# Hemagglutination and Hemadsorption of Measles Virus. (26403)

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Since the first evidence of successful isolation and propagation of measles virus in human renal cells by Enders and Peebles(1) many laboratories have reported studies on growth properties, adaptation to other host tissues, as well as serological responses in humans and laboratory animals. Recently, Peries and Chany(2) have reported their findings on yet another property of this virus, namely, the phenomenon of hemagglutination. They found that hemagglutination occurred only with monkey blood cells and that this hemagglutination could be inhibited by measles convalescent serum but not by the serum of subjects without previous measles experience. This is to report further studies on agglutination of monkey red blood cells by different measles virus preparations and inhibition of this hemagglutination by various measles antisera.

*Methods.* The Edmonston strain of measles virus, kindly supplied by Dr. J. Enders, was used in this study. In some experiments an additional measles isolate, kindly supplied by Dr. J. Frankel, was used for comparison. Complement fixation (CF) tests were performed according to the technique described by Jensen(3).

Hemagglutination (HA) tests were performed by 2-fold serial dilutions of virus in physiological saline in a volume of 0.5 ml. Five-tenths (0.5) ml of 0.5% monkey red blood cells in saline was then added to all tubes. One HA unit of virus was considered to be the highest dilution of virus showing complete hemagglutination.

Hemagglutination-inhibition (HA-I) tests were performed by mixing 4 HA units of virus in a volume of 0.25 ml and 2-fold serial dilutions of serum in 0.25 ml. The mixtures were shaken thoroughly and incubated at room temperature for one hour. Five-tenths ml monkey red blood cells (0.5%) was then added and incubation continued. Tests were read when red cell controls and serum controls were negative. Reshaking and reincubation of tubes at room temperature overnight occasionally clarified end point reactions. The HA-I titer of a serum was considered to be the highest dilution of that serum which completely inhibited hemagglutination of 4 HA units of virus.

Concentration of hemagglutinin by centrifugation was accomplished by centrifuging different measles virus harvests in a Spinco, Model L, ultracentrifuge, in a No. 30 rotor. Sedimented debris (pellets) were reconstituted in one-tenth of the original starting volumes in saline. Pellets were found to reconstitute easily.

*Results.* Hemagglutination of measles virus was demonstrated with 10× concentrated measles virus specimens only when red blood cells from monkeys were used. Cells from rhesus, cynomolgus, patas, African green or baboon all were equally sensitive. In all subsequent tests red cells from African green monkeys were used. Red blood cells from 11 other mammals: sheep, chick, guinea pig, rabbit, human types AB and O, horse, hamster, mouse, rat, cat and cow were negative.

The ability of measles virus to agglutinate monkey red blood cells varied according to the tissue in which the virus was grown. Table I lists some of the preparations used, their approximate passage history and their infectivity, CF and HA titers. We have found that measles virus propagated in one passage of primary baboon kidney cells yields higher titered infectious measles virus preparations than when grown in human amnion or monkey kidney (rhesus or cynomolgus) cells. These preparations also showed higher HA titers than other preparations. Measles virus propagated in chick fibroblasts showed virtually no hemagglutination even though infectivity titers were between $10^{4.0}$ to $10^{4.7}$. Hemagglutination was observed, although to a lower titer, with the Edmonston strain of measles virus propagated in primary amnion

TABLE I. Comparison of Serological Reactions of Measles Preparations.

| No. | Sample | Strain—known passage history | Reciprocal of titer INF.* | CF | HA† |
|---|---|---|---|---|---|
| 1 | MeM446BK | Edmonston—human amnion (prim.) baboon | 6.0 | 256 | 32 |
| 2 | MeM387BK | *Idem* | 6.0 | 256 | 32 |
| 3 | MeM38 | Edmonston—human amnion (prim.) | 3.5 | N.T. | 4 |
| 4 | MeLB4-5 | Edmonston—human amnion (prim.) chick embryo chick fibroblast | 4.3 | <4 | <4 |
| 5 | Me33G-D | Edmonston—human amnion (prim.) monkey kidney | 3.0 | N.T. | 2 |
| 6 | MeG.H. (live)‡ | Edmonston—human kidney monkey kidney Girardi heart | approx. 4.0 | 64 | 8 |
| 7 | MeG.H. (inactivated)§ | Edmonston—human kidney monkey kidney Girardi heart | — | 256 | 32 |
| 8 | Me #6‖ | Isolate —human amnion (cont.) | 4.5 | 32 | 8 |

