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Effect of DDT on Cell Population Growth, Cell Division, and DNA Synthesis in *Stylonychia notophora* (Stokes)

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Abstract. The effect of DDT on growth and DNA synthesis in *Stylonychia notophora* was investigated. DDT at a concentration of one ppm did not alter the cell population growth and the morphology of the organisms. However, 50 and 100 ppm DDT inhibited growth and cell division. Continuous treatment of the organisms with 100 ppm DDT produced several nuclear abnormalities. Cells treated with 100 ppm DDT in G_1 did not enter S-phase while DNA synthesis was blocked in those cells treated in S-phase.

The wide distribution of DDT and other chlorinated hydrocarbons in the environment, especially in the aquatic ecosystem, has been well documented (Edwards 1973, Khan 1976, Brown 1978). Among the aquatic microorganisms, protozoans, algae and bacteria, which form the broad base of the aquatic food chain, are more susceptible to DDT and accumulate it more readily than do other organisms (Gregory *et al*, 1969, French 1976, Johnsen 1976, Butler 1977, Williams 1977). The effects of DDT and other chlorinated hydrocarbons on algae and bacteria have been described (Cox 1972, Butler 1977, Williams 1977) but little has been done on the protozoans. Furthermore, data on the interaction of DDT with macromolecules such as DNA, RNA and proteins can be of immense value, because DDT has been reported to have carcinogenic, mutagenic, and teratogenic properties (Fishbein 1976).

Ciliates, due to their rapid growth rate, ease of handling and genetic stability, have been used as toxicological tools in studies relating to drug action mechanisms (Hunter 1964, Shivaji *et al.* 1975, Geike and Parasher 1976). Based on these observations, a hypotrichous ciliate, *Stylonychia notophora* was used to study the effect of DDT on cell population growth, cell division, and DNA synthesis. In hypotrichous ciliates, DNA synthesis is associated with the appearance and movement of replication bands (RB) which can be used as a morphological marker for the S-phase; i.e., the DNA synthesis phase (Gall 1959). Thus, the cell cycle in *Stylonychia notophora* can be divided into various phases of cell cycle *viz.*, G_1, S and G_2 by using RB as the marker for DNA

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synthesis. The first phase is called the G_1 phase, that lasts between the completion of division and the onset of DNA synthesis. The period of DNA synthesis is called the S-phase and the time from the end of DNA synthesis to the onset of division is termed G_2. D is the time taken for completion of cytokinesis.

Material and Methods

Stock cultures of *Stylonychia notophora,* collected from fresh water pools around Delhi in 1975, were maintained in sterilized hay infusion inoculated with *Klebsiella aerogenes* at 22 ± 1°C. Horlick's malted milk (produced in India by Hindustan Milk Food Ltd. for Horlicks Limited Brenford, Middlesex, U.K.) was added periodically to facilitate the growth of *K. aerogenes*. The purity of DDT was greater than 99% as evidenced by gas liquid chromatography. Stock solutions of DDT (1000, 10,000 and 20,000 ppm)[2] were prepared in acetone and appropriate quantities were added to the cultures to achieve the required concentration of DDT. It was confirmed that the concentration of acetone up to 0.5% did not have any toxic effect on the growth of the ciliates. Therefore, for all the subsequent experiments, acetone concentration was either 0.5% or less.

Chalkley's isotonic inorganic salt solution was used as a medium for *Stylonychia notophora*. This medium contained (w/v) 0.01% sodium chloride, 0.0004% potassium chloride and 0.0006% calcium chloride (Randall and Jackson 1958). *Stylonychia notophora* (300 animals) were transferred to 100 ml Chalkley's medium in 250 ml flasks and allowed to acclimatize for one hr, followed by treatment with the desired concentration of DDT. The number of cells in each flask was counted at regular intervals up to 36 hr. Two controls were run simultaneously *viz.*, (1) the untreated control and (2) the acetone control with corresponding concentrations of acetone as in the treated cells, but without DDT. After regular intervals of time, animals from both control and treated cultures were fixed and stained with Feulgen-light green for a study of the effect of DDT on the nuclear apparatus.

