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A METHOD FOR THE PREPARATION OF PURIFIED ANTIGENS OF COXSACKIEVIRUS B3 FROM A LARGE VOLUME OF CELL CULTURE SUPERNATANT

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Summary. – A simple procedure was used for the concentration and partial purification of coxsackievirus B3 (Nancy strain). For a large-scale production of virus, Vero cells grown in roller bottles were used. Virus concentrate from a large volume of cell culture supernatant was prepared by precipitation with 6 % (w/w) polyethylene glycol. This crude antigen was further purified by banding in cesium chloride gradient using ultracentrifugation. The infectivity and haemagglutination activity of virus were checked up during the whole procedure and the final recovery of infections virus was about 60 %.

Key words: coxsackievirus B3; concentration; purification; polyethyleneglycol

Coxsackieviruses are important human pathogens which cause a wide spectrum of diseases, ranging from minor common colds to severe myocarditis, neurological disorders, and possibly acute diabetes (Melnick, 1985; Frisk *et al.*, 1985). To study the role of viruses in infectious myocardiopathy, we chose coxsackievirus B3 as a model because of obvious significance of coxsackie B viruses in clinical cardiology (Lerner *et al.*, 1975). In the first stage of this study it was necessary to prepare a sufficiently large amount of virus and in this paper wer report a modified procedure of concentration and purification of coxsackievirus B3.

All the six types of group B coxsackieviruses multiply quite well in the epithelial cells of primates, however, non-primate host cells can be also infected successfully (Lenahan *et al.*, 1960). Nevertheles, the cultures of Vero or HeLa cells were recently used most frequently in virological studies (Chatterjee *et al.*, 1981; Mapoles *et al.*, 1985; Kandolf *et al.*, 1985; Lindberg *et al.*, 1987). In our study, the large-scale propagation of coxsackievirus B3 (Nancy strain) was

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carried out in Vero cells cultured in roller bottles in Eagle's minimum essential medium supplemented with 5 % neonatal calf serum and antibiotics. Confluent monolayers of Vero cells were infected with viral inoculum using a multiplicity of 0.1 $TCID_{50}$ per cell. Afterwards, the growth medium was changed for the maintenance Leibowitz medium supplemented with 2 % neonatal calf serum and antibiotics. When the radiolabelled virus was prepared, ^3H]-uridine (1 μCi/ml, Sigma) was added to the maintenance medium. Bottles were incubated for 24 hr at 37 °C, when 100 % cytopathic effect was evident and the majority of cells had detached from the surface. After three freeze-thaw cycles the cell suspension was clarified by centrifugation at 3000 x g for 20 min.

For the concentration of virus from a large volume of the cell culture supernatant we chose and slightly modified the technique of precipitation with polyethylene glycol (PEG) of molecular weight 6000, which had been applied previously for other viruses (Wetz *et al.*, 1983; Donta *et al.*, 1990). To the clarified supernatant 50 % PEG solution in 1.25 mol/l NaCl was added gradually to the final concentration of 6 % (w/w) and incubated overnight at 4 °C. The precipita-

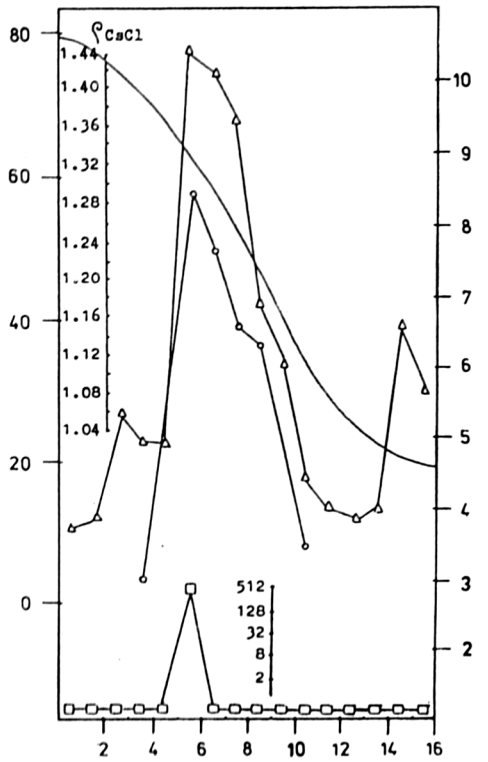

Fig. 1
CsCl density gradient centrifugation of coxsackie virus preparation
Infectivity (○); haemagglutination activity (□); radioactivity (△); CsCl density (no points). Abscissa: fraction number. Ordinate: cpm x 10^{-3} (left); -log $TCID_{50}$/ml (right); CsCl g/ml (inside up); HAU/ml (inside down).

ted virus was sedimented by centrifugation at 10 000 x g for 30 min. The pellet was resuspended in 40 ml of PBS and the suspension was clarified by centrifugation at 5000 x g for 30 min.

