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A SIMPLE METHOD OF PREPARING A COMPLEMENT-FIXING ANTIGEN FROM THE VIRUS OF HAEMORRHAGIC FEVER WITH RENAL SYNDROME (WESTERN TYPE).

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Summary. — Different methods of the preparation of haemorrhagic fever with renal syndrome (HFRS) antigen for complement-fixation (CF) test are described. The antigens were prepared from the organs of suckling white rats inoculated with the Western type of HRFS by precipitation with polyethylene glycol, by fluorocarbon treatment and or by sucrose-acetone extraction. The highest CF titre was obtained by acetone precipitation of 20% brain suspension in isotonic sucrose.

Key words: haemorrhagic fever with renal syndrome antigen western type, complement-fixation test, antigen preparation

The method generally used for diagnosis of haemorrhagic fever with renal syndrome (Western type) is the indirect immunofluorescence test (Brummer-Korvenkontio *et al.*, 1980). It was desirable to develop an another simple test (WHO 1982) such as complement-fixation with the western type of HFRS which can be used with modest laboratory equipment. The CF test is of advantage especially as a diagnostic test, in comparison with immunofluorescence.

Virus antigens used in complement-fixation test were prepared in different ways.

Appropriate amounts of solid PEG 6000 were added to the clarified liver suspension of HFRS-infected suckling rats in ice bath under vigorous stirring with a magnetic stirrer. After 2 hr incubation at 4 °C, the precipitate was pelleted by centrifugation at 10 000 x g for 20 min at 4 °C. The pellets were resuspended 1 : 10 (of the starting volume) in saline (ph 7.2) and incubated overnight at 4 °C. The supernatant of the clarified suspension was used as the antigen.

Ten per cent brain and/or lung suspension were mixed with 9 volumes of fluorocarbon according to the methods described (Hamparian *et al.*, 1985).

Complement-fixing antigen was also prepared from the brain suspension by acetone precipitation in isotonic sucrose (Casals, 1976).

Table 1. CF titres of HFRS antigens with antisera to the Eastern and Western types of HFRS

The source of antigen / treatment	The CF titres with sera to	
	Eastern type of HFRS	Western type of HFRS
Lung / fluorocarbon	16	64
Liver / PEG	32	32
Brain / acetone	16	128
Brain / fluorocarbon	16	64
Control antigen* / acetone	0	0
Control sera	0	0

PEG = Polyethylene glycol 6000
* = Chikungunya virus antigen was used in control experiments

Complement-fixing (CF) antigen for haemorrhagic fever with renal syndrome virus has been previously prepared from brain tissue of infected suckling rats (Grešíková *et al.*, 1984). The method described here was developed with the Poomala strain of HFRS (Brummer-Korvenkontio *et al.*, 1982). Suckling rats were inoculated intramuscularly and intranasally with HFRS virus. The infected suckling rats survived the 3-weeks observation period. The organs (the brain, the lungs and the liver)were harvested from infected suckling rats by 3-weeks p.i. The antigens were prepared either by acetone precipitation of a 20% suspension in isotonic sucrose either by fluorocarbon treatment and/or by precipitation with polyethylene glycol.

Antigen prepared by polyethylene glycol had only low titre (1 : 32), while those prepared by sucrose-acetone method gave higher titres (1 : 128). The antigen prepared by fluorocarbon extraction had an intermediate titre of 1 : 64. The CF titres were lower in cross-CF tests done with eastern type antisera to HFRS: 1 : 16 with fluorocarbon and acetone extracted antigen and 1 : 32 with polyethylene glycol precipitated antigen (Table 1).

We aimed to find out whether HFRS virus CF antigens can be prepared and if so, which was the optimal method for preparation of such antigen. The results showed that the sucrose-acetone method was the most effective. The sucrose-acetone method was superior to polyethylene glycol precipitation: the CF titre with the Western type of HFRS antiserum and sucrose-acetone antigen was 1 : 128; with the Eastern type of HFRS antiserum it was 1 : 16. On the other hand, the antigen prepared by polyethylene glycol precipitation reacted with the Western and Eastern types of HFRS in the titre 1 : 32.

Since the CF test is specific and easy to perform, it appears suitable for serologic screening. The CF test has great advantages: it is simple, economical, specific and need no complicated laboratory equipment. It is of interest that the antigens of the western type of HFRS had no haemagglutination activity.

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