* Negative $\log_{10}$ $TCID_{50}$/ml.
† Highest dilution showing complete hemagglutination (10× concentrates).
‡ Prepared by Microbiological Associates, Inc.
§ Commercially available from Microbiological Associates, Inc.
‖ Kindly supplied by Dr. J. Frankel, Norristown State Hosp., Norristown, Pa.
N.T. = Not tested.

TABLE II. Concentration of Hemagglutinin Activity.

| Sample | Treatment | Hemagglutination titer |
|---|---|---|
| Prep. 1 MeM38-7BK | (Centrifugation) | |
| a) Original sample | None | 1/8 |
| b) Supernate | 11,000 rpm/90 min. | 1/8 |
| c) Pellet (10×) | *Idem* | 1/32 |
| d) " —7BK control (10×) | " | 0 |
| Prep. 2 MeM38-7BK | (Centrifugation) | |
| a) Original sample | None | 1/8 |
| b) Supernate | 30,000 rpm/180 min. | <1/4 |
| c) Pellet (10×) | *Idem* | 1/64 |
| d) " —7BK control (10×) | " | 0 |

or with another measles isolate propagated in a continuous line of human amnion cells. Hemagglutination also was observed when killed measles antigen* or preparations containing live measles virus prepared in another laboratory were used. Hemagglutination was not seen in the original or concentrated suspensions of uninfected tissue cultures.

Ratios of infectious virus particles to hemagglutination units from 4 different measles concentrates (Table I) were similar and varied between 1.3 x $10^4$ to 4.0 x $10^5$.

* Microbiological Associates, Inc.

Preliminary studies have demonstrated that concentration of virus is necessary to demonstrate adequate titers of the hemagglutinin even on those preparations grown in baboon kidney (Table II). Centrifugation in a Spinco, Model L, centrifuge at 30,000 rpm/180 minutes concentrated the hemagglutinin so that hemagglutination inhibition tests could be satisfactorily performed. Hemagglutinin activity was found in uncentrifuged samples or supernatant samples of preparations centrifuged at only 11,000 rpm/90 minutes.

Stability studies on limited amounts of

TABLE III. Comparison of HA-I and CF Titers of Naturally Occurring Measles Antibodies in Simian Sera.

| Animal | Sera # | Species | Reciprocal of titer CF | HA-I |
|---|---|---|---|---|
| Baboon | 3 | Papio unibas | 256, 512 | 128 |
| | 11 | " " | 64 | 8 |
| | 6 | Papio dougeriae | 128, 128 | 128 |
| | 7 | " " | 64 | 64 |
| | 8 | " " | 32 | 64 |
| | 25 | " " | 128, 256 | 1024 |
| | 27 | " " | 64 | 128 |
| | 28 | " " | 64 | 64 |
| African green monkey | 9 | Cercopithecus aethiops | 64 | 8 |
| | 10 | " " | 128 | 128 |
| Rhesus | Standard hyp. serum | Macaca mulatta | 256 | 1024 |
| Baboon | Each of 24 sera | Papio unibas | <8 | <8 |
| African green monkey | Each of 19 sera | Cercopithecus aethiops | <8 | <8 |

10× concentrated measles preparations have shown that all hemagglutinin activity is lost in 2 hours at 56°C. There is loss after 3 to 4 days at 37°C, but the hemagglutinin is stable for at least 7 days at –20°C and 5°C, and for at least 14 days at –70°C.

Incubation of hemagglutinated measles virus at 37°C for periods up to 36 hours have shown that elution of the virus as evidenced by sedimentation of red cells did not occur. The use of tanned red blood cells according to the technique described by Arbesman, *et al.* (4), did not significantly increase the sensitivity of the hemagglutination reaction.

