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Research Note

The Plasma Membrane of *Entamoeba histolytica* Incubated with Halogenated Bisphenols in the Serum-Free Medium Is Not Disrupted

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Previous investigations on *Entamoeba histolytica* demonstrated that halogenated bisphenols like bithionol, dichlorophene and hexachlorophene inhibited respiratory activities and *in vitro* growth of the parasite (Kawasaki and Takeuchi, 1984; Takeuchi *et al.*, 1984). Subsequent observations on the basis of Weinbach and Garbus (1966) disclosed that omission of bovine serum from BI-S-33 medium (Diamond *et al.*, 1978) significantly enhanced the inhibitory action of halogenated bisphenols against the growth of the parasite.

While investigating the effect of the bisphenols under various conditions, we found that the number of amoebae, which looked undisrupted light microscopically, considerably increased by omitting bovine serum. The present communication describes morphological characteristics of *E. histolytica* incubated with the bisphenols in both complete and serum-free BI-S-33 media.

Trophozoites of *E. histolytica* (strain HM-1: IMSS) were grown in BI-S-33 medium as already described (Takeuchi *et al.*, 1977), harvested and finally suspended in the medium to yield 3×10^6 amoebae/ml. Two tenths ml of this suspension was inoculated into 15 ml of either complete or serum-free BI-S-33 medium containing 150 μg/ml of bithionol, dichlorophene or hexachlorophene, the highest concentrations of the bisphenols employed in the previous experiments (Kawasaki and Takeuchi, 1984; Takeuchi *et al.*, 1984). After incubating at 35.5°C for appropriate periods, the amoebae were observed light and electron microscopically. The specimens for electron microscopy were processed as already described (Takeuchi *et al.*, 1981) except that prefixation was done for 60 min at 4°C with 0.1 M phosphate buffer, pH 7.4 containing 2 % tannic acid as well as 2 % glutaraldehyde. The bisphenols were dissolved in 1 N NaOH to make appropriate concentrations, and subsequently diluted tenfold with distilled water.

The halogenated bisphenols were supplied by Tokyo Kasei Inc. (Tokyo, Japan). All chemicals were of the highest purity commercially available.

Figs. 1 and 2 demonstrate light microscopic views of *E. histolytica* incubated with dichlorophene for 2.5 hr in the complete and serum-free medium respectively, and show that degeneration of amoebae in the complete medium appears more conspicuous than that in the serum-free medium. Most of the amoebae in the serum-free medium do not look disrupted in contrast to those in Fig. 1.

Figs. 3 and 4 show electron microscopic views of the parasite incubated in the same manner as in Figs. 1 and 2, respectively. It is apparent that the amoebae in the complete

