Hemagglutination by Measles Virus. 4. A Simple Procedure for Production of High Potency Antigen for Hemagglutination-Inhibition (HI) Tests. (27930)

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Several authors(1-4) studying measles hemagglutination have resorted to concentration of tissue culture (TC) material as a means of obtaining hemagglutinin of sufficient potency to permit the activity margins required in various investigations. Titers of such preparations were reported to fall in the range of about 1:100. In this laboratory titers of the same order of magnitude were recorded in crude TC fluids provided that certain precautions were taken for maintenance and harvest of the cultures(5,6). In many situations titers of this order are satisfactory. For some purposes, however, higher activities and particularly maximal sensitivity of reaction might be desirable.

In studies on the nature of measles hemagglutinin(6) it was found that ether treatment resulted in a partial disintegration of virus particles with appearance of small particle hemagglutinin. Measles virus in this respect behaves like many myxoviruses. Presumably each virus particle includes a number of hemagglutinating subunits and it might have been expected that ether treatment would lead to an increase in HA titers. This was not the case. However, after addition of a detergent (Tween) prior to treatment with ether, a considerable increase in HA titer over the untreated TC fluid was observed. Since this seemed to provide a simple method for production of a satisfactory hemagglutinin a closer study of the process and its practical applicability appeared justified.

Material and methods. Virus and cell cultures. Edmonston strain of measles virus was used and in most experiments propagated in the established human embryonic lung cell line (Lu 106) described earlier(6). Cultures were seeded with undiluted material and kept at 33°C. Change of medium was made 2 to 3 days after inoculation of the cultures. After a further 3 to 4 days of incubation complete cellular degeneration had occurred and the material was harvested after 3 cycles of freezing and thawing. The virus material so obtained had titers of 64 to 128 hemagglutinating units (HAU) per 0.4 ml. The same titers were obtained with the virus adapted to growth in dog kidney TC, in which, however, rate of virus production is much slower. Material of the latter kind was used in a few experiments.

On some occasions virus material was concentrated by forced dialysis against polyethylene glycol according to the technic of McClendon *et al.*(7). By this means the volume was decreased approximately 10-fold with a corresponding increase in HA titer.

Infectivity titrations were performed in tubes of Lu 106 cells with serial 10-fold dilutions of the material and 5 tubes were inoculated per dilution. The formula of Reed and Muench was used for calculation of endpoint titers ($TCID_{50}$).

Hemagglutination (HA) tests. Two-fold serial dilutions of the virus material were set up in 0.4 ml of 0.15 M phosphate buffered saline (PBS) at pH 7.2. To each tube was then added 0.2 ml of a 0.5% suspension of erythrocytes from grivet monkeys (*Cercopithecus aethiops*). Tests were read after cells had been allowed to settle at 37°C. The highest dilution of the material, which, after addition of cells, caused either complete or partial hemagglutination was considered to contain 1 HAU.

Hemagglutination-inhibition (HI) tests. Four HAU in a volume of 0.2 ml were added to 0.2 ml of each serial 2-fold dilution of serum. Red cells were added after incubation for 1 hour at room temperature. The HI titer of the serum was taken as the dilution factor before addition of antigen and of cells.

Determination of complement fixing (CF) antigen titers. The drop technic of Fulton

and Dumbell(8) as modified by Svedmyr (9) was used. Six units of amboceptor and 2 units of complement and of a human convalescent serum, respectively, were used in the antigen titration. The dilution of antigen, before addition of other reagents, in the reaction mixture showing no or only partial hemolysis was taken as the titer.

Sera. Paired human measles sera were obtained from the Hospital for Infectious Diseases of Stockholm. Acute serum was collected within 3-4 days after appearance of the rash and convalescent serum 7-14 days later.

Human measles sera were also kindly provided by Dr. Leon Rosen, Nat. Inst. of Health, Bethesda, Md. Two or 3 sera were taken from each of 10 children. One serum specimen was taken before exposure to measles had occurred and 1 or 2 specimens at varying times after a naturally acquired infection.

Specific hyperimmune sera were produced in rabbits and in guinea pigs, injected intramuscularly with a mixture of virus material and complete Freund's adjuvant. The animals were bled by heart puncture 14 days later. High titer sera were obtained by this procedure.

All sera were inactivated at 56°C for 30 min before use.

