PENICILLIC ACID ACTION ON LIPOSOMES

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PENICILLIC acid is a toxic secondary metabolite produced by several food-borne *Penicillium* and *Aspergillus* species[1]. Penicillic acid affects various species of experimental animals causing production of malignant transplantable local tumours[2], inhibition of mammalian cell division[3] and histopathological lesions of liver, kidney and thyroid[4]. The hepatotoxicity of penicillic acid has also been reported[5].

As mycotoxin has been reported to cause lesions of the organs, it may primarily affect the membrane of the affected organs. In the present investigation an *in vitro* study has been made to see the effect of penicillic acid on membranes using liposomes.

The fungus *P. cyclopium* was grown on Raulin-Thom medium for 14 days at 20–22°C. Penicillic acid was extracted from the culture filtrate and purified[6]. The purity of penicillic acid was tested by NMR, IR and UV spectral analysis along with an authentic sample as reference. Phosphatidylcholine was obtained from the V.P. Chest Institute, New Delhi and purified by chromatography over alumina and silica gel. ^{14}C-glucose, obtained from BARC, Bombay was suitably dissolved in 0.01 M tris-HCl buffer pH 7.

Liposome of neutral charge was prepared using phosphatidylcholine and cholesterol in the molar ratio of 8:2. Phosphatidylcholine and cholesterol supplemented with stearylamine or diacetylphosphate in the molar ratio of 7:2:1 were used for preparing positively or negatively-charged liposomes respectively[7].

The lipids were dissolved in 5 ml of chloroform and dried by rotary evaporation using a vacuum pump to get a thin film. The flasks were then flushed with nitrogen. ^{14}C-glucose in tris-HCl buffer was added to the flasks and the flasks were sonicated for 20 min. The flasks were left at room temperature for 20 min for equilibration. The liposomes were then purified on Sephadex G-50 column.

Sephadex G-50 was loaded into 20 × 1.5 cm column. The column was equilibrated overnight with tris-HCl buffer (pH 7). The liposomes were loaded on the column and were then eluted with the same buffer. Subsequently samples of liposomes (1 ml) were collected. Aliquots of the fractions were counted in a liquid scintillation counter to monitor the liposomes entrapped with ^{14}C-glucose. A graph was plotted with percentage of radioactivity against volume. The liposome containing fractions were then pooled and used for studying the effect of penicillic acid. Samples of liposomes (1 ml) were incubated with various concentrations of penicillic acid for 1 hr at room temperature. After the incubation period liposomes were dialysed against 10 ml 0.01 M tris-HCl buffer (pH 7): Leakage of ^{14}C-glucose through the dialysis sac into the surrounding fluid was determined after 30 min by counting the ^{14}C-glucose in the aliquots in a liquid scintillation counter by mixing with scintillation fluid.

Scintillation fluid was a mixture of dioxane and ethylene-glycol 50:1 (v/v) containing PPO (4.0 g/l), POPOP (200 mg/l) and naphthalene (60 g/l).

Figure 1 shows the purification pattern of liposomes and figure 2 shows the effect of penicillic acid on liposomes. The leakage of the trapped marker (^{14}C-glucose) from the liposome carrying a negative charge is marginally increased when compared with neutral or positively charged membranes.

It is interesting to note that polymyxin B has been shown[8] to require negative charge for the induction of membrane sensitivity Prymnesium toxin[9] does not depend on the net charge on the membrane. Recently citrinin action on liposomes was reported by Ganesan *et al*[10]. They observed that positive charge may be essential for the higher interaction of citrinin with membrane.

Ciegler *et al*[11] reported the nucleophilic attack of –SH group from cysteine or glutathione with

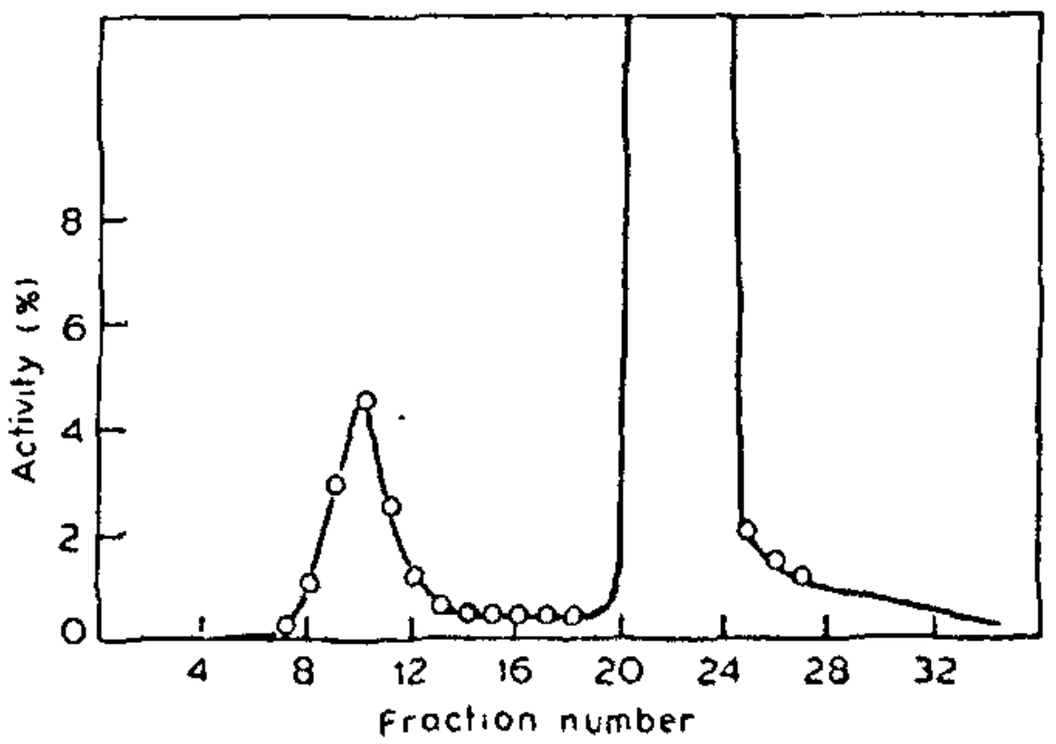

Figure 1. Purification pattern of liposome.

