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NOTES

Vacuolization in Cultured Cells Induced by Amphotericin B

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Amphotericin B induced vacuoles in one HeLa cell line and in some unrelated cell lines. Cell growth was not affected.

Amphotericin B is a polyene antimycotic drug widely used in cell culture since it is usually nontoxic in the generally used concentration range (1 to 10 μg/ml), with some exceptions (1-3).

During routine examination of cultured cell lines, we observed the appearance of vacuole-like structures in HeLa cells when amphotericin B was present in the medium. However, they seemed to have little functional significance.

The cells were cultivated in Eagle minimal essential medium supplemented with 10% calf serum or fetal calf serum and antibiotics. Amphotericin B was diluted in medium in the concentration range of 0.01 to 10 μg/ml. In control cultures, sodium deoxycholate was added.

By light microscopy, vacuole-like structures were seen in the used HeLa cell line as well as in other cell lines (Table 1) when amphotericin B (2.5 μg/ml) was present. Phase-contrast microscopy revealed circular, cytoplasmic vacuoles of different sizes (Fig. 1).

The effect was concentration dependent. No effect was seen at 0.05 μg/ml, and it was fully expressed at a concentration of 0.5 μg/ml. Of the cells, 10% were affected, but there was a wide variation among experiments; the coefficient of variation was 16.7% at 0.5 μg/ml (n = 14). No cell loss was found by trypan blue dye exclusion, nor were cell counts reduced by the drug.

After the change from control medium to medium supplemented with amphotericin B, the effect was detectable within 20 h and was maximal within 50 h (Fig. 2a). The change back to control medium reversed the number of vacuolated cells at about the same rate (Fig. 2b). Reduction of serum from 10 to 0.5% consistently resulted in a decrease in the number of vacuolated cells to about 5%. Autoradiograms showed that the number of nuclei incorporating [*methyl*-^{3}H]thymidine into DNA was equal in vacuolated and non-vacuolated cells. Mitotic cells exhibited the same proportion of vacuolated cells as did nonsynchronized cells. Thus, the vacuolated cell population seemed to proceed through the cell cycle in the same way as the main cell population. McManus, Alcian blue, Sudan black, Giemsa, and pyronin Y did not stain the vacuoles. Neutral red (0.1%) stained the vacuoles within 2 min, indicating water as a major component in the vacuoles.

Other HeLa cell lines lacked vacuoles (Table 1), whereas unrelated cell lines (amnion U cells and an embryonal lung fibroblast) were vacuolated. Therefore, the described phenomenon

TABLE 1. *Estimation of vacuolated cell ratio by using light microscopy*[a]

Cell line	Effect of amphotericin B (2.5 μg/ml)
Normal	
Human embryonic dermal fibroblast	0
Human adult dermal fibroblast	0
Human embryonic lung fibroblast	++
Human embryonic kidney fibroblast	+
BHK 21 (baby hamster kidney)	0
Vero (African green monkey kidney)	+
Neoplastic	
HeLa	++
OHIO (HeLa line)	+
KB (HeLa line)	0
Virus (HeLa line)	0
Amnion U	+++[b]
Chang liver	++
20S[c]	++
373 MG[c]	+
105 MG[c]	0
399 MG[c]	0
489 MG[c]	0
563 MG[c]	0

[a] 0, <1% Affected cells; +, occasional vacuolated cells; ++, several cells per vision field; +++, many vacuolated cells (>10%).
[b] Vacuolated cells made up 15% of total.
[c] Glioma.

