ヨトウガ(Mamestra brassicae L.)オス幼虫の培養生殖細胞による14C-標識DDTおよびパラチオンの取り込み

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Short Communication

Uptake of ^{14}C-Labeled DDT and Parathion in Cultured Male Germ Cells from the Cabbage Armyworm, *Mamestra brassicae* L.*

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The uptake of ^{14}C–DDT in cultured male germ cells derived from the cabbage armyworm, *Mamestra brassicae* L. was rapid for 3 hr after treatment, but ^{14}C–parathion was virtually not taken up by the cells. Approximately 40% of DDT taken up by the germ cells was removed from the cells by treatment with low concentrations (0.0001–0.01%) of the non-ionic detergent, Triton X–100. Only a small fraction of DDT taken up by the cells remained in the cytoskeleton structure resistant to 1% Triton X–100. Perhaps the conclusion to be drawn from the data presented in this paper is that DDT binds to outer membrane components of the germ cells.

The cytotoxicity of insecticides on cultivated cells has been evidenced by progressive inhibition of cell growth associated with cytopathic changes in cell morphology.[1–5] However the mechanisms of action of insecticides are still not well understood at the cellular level. Murakami and Fukami reported that although a large amount of DDT was taken up by human embryonic lung cells in culture,[6] only a small fraction of the insecticide accumulated in the cytoskeleton structure resistant to the detergent, Triton X–100.[7] They suggested that DDT associated mainly in lipid-rich surface membrane structures of human cells. In this study, we report that insect male germ cells in culture are similar to cultured human cells regarding the uptake of DDT and the liberation of the compound from the cells by the detergent treatment.

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MATERIALS AND METHODS

1. *Germ Cells*

Male germ cells were obtained from the non-diapausing pupae (1 day) of the cabbage armyworm, *Mamestra brassicae* L. reared aseptically on an artificial diet under a long day photoperiod at 25°C.[8]

2. *Culture Media*

Grace's culture medium (without insect haemolymph) containing ^{14}C–DDT or ^{14}C–parathion was prepared and kept at 4°C until used. DDT, universally ^{14}C ring-label, (specific activity: 29.7 mCi/mmole) and parathion, 1–^{14}C–ethyl labeled, (19 mCi/mmole) were obtained from the Radiochemical Centre, Amersham, England as benzene solutions. The labeled insecticides were dissolved in ethanol after the benzene was evaporated with nitrogen and were added to the medium to give a final concentration of 4×10^{-6} M. At this concentration, no visible cytotoxicity to the cultured human cells was observed.[7]

3. Culture Procedures

The testes taken from the sterilized pupae were ruptured with a pair of fine needles in culture vessels (5 cm in diameter) so as to release the germ cells. Then, promptly Grace's medium containing labeled insecticide (0.2 ml) was added and incubated at 25°C.

4. Uptake of Insecticides

Culture vessels were sampled at 1, 3, and 6 hr. At each period, the medium was removed and the cells were washed three times with 0.9% NaCl solution by centrifugation (2,000 rpm, 10 min). The cells were digested with 0.1 N NaOH at room temperature. Total labeled compound uptake was determined by counting an aliquot of the NaOH digest in Aquasol–2 (New England Nuclear). Total protein per culture was determined by assaying the NaOH digest according to the method of Lowry *et al.*[9]

5. Liberation of DDT from the Cells

The cells exposed to ^{14}C–DDT for 3 hr were washed as described above and then washed with 0.0001–0.01% of Triton X–100. Radioactivity of the liberated insecticide was counted. This procedure made it possible to wash out the loosely associated DDT on the cell surface.

6. Cell Solubilization

After incubation for 3 hr in the medium containing ^{14}C–DDT, the cells were washed three times with the saline and were solubilized with 0.01–1% Triton X–100 for 10 min in a water bath at 37°C. Radioactivity of each detergent-soluble phase was counted in 10 ml of Aquasol–2.

