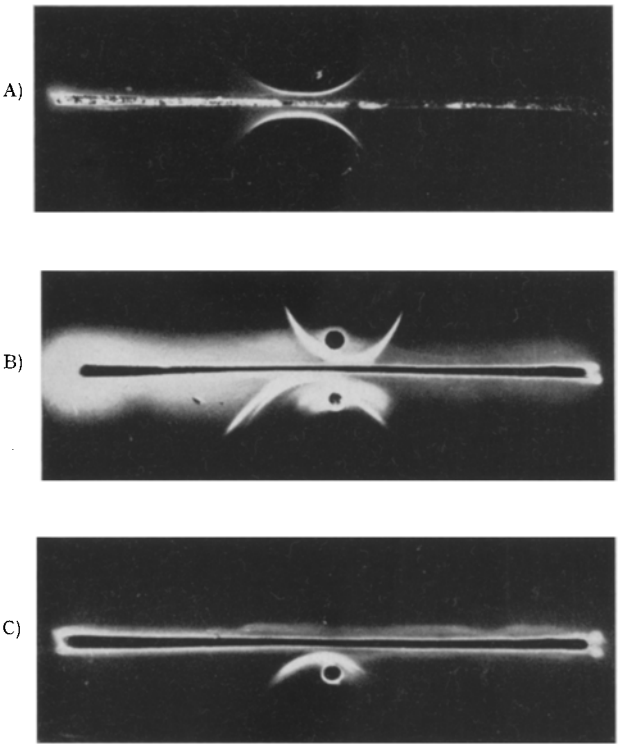

Fig. 2. Immunoelectrophoresis. Upper cell, purified CEA; lower cell, perchloric acid extract of liver metastases (see text).

compared with a reference antigen at the same concentration (BP 102, supplied by Hoffmann-La Roche), shows after labelling the same specific activity and equal binding capacity.

Immunoelectrophoresis of purified CEA shows (Figure 2): A) only one band if the antigen is tested against the antibody supplied by Roche; B) only one band if tested against nonadsorbed anti-liver metastases antiserum; C) no bands when tested against normal guinea pig anti-liver antiserum.

Résumé. Description d'une méthode de purification de l'antigène carcino-embryonnaire à partir de métastases hépatiques d'adéno-carcinomes primaires du côlon, méthode qui présente des avantages de rendement et d'application. Faite par une solution hypertonique de chlorure de potassium, l'extraction du produit préparé avec l'acide perchlorique est particulièrement intéressante. Ce procédé améliore considérablement la purification, sans nuire à la précision. On décrit également la méthode de préparation de l'antisérum de cobaye et la technique d'adsorption permettant d'en faire un produit réagissant de façon spécifique en présence du CEA.

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The Influence of Antifungal Antibiotics on Some Determinants of Virulence in *Candida albicans* and *Cryptococcus neoformans* Cells

It is generally accepted that, in a host defence against infection with yeast-like fungi, an essential role is played by the process of phagocytosis[1,2]. However, there are some observations which show that in some conditions the *Candida albicans* cells can not only survive inside phagocytes but even they may grow outside[3,4]. It was also observed that there is a relationship between the ability of engulfed *C. albicans* blastospores to undergo the mycelial transformation, and their virulence in experimental animals[4]. Similarly, but by another mechanism, the capsule of *Cryptococcus neoformans* may inhibit the phagocytosis and thus diminish the cellular defence against this fungus[5,6].

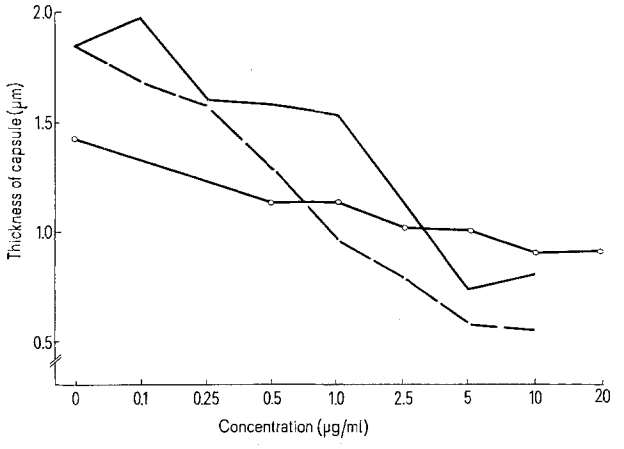

Fig. 1. Influence of various concentrations of amphotericin B on the thickness of capsule in *Cr. neoformans* cells. Each line represents a separate experiment.

In view of these findings, it seemed interesting to see the effect of some antifungal antibiotics on the process of phagocytosis of these fungi.

