Isolation of a Carcino-Embryonic-Antigen (CEA) from a Liver Metastasis of Primary Adenocarcinoma of the Colon and Preparation of the Specific Antiserum

This paper describes an attempt to improve a method of carcino embryonic antigen (CEA) purification, suitable for large quantities of material.

Materials and methods. CEA purification. The CEA titre, at the various steps, is determined by immunodiffusion against an anti-CEA antibody prepared in the Nutley, N.J., Laboratories of Hoffmann-La Roche Inc. and kindly supplied by Hoffmann-La Roche & Co. Ltd. of Basle. One kg of liver metastases of primary adenocarcinoma of the colon is homogenized without heating, together with four times its volume of distilled water, for 10 min. An equal volume of 2 *N* perchloric acid is slowly added under stirring, according to KRUPEY's technique[1,2]. The suspension is stirred for a further 30 min and is then centrifuged for 20 min at $8000 \times g$. The resulting supernatant solution is thoroughly dialyzed against distilled water, concentrated and freeze-dried; 5 g of lyophilized powder are obtained. 2,5 g of lyophilizate are suspended in 250 ml of 3 *M* KCl in 0.05 *M* NaH_2PO_4 at pH 4.2 (the pH is adjusted with 1 *N* H_3PO_4), according to the modified MELTZER's technique[3] and left, with stirring, for 20 h at +4 °C. The suspension is clarified by centrifuging at $100{,}000 \times g$ for 60 min. The supernatant is dialyzed against water, concentrated and lyophilized.

The approx. 600 mg of lyophilized powder are dissolved in 50 ml of 0.005 *M* phosphate buffer (Na/Na_2) pH 6.75 and adsorbed onto a DEAE-cellulose column (3×30 cm) (DE11-Whatman). Elution is done with the aid of a phosphate buffer discontinuous molarity gradient at pH 6.75 (0.005; 0.025; 0.05; 0.1; 0.5 *M*). The component fractions of the various peaks are reunited, dialyzed against water, concentrated and lyophilized.

The lyophilizate corresponding to the CEA peak is dissolved at a concentration of 20 mg/ml in 0.05 *M* NaH_2PO_4 + 0.9% NaCl + 0.02% NaN_3 + H_3PO_4 to adjust the pH to 4.5 and chromatographed on a Sephadex G-200 column (2.5×100 cm). The peak containing the active fractions is dialyzed against water and lyophilized. The final white lyophilizate, of a somewhat gummy and hygroscopic consistency, is kept at −20 °C in the presence of silica gel.

Preparation of antiserum. The anti-CEA and anti-normal liver antiserums were obtained from Pearbright White guinea pigs by immunization with the lyophilized perchloric acid extracts of liver metastases and of normal liver, respectively. On the 1st day, 1 mg of lyophilizate dissolved in 0.2 ml of saline and emulsified with 0.2 ml of complete Freund's adjuvant was injected s.c. The same process was repeated on the 14th, 28th, 42nd and 56th days. On the 67th day the animals were bled. At the same time 40 ml of guinea pig serum pool were treated with: 200 mg of lyophilized perchloric extract of normal liver; 4 ml of a normal serum pool; 1 ml of normal human liver homogenate (90 mg of protein); 5 ml of normal human spleen homogenate (82.5 mg of protein); 10 ml of human erythrocytes (A, B, O pool); 5 ml of human erythrocytes (A, B, O pool), 1 h after the first addition. After gentle stirring, the mixture was left for 6 h at room temperature and then for 18 h at 4 °C. It was then centrifuged, first at $2000 \times g$ for 10 min and then at $10{,}000 \times g$ for 15 min. Precipitation of the gamma-globulin was done by adding 80% sat. ammonium sulphate in a 1:1 ratio with the serum.

Experimental results. Extraction with 3 *M* KCl according to MELTZER's technique proved advantageous, since it removes about 75% of inert substances with no appreciable loss of CEA. The CEA is eluted at the molarity of 0.1 from DEAE-cellulose; CEA is detectable, in small quantities, also in the fraction eluted at 0.05 molarity. Elution on Sephadex G-200 gives three peaks: the CEA is detectable only in the middle one of the three. The yield of the outlined purification method is 100–120 mg of CEA/kg liver metastases. The whole purification process takes about three weeks. CEA, thus purified (Figure 1),