RESULTS AND DISCUSSION

Cellular uptake of DDT and parathion is illustrated in Fig. 1. For the first hr after treatment, the uptake of DDT in the germ cells was rapid, after which the rate of uptake leveled off, while very little amount of parathion was taken up by the cells. DDT taken up by the cells was easily extracted with 70% ethanol (unpublished data).

Figure 2 shows morphological effects of various concentrations of Triton X–100. At a concentration of 0.01%, cysts of the germ cells are largely intact (Fig. 2A). Exposure of the cultures to 0.1% Triton X–100, the cysts are disrupted and cytes are released from the cysts (Fig. 2B). Treatment with the detergent at a concentration of 1% leads to the complete solubilization of cells. The detergent-resistant structures such as nuclei seem to remain well (Fig. 2C).

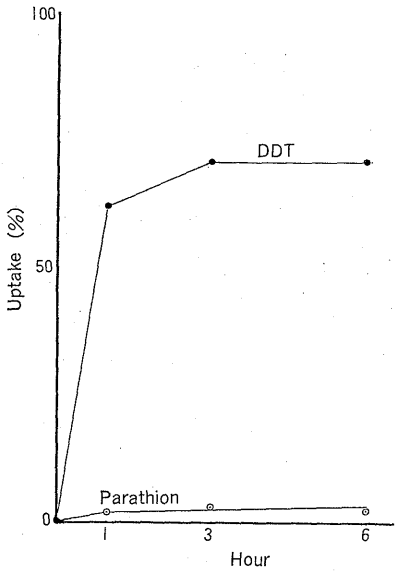

Fig. 1 Uptake of DDT and parathion into cultured male germ cells.
The germ cells were cultured in Grace's medium containing ^{14}C–labeled DDT or parathion for 1, 3, and 6 hr. The uptake is expressed as the percentage of the amount of radioactivity added into the culture vessel.

Liberation of ^{14}C–labeled DDT from the cells by treatment with various concentrations of Triton X–100 is summarized in Table 1. When treated with 0.0001–0.01% Triton X–100, the amount of label bound to the cells decreased to 50.4% of its initial amount. When cultures were treated with 0.01% Triton X–100, cysts of the germ cells were largely intact (Fig. 2A). Therefore, it is considered that the insecticide removed with 0.0001–0.01% Triton X–100 associates loosely with the cell surface. Approximately 40% of DDT that was added in the medium was removed with the detergent at these concentrations. In contrast, exposure of cultures to 0.1% Triton X–100 completely