Experimental. Influence of variation of conditions on effect of Tween and ether treatment of hemagglutinin. Time and temperature of treatment. In principle the treatment consisted of incubation of the material with Tween 80 (L. Light & Co., Ltd., Colnbrook, England), followed by addition of ether ad narcosin. The two phases of treatment were performed with continuous shaking or magnetic stirring and different times of exposure up to 60 min were tested in each case. Tween was used in a final concentration of 0.125% and ether was added equivalent to half the volume of the material. Some tests were performed at room temperature and some in the cold, after incubation samples were centrifuged at 3000 rpm for 20 min. The aqueous phase was withdrawn and on some occasions a sample was also taken from the interphase. Residual ether was eliminated by cautiously bubbling nitrogen gas through the sample. Increase in HA-titer was taken as an indicator of the effectiveness of the treatment.

Results suggested that optimal conditions consisted in pretreatment of virus material with Tween for a few minutes either at room temperature or in the cold followed by treatment with ether for about 15 min in the cold. This technic gave the most reproducible results, but simultaneous addition of the two agents and only 5 min incubation very often were enough to effect an optimal increase in HA titer. There was no advantage in prolonging treatment beyond 15 min. Samples taken from the interphase and the aqueous phase exhibited the same titer, indicating that hemagglutinin did not accumulate in the interphase.

Concentrations of agents. The amount of ether seemed to be of no great importance within moderate ranges and therefore an amount equivalent to half the volume of the virus material was adopted for use in all tests. The effect of different concentrations of Tween alone, and in combination with ether was determined, employing the optimal conditions described above. Two tests were performed with each concentration of the reagents and mean values obtained are graphically shown in Fig. 1. As described earlier (6) Tween in high concentrations can bring about a conversion of a large into a smaller hemagglutinin and a slight increase in HA-titer can also be obtained. However a greater increase can be obtained with lower concentrations of Tween when the material is submitted to subsequent treatment with ether. Optimal final concentration of Tween under these circumstances is 0.125%. At and above this concentration of Tween recovery of the aqueous phase is technically simple, due to a jelly-like appearance of the organic solvent phase which sometimes forms a plug in the upper part of the tube.

Freezing and thawing. An incidental observation of increase in HA titer after freezing and thawing of material containing Tween justified a more quantitative study of this phenomenon. Results are also illustrated in Fig. 1. Samples were frozen and thawed twice and each point in the curve represents

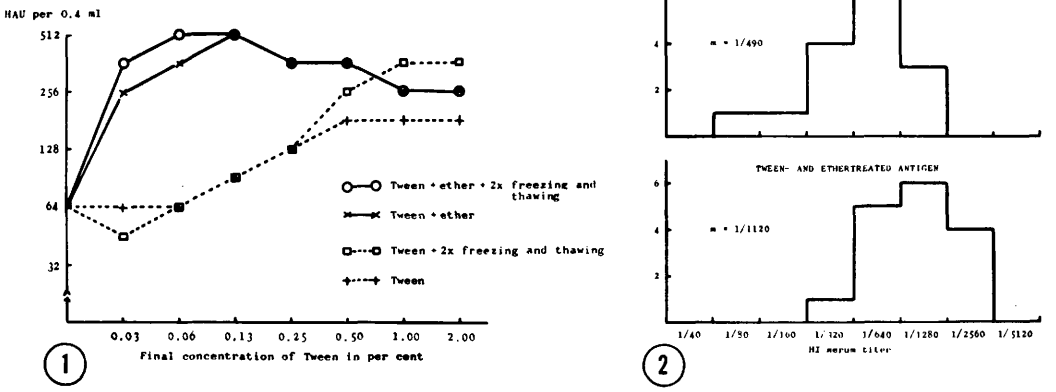

FIG. 1. HA titers of samples treated with Tween only or in combination with ether. One half of each sample was, in addition, frozen and thawed twice. Each point represents mean value of 2 samples.

FIG. 2. Distribution of HI serum titers of 16 measles convalescent sera by use of untreated and Tween and ether treated material, respectively, as antigens. m = geometrical mean HI titer.

the mean value of 2 samples. Increase in titers is most marked at high concentrations of Tween and also slightly apparent with material treated with low concentrations of Tween and with ether. However, it has been observed occasionally that Tween and ether treated material after storage in the frozen state for a long time has a tendency to become cloudy and exhibit diminished HA activity. Probably this is due to partial denaturation. Since HA titer remains unchanged for several months of storage in the cold there is no need to freeze the material.