The average generation time (interval between two fissions) for *Stylonychia notophora* was 9 ± 1 hr. The RB was used as marker to indicate DNA synthesis during various phases of cell cycle in *Stylonychia*. Cytokinetic forms from log phase cultures were isolated and allowed to complete division. Daughter cells thus obtained were allowed to grow, and some cells were fixed at an hourly interval and stained in Feulgen-light green. It was observed (from permanent slides) that daughter cells fixed between 0 and 2 hr from the last division did not show any RB. Such cells were taken to be in G_1 phase. The animals fixed between 2 and 8 hr showed RB, one in each of the macronuclei, and its position was time-dependent. These cells were considered in S-phase which lasted for six hr. The interval between the disappearance of the RB and the first sign of cytokinesis was about 30 min and this duration was taken as G_2 phase. In all the subsequent age dependent experiments, daughter cells of known age (and thus in known phase of cell cycle) were used.

The effect of DDT on different age groups was examined; 25 animals of the same age group were transferred to Chalkley's medium, acclimatized in Chalkley's medium for one hr and treated with 100 ppm DDT for six hr, fixed and stained. Similarly, in recovery experiments animals (25) of the same age group were taken each time, treated with 100 ppm DDT for six hr, washed with fresh Chalkley's medium and transferred to toxicant-free medium. The time necessary for division of animals was recorded.

Autoradiographic studies were made, to confirm the inhibition of DNA synthesis. *Tetrahymena pyriformis,* a ciliate protozoan, incubated with ^{3}H-thymidine (20 μCi/ml, specific activity 5.4 Ci/mM, obtained from BARC, Bombay, India) for 72 hr, was used as a DNA precursor source for *Stylonychia*. Animals (25) in known phase of cell cycle were transferred to Chalkley's medium

[2] The solubility of DDT in water, under the most ideal conditions, is about 1.2 ppb (1.2 μg/L) or less at 25°C.; natural waters will contain salts, colloidal material, and suspended particulate matter, which may increase the apparent solubility of the chemical (Bevenue 1976). In the above study, low dosages of DDT did not affect *Stylonychia;* therefore, it was necessary to use the minimum effective dose which would cause appreciable cytological changes during the short experimental time period. The role of ciliates, especially *Stylonychia,* as toxicological tools to study drug actions is emphasized in this report.

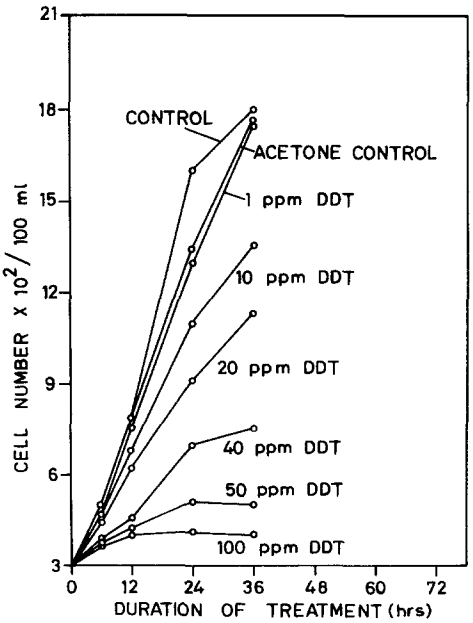

Fig. 1. Growth response of *Stylonychia notophora* to different concentrations of DDT

for one hr for acclimatization and were treated with 100 ppm DDT. Simultaneously, they were provided with *Tetrahymena* prelabelled with ^{3}H-thymidine. After six hr of treatment, the animals were fixed in Carnoy's fixative and processed for autoradiography. The slides were treated with cold trichloroacetic acid, to remove the unincorporated isotope prior to coating with the Kodak NTB-3 emulsion. These slides were exposed for 3 to 4 weeks and developed in Kodak D-19 developer.

Results

Figure 1 shows the effects of different concentrations of DDT on cell population growth of *Stylonychia*. One ppm DDT did not affect the cell population growth and morphology of the organisms as observed under binocular microscope and from the Feulgen-stained preparations. However, 40, 50 and 100 ppm DDT markedly inhibited cell population growth.

Stylonychia notophora has two macronuclei and four micronuclei (Figure 2). During S-phase in *Stylonychia,* RB appear in each of the two macronuclei and move inwards along the length of each macronucleus (Figure 3). The two macronuclei in the G_2 phase fuse to form a composite body (Figure 4), which subsequently during division phase (D) undergoes primary and secondary divisions, thus resulting in the formation of four macronuclei (Figure 5). Cytokinetic constriction, which appears at this stage, gradually deepens and ultimately the two daughter cells are formed. Micronuclei enter metaphase after RB have passed through one-half of each macronuclear length. With the disappearance of RB, micronuclear mitosis is also completed.