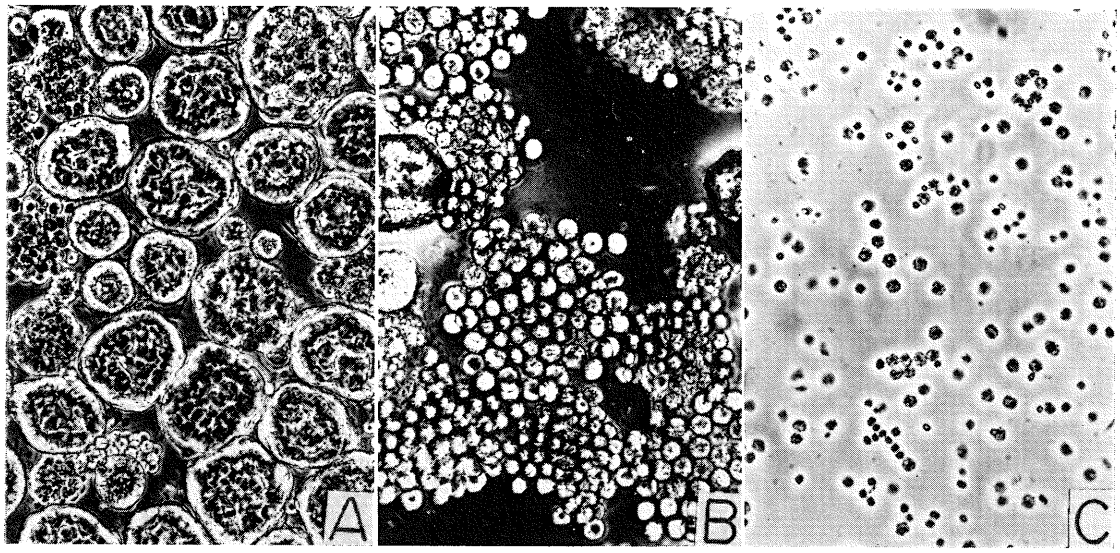

Fig. 2 Photomicrographs of cysts of the germ cells treated with various concentrations of Triton X–100 at 37°C for 10 min.
Photographs were taken with a phase-contrast microscope.
A: 0.01% Triton X–100 (×160), B: 0.1% Triton X–100 (×240), C: 1% Triton X–100 (×240).

Table 1 Liberation of ^{14}C–DDT from cells after treatment with various concentrations of Triton X–100.

A) Exposure of cultures to 0.0001–0.01% Triton X–100.

Concentration of Triton X–100 (%)	Radioactivity liberated	
	(cpm)	(%)
(Medium)	1,108	9.9
(0.9% NaCl)	40	0.4
0.0001	412	3.6
0.0005	228	2.0
0.001	348	3.1
0.005	784	6.9
0.01*	2,686	23.8
Radioactivity remained in the cells**	5,694	50.4

B) Exposure of cultures to 0.05–1% Triton X–100.

Concentration of Triton X–100 (%)	Radioactivity liberated	
	(cpm)	(%)
0.05	4,506	32.3
0.1	3,952	28.3
0.5	3,456	24.7
1	1,370	9.9
Radioactivity remained in the cytoskeleton structure***	684	4.9

* This dose of Triton X–100 caused no visible disruption to the cells (see Fig. 2A).
** Protein content digested with 5 ml of 0.1 N NaOH at room temperature was 375 μg/culture vessel.
*** Protein content in the cytoskeleton structure digested with 5 ml of 0.1 N NaOH at room temperature was 275 μg/culture vessel.

solubilized outer membranes of the cysts (Fig. 2B). Accordingly, it is thought that DDT removed by treatment with the detergent at concentrations of 0.05–0.1% is more closely associated with membrane structures. Only a small fraction of DDT taken up by the cells remained in the cytoskeleton structure resistant to 1% Triton X–100.

The data presented in this paper suggest that DDT associates mainly in outer membrane

components of the germ cells. Recently, Krause reported the damage to spermatogenesis in mice and juvenile rats which were treated with DDT.[10] We are now investigating the effects of DDT on spermatocysts by using a suitable culture system in which spermiogenesis of *M. brassicae* can be observed *in vitro*.[11]

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要　　約

ヨトウガ (*Mamestra brassicae* L.) オス幼虫の培養生殖細胞による ^{14}C–標識 DDT およびパラチオンの取り込み

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ヨトウガ (*Mamestra brassicae* L.) 雄，蛹1日目の個体から得た生殖細胞を ^{14}C–標識 DDT およびパラチオンを含む Grace の培地で培養し，取込み率を調べた．その結果，生殖細胞による DDT の取込みは，培養開始後 1～3 時間で，非常に急速に取り込まれたが，パラチオンの場合には，取り込み率は非常に低かった．顕微鏡下で，外観的に生殖細胞に影響がない低濃度 (0.0001～0.01%) の界面活性剤 (Triton X–100) を使って，DDT を取り込ませた細胞を洗うと全体の 39.8% が洗い出された．また細胞内に取り込まれた DDT が 0.05, 0.1, 0.5, 1% の濃度の Triton X–100 で細胞を可溶化した場合，どれだけ溶出されるかも調べられた．1% の濃度で可溶化されない画分に残っている DDT は，0.01% の界面活性剤処理で洗い出されなかった DDT の量の 4.9% であった．

以上の結果から，生殖細胞によって取り込まれた DDT は脂質に富む細胞表面（あるいは膜構造）に多く結合していることが示唆された．