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Received September 26, 1966. P.S.E.B.M., 1967, v124.

## Agglutination of the H-Viruses with Various Types of Red Blood Cells.* (31685)

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Although it has been noted that the H-viruses(1), including Kilham's RV(2), are able to agglutinate several types of red blood cells(3), no comprehensive data on this phenomenon has been reported. Recently, the discovery of a number of small DNA viruses similar to the H-group, such as the adenovirus-associated agents(4,5,6,7,), and Crawford's MVM(8), have focused considerable attention on these minute viruses. Type of hemagglutination (HA) has been used as one method of differentiation between the various small agents and between these viruses and other agents such as polyoma(8). In some instances where investigators have not been fully aware of the range of hemagglutination capability of the H-viruses, greater faith has been placed on HA or lack of HA with one or two types of red cells as a definite diagnostic aid, than is warranted. A detailed survey of the HA patterns of the H-viruses should be of value, therefore, in future work with these particular agents as they have consistent and individual HA patterns which are reproducible. Designation as a member of the H-virus group, *per se*, should, of course, be determined by pathogenicity studies in newborn hamsters, *i.e.*, death or production of a "mongoloid-type" deformity(1,2).

*Materials and methods.* Five H-viruses (H-1, HT, H-3, RV and HB) derived from distilled water filtrates of infected baby hamster litters(9) were employed for this study. (H-viruses prepared from infected tissue culture stocks have given similar re-

* Aided by Grant E343A from Am. Canc. Soc., and Grant CA-07826-03 from Nat. Cancer Inst., N.I.H., USPHS.

TABLE I. Agglutination of the H-Viruses with Various RBC's.

| | | Red cell type | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Virus | Test # | G.P. | Ham. | Human | Rat | Mouse | Agouti | Rabbit | Monkey | Dog | Cat | Horse | Gerbil | Sheep | Duck | Goose | Chicken |
| H-1* | 1 | 8† | 7 | 6 | 3 | 6 | 8 | 0 | 5 | 2 | 3 | 3 | 3 | 0 | 2 | 3 | 0 |
| | 2 | 8 | 7 | 6 | 4 | 6 | 7 | 0 | 3 | 3 | 1 | 3 | 1 | 0 | 3 | 2 | 0 |
| | 3 | 8 | 8 | 7 | 5 | 6 | 7 | 0 | 4 | 6 | 4 | 4 | 2 | 0 | 2 | 2 | 0 |
| HT | 1 | 8 | 8 | 0 | 0 | 0 | 8 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 3 | 0 | 0 |
| | 2 | 8 | 8 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 3 | 8 | 8 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| H-3 | 1 | 8 | 5 | 4 | 8 | 7 | 8 | 5 | 7 | 0 | 4 | 7 | 0 | 0 | 8 | 0 | 0 |
| | 2 | 8 | 5 | 3 | 8 | 6 | 7 | 4 | — | 0 | 3 | 6 | 0 | 0 | 6 | 0 | 0 |
| | 3 | 8 | 4 | 5 | 7 | 5 | 8 | 4 | 7 | 2 | 3 | 7 | 0 | 0 | 7 | 0 | 0 |
| RV | 1 | 8 | 6 | 8 | 5 | 8 | 8 | 0 | 7 | 4 | 2 | 8 | 0 | 0 | 7 | 0 | 0 |
| | 2 | 8 | 5 | 8 | 5 | 7 | 7 | 0 | 7 | 5 | 0 | 7 | 0 | 0 | 7 | 0 | 0 |
| | 3 | 8 | 4 | 7 | 4 | 7 | 7 | 1 | 5 | 3 | 0 | 7 | 0 | 0 | 4 | 0 | 0 |
| HB | 1 | 8 | 6 | 0 | 6 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| | 2 | 8 | 5 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 3 | 8 | 5 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |

* Antiserum to H-1 virus cross reacts with HT; antiserum to H-3 cross reacts with RV. HB antiserum does not cross react with any of the other H-viruses.

† Titers were read as that dilution of virus which completely agglutinated all the red cells. 1 = 1:10; 2 = 1:20; 3 = 1:40; 4 = 1:80; 5 = 1:160; 6 = 1:320; 7 = 1:640; 8 = 1:1280. 0 = no agglutination (<10).

sults.) Three different passages of each virus were tested; one passage was used for each of 3 large tests. All of the viruses had a similar HA titer of 1:1280 with guinea pig cells in order that a base line might be provided for the relative values of HA with other cell types.

Hemagglutination tests were run on plastic spot plates. Phosphate buffered saline, pH 7.2, was used as the diluent and suspending fluid. Equal parts of dilutions of virus and washed 0.4% red cells were mixed and stored overnight in a cold room at 5°C. End points were read the next morning as that dilution of virus which completely agglutinated all the red cells.

Animals were bled without anesthesia and the red cells prepared and used within the next 24 hours.

*Results.* All the 5 viruses tested agglutinate guinea pig and hamster cells (Table I). When, in addition, human and rat cells are employed, as is routinely done in this laboratory, it is possible to differentiate the 5 virus types, preliminary to tests with specific antisera. Neither HB nor HT agglutinate human cells but, in addition, HT does not agglutinate rat cells. This virus also has a great and equal avidity for guinea pig and hamster cells. H-1 agglutinates guinea pig, hamster, human and rat cells, respectively, in descending order. H-3 agglutinates rat cells in as high titer as cells from the guinea pig, while RV preferentially agglutinates guuinea pig and human cells. Further differences between the various viruses become apparent as other red cell types are tested. HB and HT both have a narrow agglutinating range though the former, in common with most of the H-viruses, agglutinates mouse cells and the latter agglutinates agouti cells. Only H-3 regularly agglutinates rabbit cells though some weak HA reactions between RV and rabbit cells have been seen. H-1 has the widest HA range of all the virus strains. It is the only one which agglutinates gerbil and goose cells. In contrast to polyoma, none of the H-viruses react with chicken cells. They all show some reaction, however, with duck cells.

It is apparent that knowledge of the HA capability of the individual H-viruses facilitates preliminary differentiation between these agents. In the case of the H-viruses, all the agents remain true to their HA type. HT, for example, although it cannot be differentiated from H-1 serologically, has retained its own HA pattern after numerous passages in newborn hamsters or tissue culture. The same finding applies to all the H-viruses. Routine use of 4 types of red cells for all HA tests has proved most valuable and an argument against the continual use of only one kind of red cell, *e.g.*, guinea pig, with the occasional employment of a presumed "non-agglutinating" cell. The latter practice could lead to errors of diagnosis.

*Summary.* The hemagglutinating capabilities of 5 H-viruses with 16 different kinds of red cells are presented. Knowledge of such HA patterns offers an aid to determination of the H-virus strain, as well as an accessory method of differentiation between these agents and other hemagglutinating viruses of similar size.

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Received September 26, 1966. P.S.E.B.M., 1967, v124.