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HAEMAGGLUTINATION
BY MOUSE HEPATITIS VIRUS TYPE 3

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RÉSUMÉ

L'HÉMAGGLUTINATION PAR LE VIRUS TYPE 3
DE L'HEPATITE DE LA SOURIS

Une suspension partiellement purifiée de virus de l'hépatite de la souris (MHV₃) agglutine les érythrocytes de poule, poussin d'un jour, furet, hamster, et les érythrocytes humains du groupe O, aux températures de 4, 20 et 37° C, tandis que les érythrocytes de souris ne sont agglutinés qu'à 4° C.

Mots-clés : Hépatite de la souris, MHV₃; Hémagglutination, Coronavirus.

INTRODUCTION

Heretofore, mouse hepatitis virus type 3 (MHV₃), a coronavirus causing a fatal infection of mice, has not been observed to agglutinate erythrocytes [4, 7, 9]. However, other coronaviruses do cause agglutination of red cells from several species, including rat, chicken, hamster and mouse, but some, such as avian infectious bronchitis virus (IBV) must first be purified in sucrose gradients [6, 8, 1, 5]. Previous preliminary work in this laboratory had shown that untreated extracts from mouse livers containing MHV₃ gave inconsistent haemagglutination and thus, in the present study, partially purified preparations of the virus were tested for haemagglutinating properties.
MATERIALS AND METHODS

Culture and purification of virus.
MHV₃ (obtained from Dr. J. S. Porterfield, Sir Williams Dunn School of Pathology, Oxford, England) was propagated by the intraperitoneal inoculation of 21-day old Manchester strain mice. Livers were removed 72 h after infection, macerated in 1 ml of Dulbecco [1] phosphate buffered saline "A" (PBS-A) per liver, and centrifugated at 1,500 g for 10 min. Supernatant fluids (3 ml) were overlaid onto 20 to 60 % (v/v) sucrose gradients (55 ml) prepared in distilled water and centrifuged at 70,000 g for 48 h. The gradients were fractionated in 3-ml aliquots which were diluted four fold in PBS-A and centrifuged for 2 h at 30,000 g. Sedimented material from each fraction was resuspended in PBS-A and screened for infectivity in mice. Control preparations of uninfected mouse livers were processed as above.

Erythrocytes.
Erythrocytes were collected into Alsever's solution, washed in PBS-A and resuspended to 8 % (v/v) by haematocrit.

Haemagglutination tests.
Haemagglutination tests were carried out as described by Clarke and Cassals [2], except that each well (WHO plates) contained 0.2 ml of virus dilution and 0.2 ml of a 0.2 % (v/v) erythrocyte suspension. Fractions containing infectious virus were pooled and tested at 4, 20 and 37°C, using pH values 5.75, 6.0, 6.2, 6.4, 6.8 and 7.2.

RESULTS AND DISCUSSION

Coronavirus-like particles and small amounts of amorphous material were seen by electron microscopy in pooled materials recovered from virus-containing fractions detected towards the middle of the sucrose gradients. Haemagglutination was detected only in those fractions which contained infectious virus (titre of pooled material 10⁵.₃ LD₅₀/ml in mice).

Optimal haemagglutination titres using mouse and ferret erythrocytes were obtained at pH values between 5.75 and 6.4, and erythrocytes from all species were thence tested at pH 6.2 (table I). Haemagglutination was not observed with erythrocytes from the following species: sheep, rabbit, rhesus monkey, rat, gerbil, pigeon, cat and guinea-pig. Haemagglutination was not detected in control material from fractions of sucrose gradients used to "partially purify" uninfected liver material.

In common with other coronaviruses, MHV₃ agglutinates erythrocytes from several species and, as with IBV, it is necessary to partially purify the preparations of virus to obtain consistent results. Haemagglutination

IBV = infectious bronchitis virus.
MHV₃ = mouse hepatitis virus type 3.
PBS-A = phosphate-buffered saline "A".
TABLE I. --- Haemagglutination (*) by MHV₃ of erythrocytes from different species, titre of haemagglutination.

| Erythrocytes | 4°C    | 20°C   | 27°C   |
|--------------|--------|--------|--------|
| Human O      | 1/4    | 1/16   | 1/32   |
| Fowl         | 1/16   | 1/32   | 1/16   |
| Mouse        | 1/16   |        |        |
| Day-old chick| 1/32   | 1/64   | 1/16   |
| Ferret       | 1/8    | 1/32   | 1/32   |
| Hamster      | 1/8    | 1/8    | 1/8    |

(*) Using 0.2 % (v/v) erythrocytes at pH 6.2.

was observed at 4, 20 and 37°C except with mouse erythrocytes, which did not agglutinate at 20 and 37°C. Erythrocytes did not elute from agglutinated preparations left for several h at 37°C.

The preliminary studies reported here indicate that MHV₃ may be included among those coronaviruses causing haemagglutination. Haemagglutination inhibition studies and infectivity assays are now being undertaken to confirm the above results and establish a rapid method of measuring antibody levels to MHV.

SUMMARY

Partially purified preparations of mouse hepatitis virus type 3 (MHV₃) agglutinated fowl, day-old chick, ferret, hamster and human O erythrocytes at 4, 20 and 37°C and mouse erythrocytes at 4°C.

KEY-WORDS: Mouse hepatitis, MHV₃; Haemagglutination, Coronavirus.

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