Materials and methods. The *Cr. neoformans* cells were incubated for 96 h at 37 °C in Littman's liquid medium in modification of BULMER[6] to which amphotericin B or polyfungin in various concentrations were added. After washing the suspensions of cryptococcal cells containing 10^6 cells were mixed with the same number of mouse peritoneal macrophages in 1 ml of culture fluid (Parker solution + 40% of normal mouse serum) and the resulting suspensions were distributed in plastic chambers with cover slips. After 1 h incubation at 37 °C the slips were removed and stained by the PAS method. The

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percentages of actively phagocyting macrophages were calculated. Similar experiments were carried out with *C. albicans* and nystatin and water-soluble derivative of this antibiotic. After washing the *C. albicans* blastospores cultured previously on Sabouraud's dextrose agar were mixed with mouse peritoneal macrophages and distributed in chambers with cover slips. The slips were removed after 30 min of incubation at 37 °C and washed with warm Parker solution to get rid of non-ingested fungal cells. The slips were placed in fresh culture medium containing appriopriate concentrations of nystatin and at different time intervals they were removed and stained by Giemsa method. The percentages of engulfed *C. albicans* cells actually forming germ-tube were calculated. All tests were performed in triplicate.

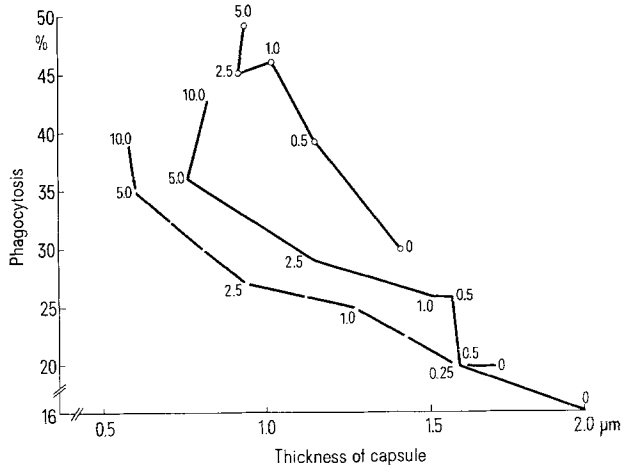

Fig. 2. Correlation between the thickness of capsule in *Cr. neoformans* cells and phagocytosis of these cells by mouse peritoneal macrophages. The numbers at the points of the curve indicate the concentrations (µg/ml) of amphotericin B in the medium in which cryptococcal cells had previously been grown. Each line represents a separate experiment.

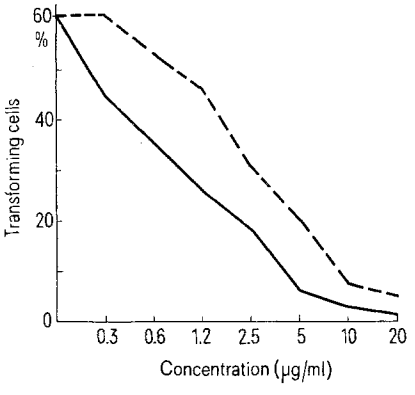

Fig. 3. Influence of various concentrations of nystatin (solid line) and its water-soluble derivative (dashed line) on the formation of pseudomycelia by phagocytized *C. albicans* cells.

Results. The experiments have shown that amphotericin B used in subinhibitory concentrations was able to inhibit the process of capsule formation in cryptococcal cells (Figure 1). It was clearly evident (Figure 2) that such poorly encapsulated cells were more readily phagocytized. Similar results were obtained with polyfungin.

A strict correlation has been observed between the concentration of nystatin in culture fluid and the degree of inhibition of germ-tube formation by engulfed *C. albicans* cells (Figure 3) and as a rule the germ tubes were shorter. This antibiotic showed its action in early as well as in later stages of germ-tube production by *C. albicans* cells while in macrophages. Nystatin in concentration of 10 µg/ml produced complete inhibition of this process independent of whether it was added at the start of the experiment or after 30 and 60 min of incubation.

Discussion. In our previous paper[7] we have shown that nystatin used in comparatively low concentration has the ability to inhibitit the mycelial transformation of *C. albicans* cells suspended in serum. From the experiments presented here, it is clearly seen that nystatin may penetrate to mouse macrophages and inhibit there the process of germ-tube formation by phagocytized *C. albicans* cells. Two other polyene antibiotics inhibited the formation of capsule in *Cr. neoformans* cells thus rendering them more susceptible to phagocytosis. As the ability of *C. albicans* cells to produce germ-tube and ability of *Cr. neoformans* cells to produce the capsule are the determinants of the virulence of these fungi[4,6,8], it is clearly evident that these determinants may be partly or completely abolished by antifungal antibiotics used in comparatively low concentrations. It may be assumed that when thus 'prepared' by an antibiotic fungal cells may be more susceptible to intracellular killing. The results presented might to be an example of a strict collaboration between the mechanism of host immune defence and the activity of antifungal antibiotics.

Zusammenfassung. Nachweis, dass die Kapselbildung von *Cryptococcus neoformans* durch Inkubation mit niedrigen Polyenantibiotika-Konzentrationen gehemmt und die Aufnahme solcher kapselarmer Formen des *Cryptococcus* durch Phagozaten verstärkt wird. Die Hemmung durch das Antibiotikum betraf die Pseudomyzelbildung der intrazellulären *Candida albicans*-Zellen.

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Distribution of Radioactivity after Administration of ^{125}I-Labelled Luteinizing Releasing Hormone in Rats

Attention has recently been focused on the entity and duration of permanence of the synthetic gonadotropin releasing hormone (LH-RH) in the pituitary, and also on its possible localization in the cerebral cortex and peripheral organs (e.g., the gonads). Data has already been obtained in similar studies using TRH[1], while, to our knowledge, no study of this type has been carried out using synthetic LH-RH.