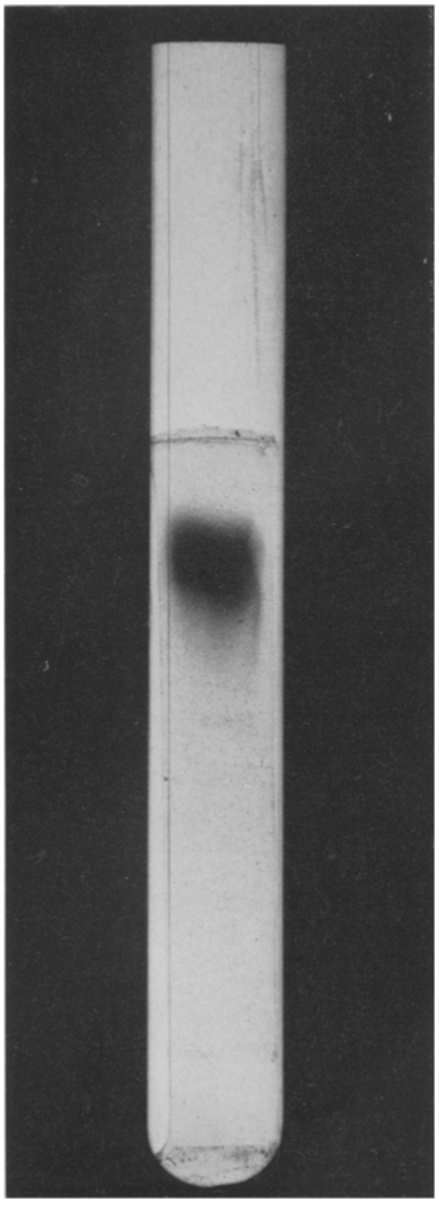

Fig. 1. Polyacrylamide gel electrophoresis.

[1] J. KRUPEY, T. WILSON, S. O. FREEDMAN and P. GOLD, Immunochemistry *9*, 617 (1972).
[2] J. KRUPEY, P. GOLD and S. O. FREEDMAN, J. exp. Med., *128*, 387 (1968).
[3] M. S. MELTZER, E. J. LEONARD, H. J. RAPP and T. BORSOS, J. natn. Cancer Inst., *47*, 703 (1971).

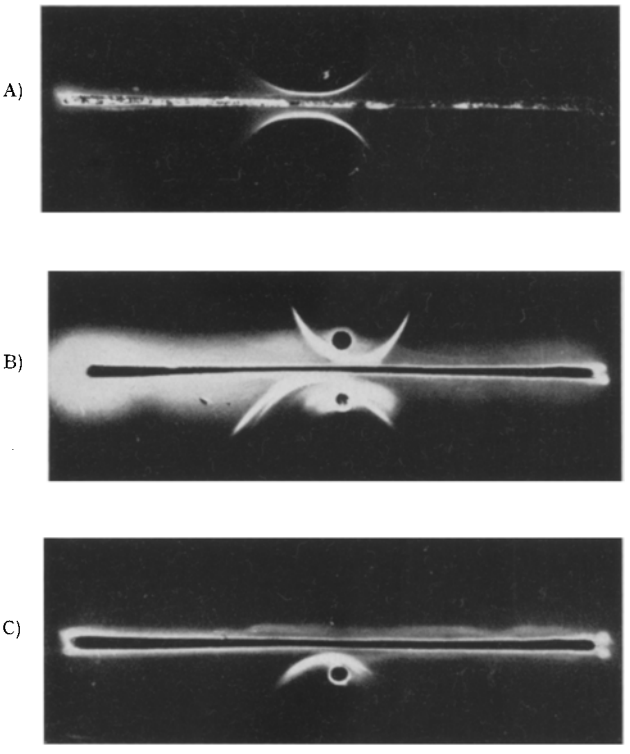

Fig. 2. Immunoelectrophoresis. Upper cell, purified CEA; lower cell, perchloric acid extract of liver metastases (see text).

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].

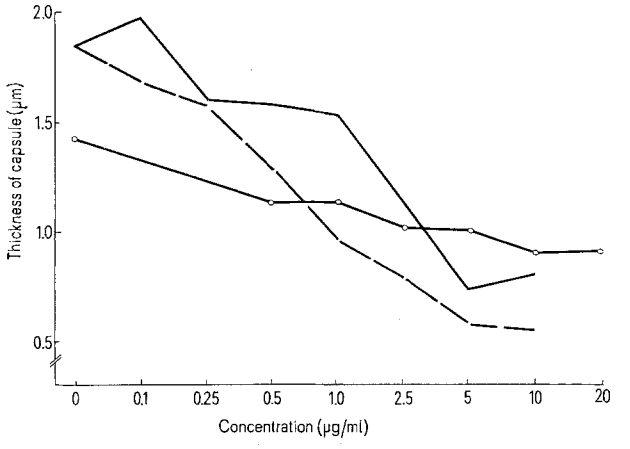

Fig. 1. Influence of various concentrations of amphotericin B on the thickness of capsule in *Cr. neoformans* cells. Each line represents a separate experiment.

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

[1] R. D. DIAMOND, R. K. ROT and J. E. BENNET, J. infect. Dis. *125*, 367 (1972).
[2] R. I. LEHRER and M. J. CLINE, J. Bact. *98*, 996 (1969).
[3] D. B. LOURIA, R. G. BRAYTON and G. FINKEL, Sabouraudia *2*, 271 (1963).
[4] D. B. LOURIA and R. G. BRAYTON, Proc. Soc. exp. Biol. Med. *115*, 93 (1964).
[5] G. S. BULMER and M. D. SANS J. Bact. *94*, 1480 (1967).
[6] G. S. BULMER and M. D. SANS, J. Bact. *95*, 5 (1968).