Infected stationary tubes of various cell types infected with measles virus were also examined for direct tissue culture hemadsorption. Areas of positive hemadsorption patterns in measles inoculated kidney cultures were clearly evident, whereas control cultures were negative. When measles infected tubes were tested with red blood cells from animals other than monkeys, no positive patterns of hemadsorption could be seen. However, when these red cells were washed off and monkey cells added, positive hemadsorption again was noted.

A study was made of the HA-I titers in a limited number of sera from monkeys with naturally acquired measles, artificially induced hyperimmune measles sera, and human measles sera to determine whether correlation existed between measles antibodies from those sources.

Table III shows CF and HA-I titers in 10 simian sera against measles occurring naturally. These 10 sera contained CF as well as HA-I antibodies. A total of 43 negative simian sera by CF procedures were also negative by HA-I.

HA-I tests performed with hyperimmune measles antisera (Table IV) show that all sera obtained from animals immunized with measles antigens contained high levels of HA-I antibodies. Inhibition of hemagglutination by these antisera was observed when any antigen was used. Of particular interest is the fact that antisera prepared against measles virus grown in chick fibroblasts inhibited hemagglutination of measles virus even though little or no hemagglutinin activity could be demonstrated with chick grown virus. Specific human gamma globulin in high dilution also inhibited hemagglutination of measles virus prepared in our laboratory. Normal sera and sera from animals immunized with control tissue culture cells contained no antibodies.

Antisera against a number of viruses, mumps, respiratory syncytial, herpes simplex, parainfluenza, Newcastle disease, vaccinia, polio types 1, 2, and 3, influenza strains Lee and Jap 305, sendai, and canine distemper failed to inhibit measles hemagglutination.

Analyses of the HA-I and CF titers of 4 samples of paired human sera obtained in the Philadelphia area during an epidemic of measles in 1957, as well as 8 samples of convales-

TABLE IV. Hemagglutination-Inhibition Titers of Measles Hyperimmune Sera.

| | Measles virus* grown in | Antisera prepared in | Reciprocal of highest dilution showing complete inhibition of hemagglutination† |
|---|---|---|---|
| 1. | Girardi human heart‡ | Monkey | 2560 |
| | | Control monkey serum | 8 |
| 2. | Chick embryo fibroblasts | Rabbit | 256 |
| | | Control rabbit serum | <4 |
| 3. | Continuous human amnion | Rabbit | 512 |
| 4. | *Idem* | Guinea pig | >4096 |
| | | Control guinea pig | 8 |
| 5. | Baboon kidney | Guinea pig | 4096 |
| | | Control guinea pig | 16 |
| 6. | γ-globulin§ | Human | 6400 |

* Edmonston strain of measles.
† Against 4 HA units of concentrated measles virus.
‡ Killed CF antigen—Microbiological Associates, Inc.
§ American Cyanamid Co.

cent measles sera, are presented in Table V. Significant increases in HA-I antibodies were found in 3 out of 4 of the paired convalescent serum samples over that in the acute specimen. One matched pair showed only a 2-fold increase in titer in the convalescent serum over the acute samples. The 8 convalescent measles samples showed comparable CF and HA-I activity.

TABLE V. Comparison of Hemagglutination-Inhibition and CF Titers of Human Sera against Measles Virus.

| Human sera No. | | Titers† CF Acute | CF Conv. | HA-I Acute | HA-I Conv. |
|---|---|---|---|---|---|
| 1 | | <8 | 128 | 8 | 256 |
| 2 | | 8 | 256 | N.A.‡ | 256 |
| 5 | | 4 | 64 | <8 | 64 |
| 7 | | <8 | 64 | N.A. | 512 |
| 11 | | <4 | 256 | 8 | 128 |
| 12 | | <8 | 128 | 64 | 128 |
| 60-2749* | 1 | | 256 | | 32 |
| | 2 | | 256 | | 32 |
| 60-1279* | 1 | | 256 | | 256 |
| | 2 | | 128 | | 256 |
| 60-1091* | | | 64 | | 512 |
| 60-1210* | 1 | | 128 | | 256 |
| | 2 | | 128 | | 512 |
| 60-1204* | | | 64 | | 128 |
| Standard monkey hyp. sera | | 256 | | 1024 | |

* Kindly supplied by Dr. K. Hummler, Children's Hospital, Philadelphia, Pa.
† Reciprocal of dilution.
‡ None available.