Methods for the purification of coxsackie B viruses using cesium chloride gradient centrifugation were described previously (Crowell *et al.*, 1971; Chatterjee *et al.*, 1981), nevertheless, we slightly modified them in the present study. The crude viral antigen prepared in the previous step was pelleted by centrifugation at 150 000 x g for 2 hr at 6 °C in the Beckman 70 Ti rotor. The pellet was resuspended in 5 ml of PBS containing 0.15 % Nonidet P-40, incubated overnight at 4 °C, homogenized, and centrifuged at 5000 x g for 30 min to remove insoluble debris. This clarified virus suspension was layered over a 10.5 ml discontinuous gradient of CsCl in PBS (three layers by 3.5 ml: 1.25–1.35–1.45 g/ml) and centrifuged at 120 000 x g for 12 hr at 8 °C in the Beckman SW 28.1 rotor.

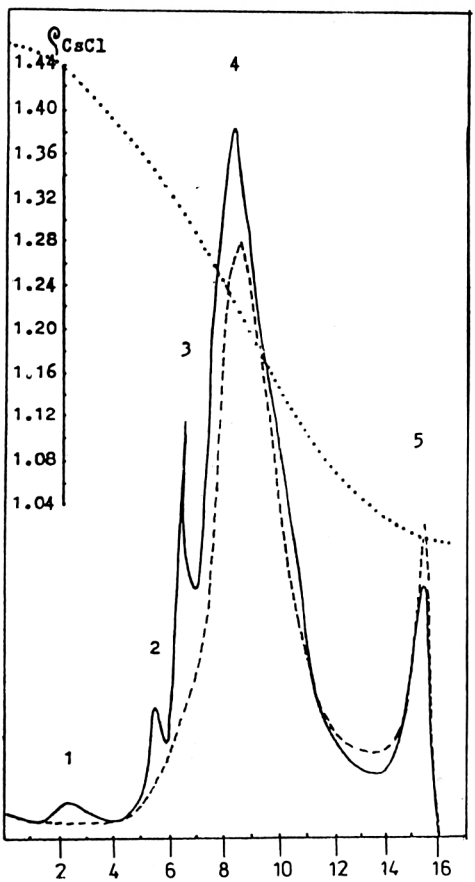

Fig. 2
CsCl density gradient centrifugation of coxsackie virus and mock-infected control preparations
Virus – solid line; control – broken line; CsCl density – dotted line. Abscissa: fraction number. Ordinate: A_{280} (main); CsCl g/ml (inside).

1 ml fractions were collected from the bottom of the tube and assayed for the infectivity, haemagglutination activity and radioactivity (Fig. 1). The infectious titer of selected fractions in the area of virus particles was determined in microtiter plates with Vero cells. For haemagglutination activity virus samples were titrated in microtiter plates using human erythrocytes (Melnick, 1969). The absorbance at 280 nm was also recorded (Fig. 2).

Two common absorption maxima were found in preparations of both virus and mock-infected control (peak 4 and 5 in Fig. 2). In the case of virus there appeared another three dense peaks; two of them (peak 2 and 3) evidently corresponded in density to the infective and defective virus particles (1.34 and 1.30 g/ml, respectively). These findings were confirmed by checking the infectivity, haemagglutination activity and radioactivity of all samples (Fig. 1), and are in good agreement with the data published for other coxsackieviruses (Chatterjee *et al.*, 1983; Muir *et al.*, 1990). In other experiments we confirmed that only the complete infections virions were able to agglutinate human erythrocytes, whereas the defective virus particles of lower buoyant density lacked this agglutination capacity (data not shown).

To remove CsCl, viral material from the fractions 6 and 7 was diluted in 40 ml of PBS and centrifugated at 150 000 x g for 2 hr at 6 °C in the Ti 70 rotor. The pellet was resuspended in a small volume of PBS and clarified by centrifugation at 5000 x g for 30 min. If necessary, this virus suspension can be recentrifugated in the CsCl gradient in the same way as described above.

The efficiency of this concentration and purification procedure was checked by titration of the infectivity and haemagglutination activity of the initial material, the PEG precipitate and the final virus preparation. Virus titration was carried out by the plaque method.

From the results shown in Table 1 it can be seen that the yield of infections virus achieved by this method is sufficient enough, the final recovery being about 60 %. Additional advantage of described procedure consists in a relatively

Table 1. The efficiency of virus concentration procedure

	Initial material (520 ml)		PEG precipitate (38 ml)		Final concentrate (7 ml)	
	per ml	Total	per ml	Total	per ml	Total
Infectious titer (PFU)	$2x10^8$	$1.04x10^{11}$	$2x10^9$	$7.60x10^{10}$	$9x10^9$	$6.30x10^{10}$
(%)		(100)		(73.1)		(60.6)
Haemagglutination titer (HAU)	2	1040	32	1216	128	896
(%)		(100)		(107.9)		(86.2)

easy and reliable detection of the area of virus particles after fractionation in the CsCl gradient, based on the mere recording of absorbance at 280 nm and the measurement of buoyant density (refractive index), respectively.

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