Organisms treated with 100 ppm DDT revealed a large number of abnormalities in the nuclear apparatus. Continuous treatment of organisms with 100 ppm DDT for nine hr resulted in deep incisions in the macronuclei (Figure 6).

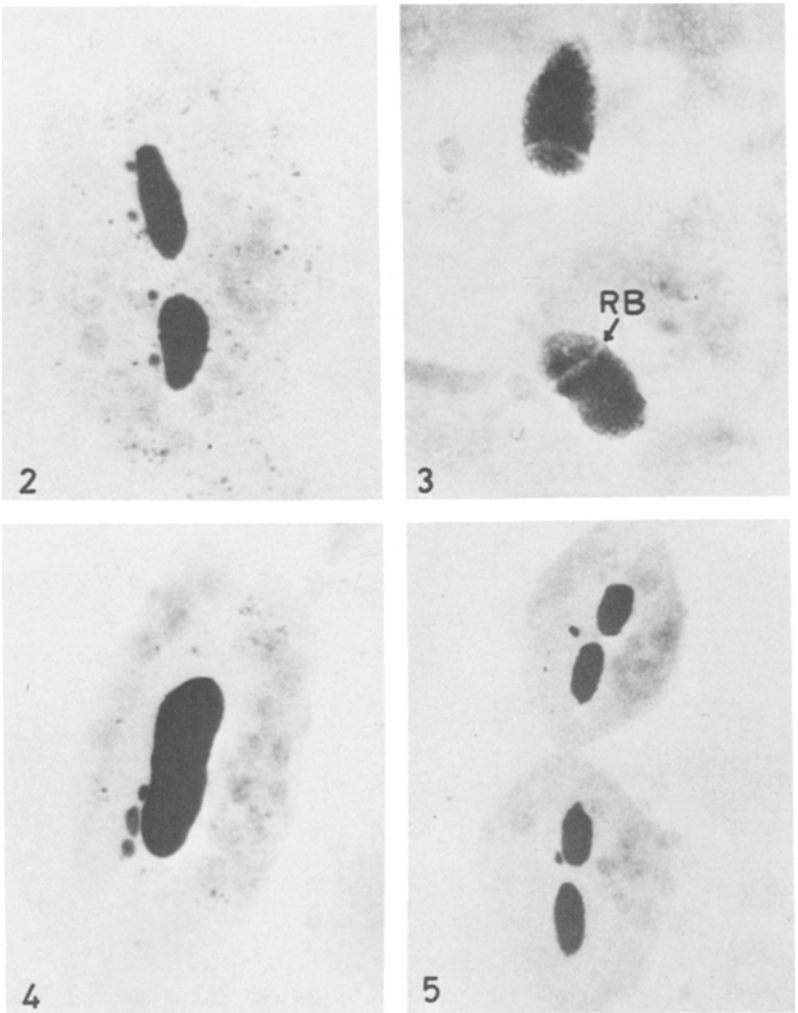

Fig. 2. *Stylonychia notophora* in G_1 phase showing two macronuclei (ma) and four micronuclei (mi) ×450.
Fig. 3. *Stylonychia* in S phase, showing RB in macronuclei. ×450.
Fig. 4. *Stylonychia* showing compact macronucleus (G_2 phase). ×450.
Fig. 5. *Stylonychia* undergoing cytokinesis. ×450.

The relationship between macro- and micronuclear division was also disturbed (Figure 7). After 18 hr of treatment with 100 ppm DDT, macronuclei showed loose chromatin (Figure 8). When the treatment was continued beyond 18 hr, the macronuclei fragmented in small spherical or irregular bodies (Figure 9).

DDT (100 ppm) completely inhibited cell division in G_1 and S-phase organisms (Table 1). However, the G_2 animals divided, even though the division was delayed by 25 min.

Autoradiographic studies confirmed the inhibition of DNA synthesis observed cytochemically. Animals treated with 100 ppm DDT in G_1 phase, fixed after six hr and autoradiographed, did not show any incorporation of ^{3}H-