*Discussion.* An interesting observation was the apparent failure of chick TC grown measles to hemagglutinate. This was strikingly similar, at least in our hands, to the failure of these preparations to demonstrate complement-fixing antigen. CF measles antigen was demonstrated when primate tissue was used as the host tissue even when infectivity titrations showed that titers were below $10^{-4.0}$. Peries and Chany(2) have pointed out that in their system of KB infected measles cells, $10^{5.0}$ to $10^{6.0}$ infectious particles per ml must be attained in order to demonstrate hemagglutination. It might be that more potent chick grown measles virus preparations will demonstrate hemagglutination; however, it is conceivable that this virus present in supernatant fluids may be bound in such a manner that hemagglutination does not occur. These points are now being investigated.

As hemagglutination and hemadsorption are related manifestations in the case of certain other viruses, it was interesting to find that positive hemadsorption patterns could be observed with monkey red blood cells and tubes infected with measles virus. Hemadsorption technics, therefore, should offer a

more rapid means of attempting to isolate measles virus as well as studies on adaptation of the virus to other tissues.

The observations that CF and HA-I titers were comparable in measles sera from simian, rabbit, guinea pig, and human (the latter small in number), and that no CF or HA-I antibodies were found in a larger number of measles negative sera would indicate that hemagglutination-inhibition may be useful in evaluation of antigenic responses in man and animals, assay of virus pools, and standardization of hyperimmune sera.

*Summary.* Evidence has been presented to show that concentrated measles virus in presence of monkey red blood cells will demonstrate the phenomenon of hemagglutination. Baboon kidney propagated measles virus yielded higher levels of hemagglutinin than measles virus grown in other primate tissue. Chick tissue propagated measles virus did not demonstrate hemagglutination. Specific inhibition of hemagglutination was demonstrated by rabbit and guinea pig antisera prepared against measles virus in our laboratory and in another laboratory, by naturally occurring simian measles antisera, by human gamma globulin and by human convalescent measles antisera. Inhibition was not observed when measles CF negative simian sera, measles acute human sera and a number of viral antisera were tested.

ADDENDUM: After this paper was submitted for publication, an article entitled "Hemagglutination and Hemagglutination-Inhibition with Measles Virus" by Leon Rosen appeared in *Virology*, 1961, v13, 139. The work reported herein is in complete agreement with his observations.

Technical assistance of Robert Jakubowski is gratefully acknowledged.

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Received January 3, 1961. P.S.E.B.M., 1961, v106.

## Experimental Growth of Mammary Gland in Male and Female Mice.* (26404)

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Estrogen (E.B.) and progesterone (P) (1,2) or relaxin(3) have been shown to synergize in stimulating lobule-alveolar growth in male and female mice in short-time experiments. Recently, normal growth of mammary glands of mice pregnant 18 days was determined using total DNA (desoxyribonucleic acid) as an index of growth(4). Levels of E.B. and P. which produce maximum mammary gland growth in ovariectomized rats(5) have been reported. The present study is concerned with levels of E.B. and P. which stimulate maximum growth of the mammary gland of male and ovariectomized female mice during period of 19 days.

*Materials and Methods.* Mature male and female Webster-Swiss mice were maintained on standard laboratory feed at constant temperature of 78±1°F. Ovariectomized mice received subcutaneous injections beginning day 14 postoperatively. Male mice were pretreated 28 days with 1.23 mg diethylstilbestrol per kg ground lab feed to insure good duct development before injection(6). E.B. and P. were dissolved in olive oil carrier at

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2249. Approved by the Director. Aided in part by research grants from P.H.S. and Am. Cancer Soc.

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