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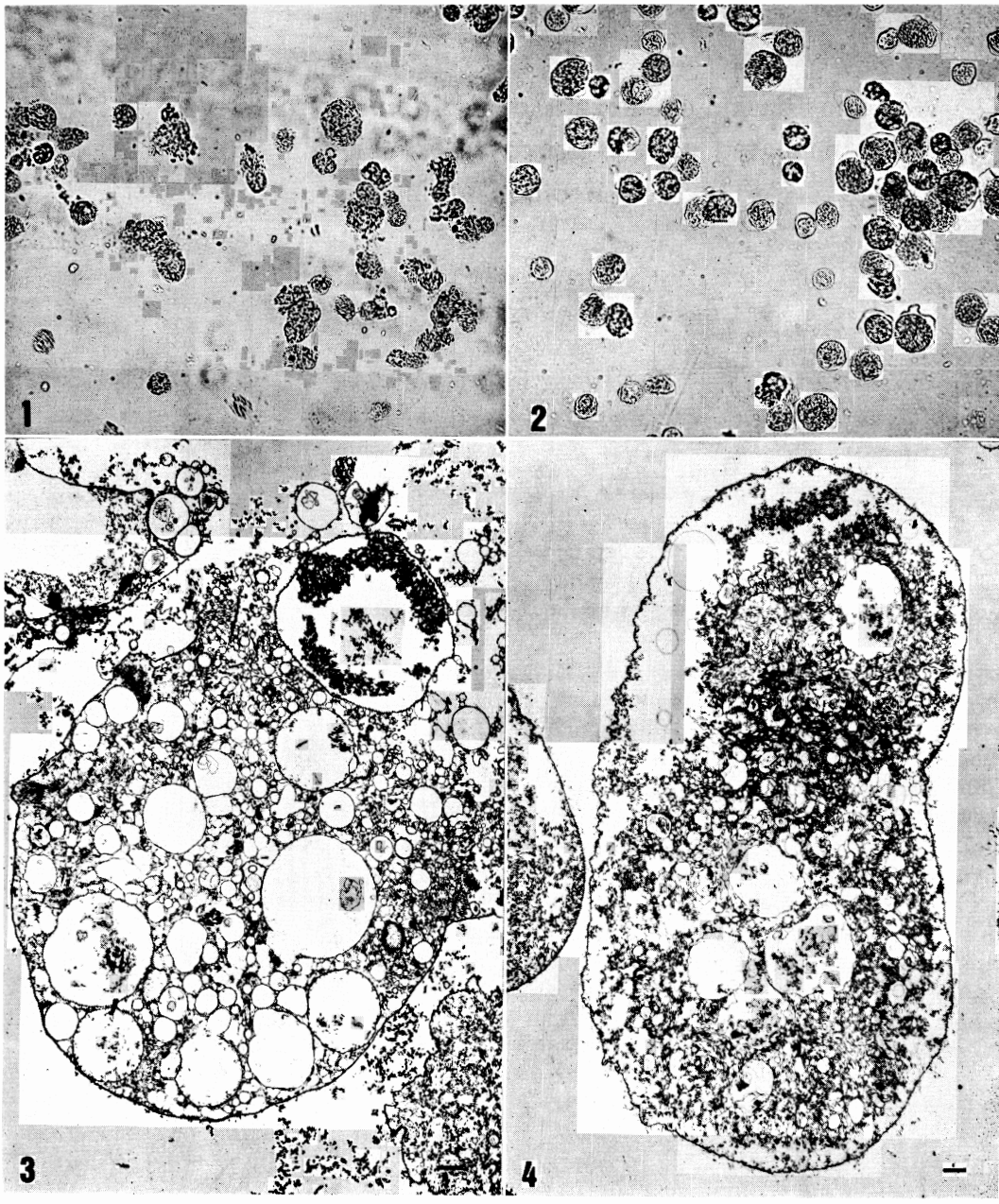

Fig. 1 A light microscopic view of *Entamoeba histolytica* (strain HM-1 : IMSS) incubated in complete BI-S-33 medium containing 150 μg/ml dichlorophene for 2.5 hr at 35.5°C. (×200)

Fig. 2 *E. histolytica* incubated in serum-free medium containing dichlorophene. Other details as in the legend to Fig. 1.

Fig. 3 An electron microscopic view of *Entamoeba histolytica* (strain HM-1 : IMSS) incubated as in Fig. 1. Bar=1 μm.

Fig. 4 *E. histolytica* incubated as in Fig. 2. Bar=1 μm.

medium exhibit extensive degeneration of the cytoplasmic organelles including the nucleus, and disruption of the plasma membrane (Fig. 3). In contrast, the plasma membrane of the parasite in the serum-free medium does not seem to be disrupted, although the cytoplasmic organelles are damaged (Fig. 4).

In the presence of lower concentrations of dichlorophene, e.g., 50 and 100 μg/ml, omission of bovine serum showed essentially the same degenerative changes of amoebae as above. Bithionol and hexachlorophene also functioned in the same manner as dichlorophene.

These findings suggest that bovine serum appears to enhance the disruption of the plasma membrane of *E. histolytica* by the halogenated bisphenols. Since disruption of the plasma membrane is also lowered by removing bovine serum at lower concentrations of dichlorophene as noted above, it seems unlikely that dichlorophene is able to disrupt the plasma membrane of amoebae only at low concentrations, e.g., 50 μg/ml. It is plausible, therefore, that binding of these bisphenolic derivatives to serum component(s), probably serum albumin as Weinbach and Garbus (1966) demonstrated on halogenated and nitrated monophenols, may not only diminish the inhibitory action of the compounds as described in our previous reports (Kawasaki and Takeuchi, 1984; Takeuchi *et al.*, 1984) but also alter their mode of action.

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血清無添加培地におけるハロゲン化ビスフェノールの赤痢アメーバに対する作用

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ウシ血清添加，無添加 BI-S-33 培地における赤痢アメーバ（HM-1 : IMSS 株）に対するハロゲン化ビスフェノールの作用を光顕的，電顕的に調べた結果，前者においてはオルガネラの変性のみならず細胞膜が破壊されるのに反し，後者ではオルガネラの変性はあるが，細胞膜は殆んど破壊されずに残るらしいことが明らかとなつた．ハロゲン化ビスフェノールが血清成分と結合した結果，作用機序が変化することが示唆された．