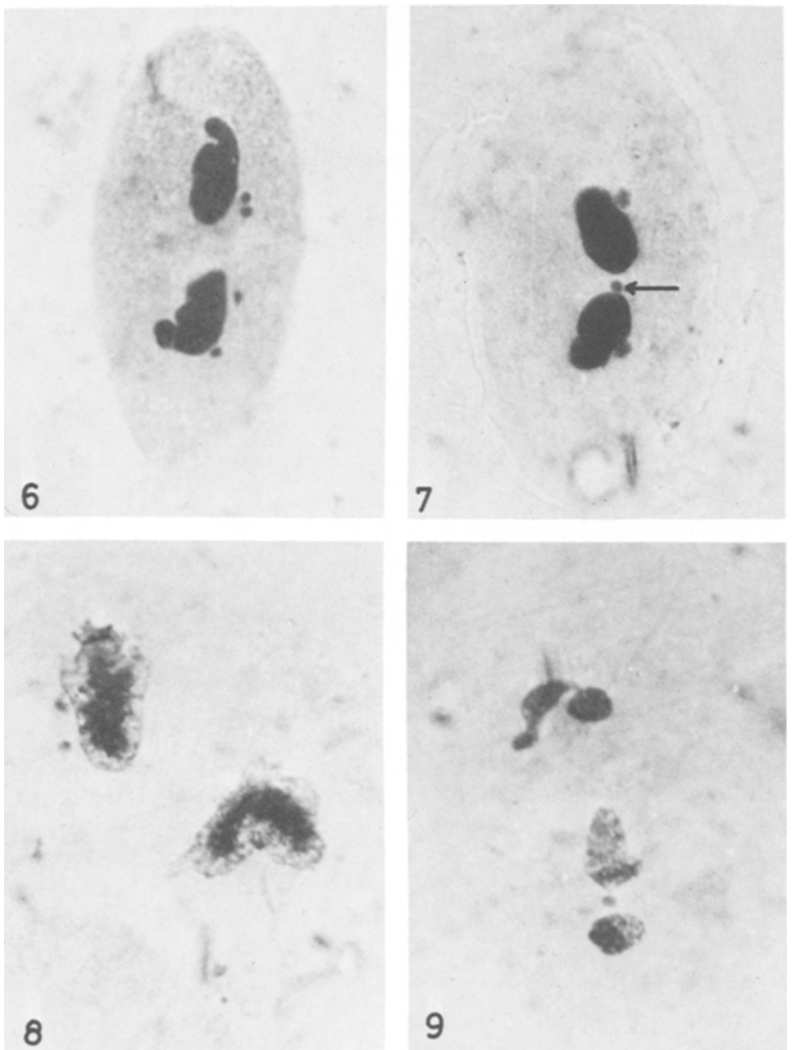

Fig. 6. *Stylonychia* treated with 100 ppm DDT for nine hr, showing macronuclear incisions. ×450.
Fig. 7. *Stylonychia* treated with 100 ppm DDT. No RB are seen in the macronuclei, although the micronuclei are in metaphase. Note a micronucleus (→) still in interphase. ×450.
Fig. 8. *Stylonychia* treated with 100 ppm DDT for 18 hr, showing chromatin in loose form. ×450.
Fig. 9. *Stylonychia* treated with 100 ppm DDT for 18 hr, showing fragmentation of the macronuclei. ×450.

thymidine in the macronuclei. These animals remained in G_1 phase during the treatment period and did not enter S-phase. However, in the corresponding controls which were in late S-phase by that time, the macronuclei showed dense incorporation (Figure 10). Similarly, animals treated in S-phase remained in S-phase by the end of six hr of treatment and did not show any incorporation in the macronuclei (Figure 11).

Organisms treated with 100 ppm DDT for six hr and then transferred to DDT free medium recovered from the effect of the drug. However, recovery

Table 1. Effect of six hr treatment of 100 ppm DDT on the cell cycle of *Stylonychia notophora*

Age of animals at time of treatment (min.)	Experimental			Control
	Macronucleus		Average time taken by the animals to divide (min.)[a]	Average time taken by the animals to divide (min.)[a]
	Phase before treatment	Phase after treatment		
60	G_1	G_1	—	465
240	S	S	—	285
410	S	S	—	105
450	late S	late S	—	65
475-530[b]	Fusion stage (G_2)	divided	80	55
	Primary division (D)	divided	40	30
	Secondary division (D)	divided	20	15

[a] The average time is exclusive of the age of the animal at the time of treatment
[b] The division of the macronuclei and cytokinesis occurred during the period of treatment

time was dependent on the age of the animals at the time of treatment (Table 2). Animals when treated in G_1 phase for six hr and then transferred to toxicant-free medium required 95 min. to recover, while ciliates treated in S-phase (4 hr old) showed a recovery time of 135 min. Animals treated in late S-phase (7 hr old) took 40 min. to recover from the effect of the drug.