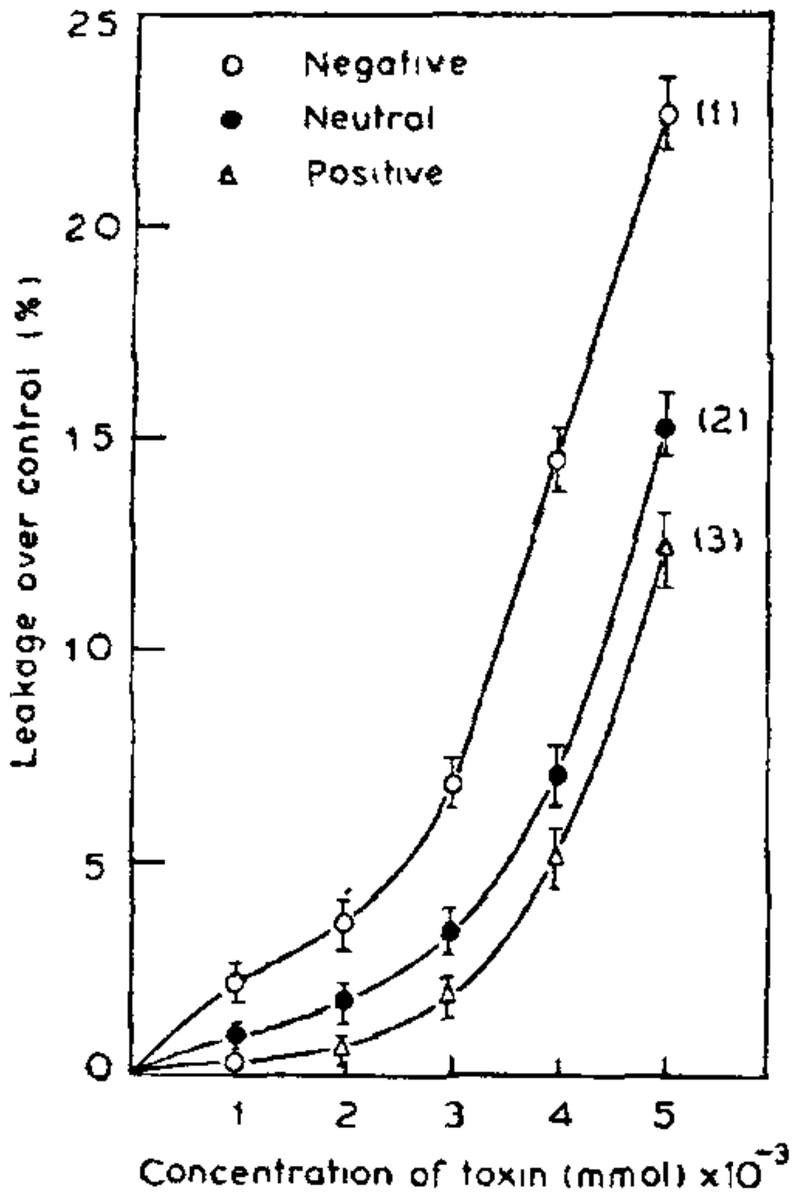

Figure 2. Effect of penicillic acid on liposomes.

penicillic acid, forming S-alkylated derivatives. Rapidly metabolising embryonic and developing systems are found to react with these adducts. In our experiment liposome carrying a net negative charge may act as a nucleophile; hence the affinity of penicillic acid will be greater towards the liposome carrying a net negative charge, resulting in the higher leakage of entrapped ^{14}C-glucose.

In other words membranes carrying a net negative charge may be more vulnerable to the action of penicillic acid.

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A CONVENIENT LABORATORY SYNTHESIS OF METHYL ISOCYANATE

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METHYL isocyanate (MIC) is an important synthetic intermediate particularly in pesticide synthesis[1]. Amongst the several procedures[2] reported for the synthesis of aliphatic isocyanates, the Curtius rearrangement of acylazides[3] remains the method of choice due to the ready availability of acylazides. Acylazides are commonly prepared by reacting acylchloride with sodium azide. However, the insolubility of sodium azide in common organic solvents results in heterogeneous reaction conditions, leading to irreproducible results. The use of water-containing solvent mixture e.g. with aromatic acid chlorides is precluded due to the high sensitivity to hydrolysis of MIC. Several soluble azide equivalents for the preparation of aliphatic isocyanates have been described including tetramethylguanidinium azide[4], trimethylsilyl azide[5], tributylstannyl azide[6] and tetrabutylammonium azide[7]. Although these reagents are soluble in organic solvents, they have to be prepared separately prior to their reaction with acyl chlorides. A laboratory procedure is reported here for the preparation of MIC.

The reaction of acetyl chloride and sodium azide in refluxing toluene in the presence of tetrabutylammonium bromide (TBAB) gave MIC in 70% yield. Comparable yield (73%) was obtained when reaction was conducted with 18-Crown-6 (18-C-6) in place of TBAB, while in their absence MIC resulted in only 20% yield, demonstrating definite advantage of phase transfer reagents in Curtius reaction. The