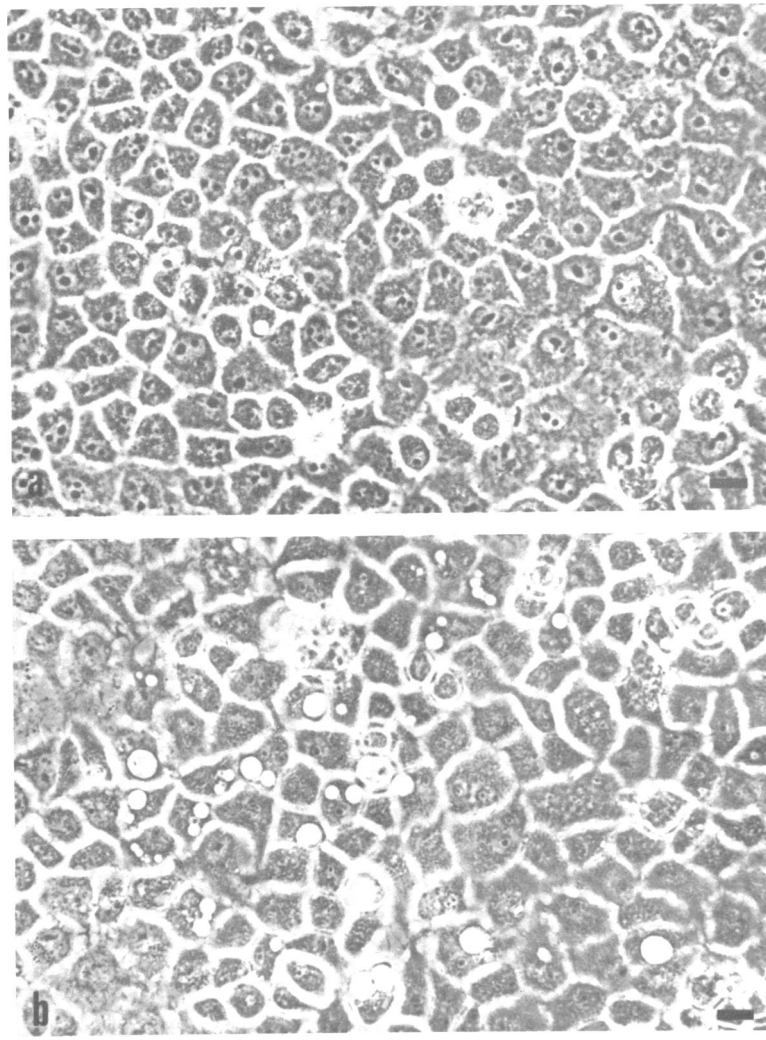

FIG. 1. *Phase-contrast microscopy of HeLa cells (a) Controls; (b) amphotericin B, 2.5 μg/ml. Bars represent 20 μm.*

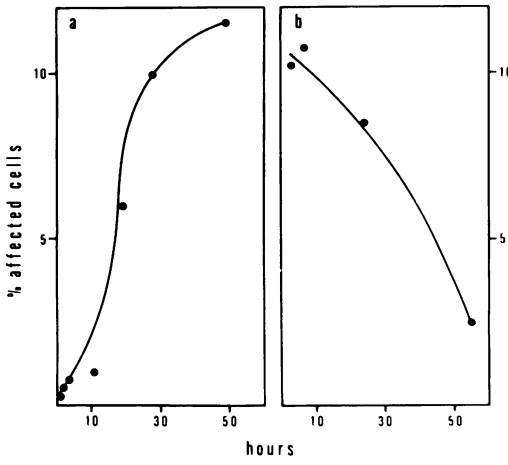

FIG. 2. *Percent affected cells at various times after (a) adding and (b) removing amphotericin B (2.5 μg/ml). At least 400 cells were counted. No vacuoles were seen in control cells.*

seemed to be associated with individual cell lines rather than cellular origin.

Amphotericin B has been shown to be a potent affector of cell membrane functions, most probably by binding to sterols in the cell membrane (4, 10). Many authors have reported synergism between amphotericin B and quite unrelated drugs, both in vivo and in vitro (6–9, 14, 16). This synergism has been supposed to be due to increased permeability of the active substance.

The vacuoles were observed within the low concentrations recommended by the supplier, and no effects on cell growth were observed.

They may be a consequence of a reported colloid osmotic mechanism (5), but their significance for membrane transport is not known. However, the possibility of interaction with different experimental situations must be kept in mind.

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