Discussion

The cytotoxicity of DDT to *Stylonychia notophora* is evident from the progressive inhibition of growth and associated alterations in the nuclear morphology. Inhibition of cell growth by DDT and other chlorinated insecticides has been reported in *Euglena* (Jeanne-Levain 1974), *Tetrahymena pyriformis* (Geike and Parasher 1976, Rup Lal and Saxena 1979), *Euplotes vannus* (Perssone and Uyttersprot 1975) and *Crithidea fasciculata* (French 1976). The effect of DDT

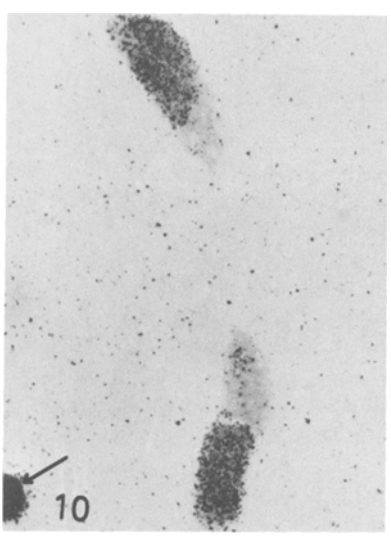

Fig. 10. Autoradiograph of untreated *Stylonychia notophora*. Animals were picked up in G_1 phase and provided with *Tetrahymena* prelabelled with ^{3}H-thymidine. After six hr of feeding, animals were fixed and processed for autoradiography. At the time of fixation, the animals were in S-phase. The macronuclei clearly show incorporation of the precursor. The arrow indicates the food vacuole formed by ingested *Tetrahymena*. ×450.

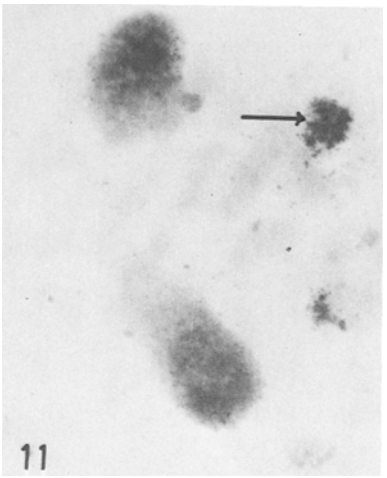

Fig. 11. Autoradiograph of *Stylonychia notophora*. Animals were picked up in S-phase and treated with 100 ppm DDT for six hr. Simultaneously, they were provided with *Tetrahymena* prelabelled with ^{3}H-thymidine. At the end of six hr of treatment and feeding, animals were fixed and processed for autoradiography. No incorporation of ^{3}H-thymidine is seen in the macronuclei (although the RB are present). The arrow marks the ingested *Tetrahymena*. ×450.

on nuclear apparatus is similar to that produced in sperms of mammals (Wyrobek and Bruce 1975). Polikarpov *et al.* (1975) observed that ^{14}C-DDT was localized mainly in the nucleus in algae, *Procentrum micans*, suggesting its direct interaction with nuclear material.

The synthesis of DNA in *Stylonychia notophora* was inhibited by DDT. Chlorinated hydrocarbons, such as DDT and BHC, are known to inhibit DNA synthesis in other systems also, *e.g.*, rats (Anina 1975), HeLa cells (Chung *et al.* 1967, Litterst *et al.* 1969), sea urchin embryos (Bresch and Arendt 1977), and plants (Anderegg *et al.* 1977). Recently, French (1976) reported the inhibition of DNA synthesis in DDT treated flagellate protozoan, *Crithidia fasciculata*. The exact mechanism of DDT interaction with DNA is not known. The effect of DDT as suggested for PCBs by Blakemore (1978) on DNA may involve its interaction with complex regulatory processes associated with the transport of precursors necessary for DNA synthesis. Alternatively, DDT may interfere specifically with the enzymes required for synthesis of nucleic acids from nucleotide precursors as postulated for PCBs by Blakemore (1978). Recently, DDT has been suggested to exert its toxic action by modifying certain mechanisms in the cell membrane (Antunes-Madeira and Madeira 1979), which could result in inhibition of many cellular activities including those involved in DNA synthesis.

Table 2. Recovery time of *Stylonychia notophora* treated with 100 ppm for six hr

Age of animals at time of treatment (min.)	Average time (min.) taken by the animals for completion of division during recovery[a] (A)	Average time (min) taken by the control to divide[a] (B)	Recovery time (min.) (A-B)
60 (G_1 phase)	560	465	95
240 (S phase)	430	295	135
420 (late S phase)	140	100	40

[a] Average time recorded is exclusive of the age of the animals at the time of treatment

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