in 1.0 ml of ethanol, but that with 0.1 ml of ethanol or no ethanol, DDT had no effect.

The inhibited cultures recovered after 3 days probably after the ethanol had been utilized. This observation draws attention to the possibility of other environmental factors influencing the apparent toxic effects of DDT.

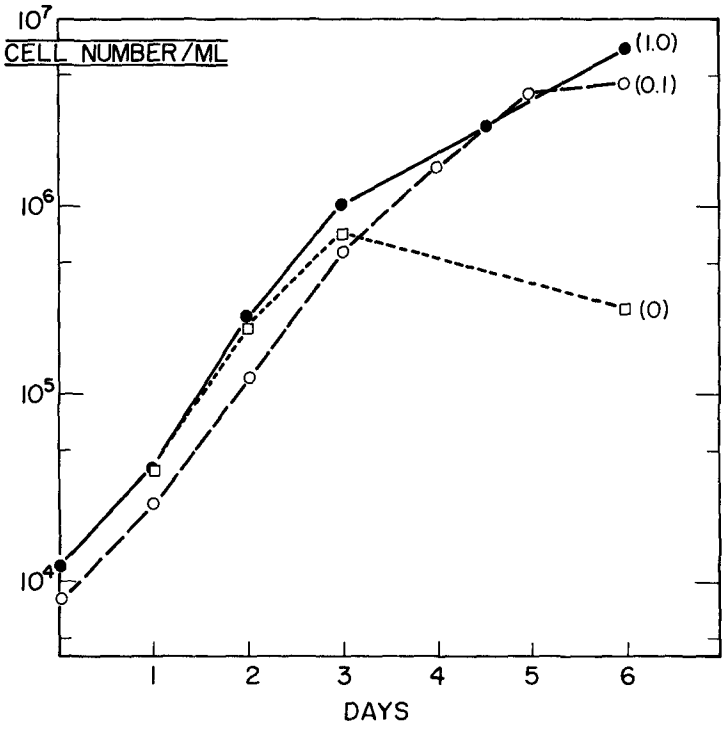

Figure 1 - Effect of 1.0 ml, 0.1 ml and no ethanol on the growth (cell number) of *Euglena gracilis* cultures (70 ml).

Table I

Effect of DDT and ethanol on growth of *Euglena gracilis*[1]
Cell number in control flask = 100

Day	10 µg DDT 1.0 ml ethanol	10 µg DDT 0.1 ml ethanol	10 µg DDT ---
1	73	97	104
2	69	104	105
3	74	106	110
4	99	110	103

[1] All cultures initially 70 ml in 125 ml flasks.

The movement of DDT into Euglena cells was followed by extracting aliquots of a growing culture with hexane, discarding the aqueous layer, and analysing both cells and hexane. The assumption was that DDT within the cells would not be extracted under the conditions described above but that DDT in the medium and on the cell surface would appear in the hexane. Data from

these analyses are plotted in Figure 2. Uptake of DDT at zero time is very rapid. During exponential growth of the culture, cell division seems to dilute the DDT concentration in the cells. In the later stages when cell growth slows, DDT uptake parallels cell growth. It is clear that DDT accumulation by Euglena does not have an observable effect on cell division, confirming earlier observations by Gregory (3).

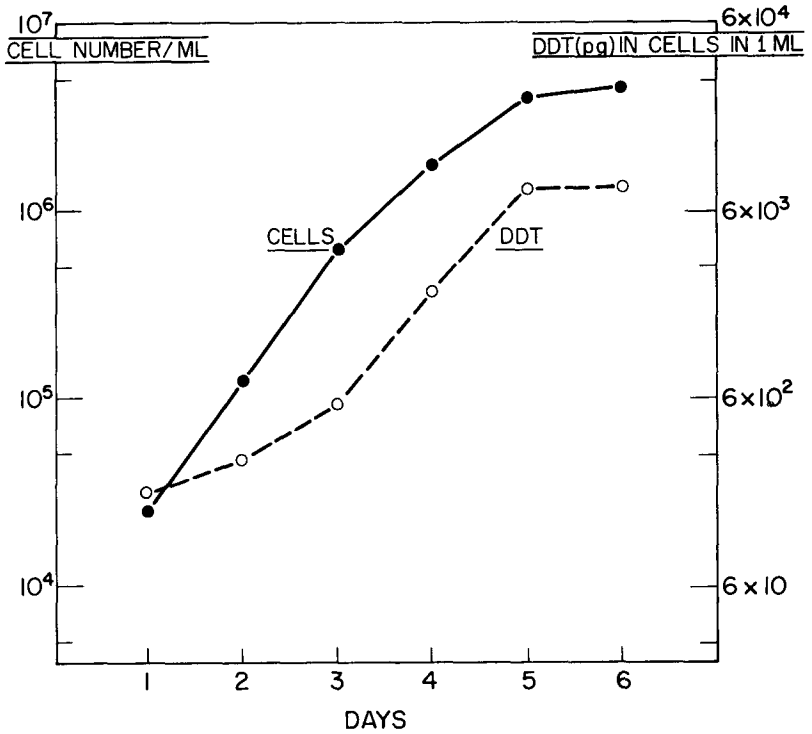

Figure 2 - Accumulation of DDT by Euglena cells in relation to cell number following one addition of 10 µg of ^{14}C-DDT in 0.1 ml of ethanol on day 1.

The loss of DDT from the medium was followed concurrently with the uptake by the cells. The actual values for the daily samples from a single experiment are shown in Table II.

The DDT in the hexane washings of the culture samples (free DDT) was determined both by radioactivity measurements and by gas chromatography, with good agreement. Recovery totals at the end of the experiments accounted for about 60% of the added DDT; less than 1% of this had been degraded.

As an extension of the latter observation, a quantity of Euglena cells containing a known amount of DDT, was washed and resuspended in fresh medium. The cells recovered after 5 days growth still contained all of the DDT of the original inoculum and degradation products were not detected.

Table II

DDT concentrations in cells and medium of a culture of *Euglena gracilis*[1]

Day	Cell number	DDT/10^3 cells	DDT/ml medium
0	5x10 /ml	324×10^{-12} g	120×10^{-9} g
1	25	77	192
2	125	23	78
3	615	9	45
4	1800	13	26
5[2]	4100	17	66
6	4700	17	24

[1] 10 µg DDT added to 140 ml of culture

[2] an additional 10 µg of DDT and 70 ml of fresh medium added to 70 ml of culture.

The chromatogram charts of the hexane washings showed traces of PCB (polychlorinated biphenyl) on the 3rd day and increasing amounts on succeeding days. The PCB is thought to have come from the foam plugs (Eurethane) stoppering the culture flasks and is probably PCB type 1254. The most significant observation here is that the PCB appeared only in the Euglena medium, the separated and extracted cells yielding DDT alone. Since the column cleanup of the cell extracts did not separate DDT from the PCB's, our observations suggest that the cells selectively absorb DDT and that the process is under some physiological control.

References

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