Importance of different TC as source of virus material. As described above crude infective TC fluid from Lu 106 cells regularly reveals a 4- to 8-fold increase of titer after Tween and ether treatment when this is performed under optimal conditions. For comparison, virus material harvested from dog kidney TC was treated in the same way. With material from this source treatment seemed to be even more effective. Regularly 8- to 16-fold, and occasionally 32-fold increases in titer were obtained. HA titers of unconcentrated material as high as 1:4096 were recorded after treatment.

Uninfected TC materials treated with Tween and ether did not exhibit hemagglutinating activity.

Effect of Tween and ether treatment on different activities of measles virus. This is illustrated by results of 2 experiments summarized in Table I in which material concentrated by forced dialysis was used. Hemolyzing activity was determined only qualitatively. Evidently the treatment completely destroyed infectivity and hemolyzing activity. Furthermore a marked reduction in CF activity was obtained. In contrast a four-fold increase in HA titer was recorded.

Use of Tween and ether treated material as antigen in HI tests. HI tests were performed employing 4 HAU per serum dilution. There was a general tendency for serum titers both with hyperimmune sera and with convalescent sera to reach a 2-fold higher level when Tween and ether treated antigen was used. This is partly exemplified in Table II and in Fig. 2. The former gives examples of HI titers of sera taken from patients with clinically manifest measles and shows good correspondence of titers obtained with different antigens. Fig. 2 illustrates the distribution of HI titers of sera collected from children who had just passed through an attack of measles. Serum samples taken before the attack revealed no HI activity in dilution of 1:10 with either of the antigens.

It may be remarked that patterns formed

TABLE I. Effect of Tween and Ether Treatment on Activities of Measles Virus Preparations.

Activity measured	Unit of expression	Treatment of virus material: Exp I None	Exp I Tween and ether	Exp II None	Exp II Tween and ether
Infectivity	Log_{10} $TCID_{50}$/.1 ml	5.6	≦-.5	6.2	≦-.5
HA	HAU/.4 ml	512	2048	1024	4096
Hemolysis	Qualitative test	+	—	+	—
CF	CF antigen/.02 ml	8	1	8	2

by agglutinated RBC often were more clear-cut and easier to read with Tween and ether treated antigens as compared with crude tissue culture material.

Discussion. It was demonstrated(6) that ether treatment can bring about a degradation of virus particles, resulting in release of a free, smaller hemagglutinin. It was shown that pretreatment of virus material with Tween 80 had a protective effect during ether treatment with an increase in HA titers as result. The present paper describes the usefulness of such preparations as antigens in HI tests. The main advantage consists in the saving of TC hemagglutinating material, the activity of which is often of low order.

Increased sensitivity of the HI tests is another advantage, as for example when screening for HI antibodies in vaccinated persons. The HI test probably is the one of choice for such purposes since a good correlation has been demonstrated between measles neutralizing and HI antibodies(10) and as found in this laboratory. Serological responses to an influenza virus vaccine have been analyzed with good results by a similar technic in which ether treated virus material was used as antigen in HI tests(11).

TABLE II. HI Serum Titers of Sera Collected from Patients with Clinically Manifest Measles Infection: Comparison of untreated and Tween and ether treated materials as antigens.

Patient	Age (yr)	Time of collection of sera after appearance of rash (days)	HI serum titers with antigens: Untreated	Tween and ether treated
S.R. ♀	35	4	1:80	1:160
		18	1:640	1:1280
R.E. ♂	5	1	1:10	1:10
		9	1:640	1:1280
C.S. ♀	5	4	1:160	1:160
		16	1:640	1:1280
B.S. ♂	11	2	1:40	1:80
		10	1:1280	1:2560
M.N. ♂	12	2	1:20	1:40
		16	1:320	1:1280

If there is a correlation between antibody adsorbing sites and size of hemagglutinating particles one would expect to obtain higher HI serum titers with Tween and ether treated material. Thus, a smaller amount of antibody should be required to inhibit one HAU. This theoretical consideration is supported by the results obtained.

Freezing and thawing of material in the presence of relatively high concentrations of Tween also caused an impressive increase in hemagglutinating activity. However these preparations are not suited for serological purposes due to their hemolyzing activity.

Summary. A simple method for increasing hemagglutinating activity of measles virus tissue culture material is described. By treatment with Tween 80 and ether a 4- to 8-fold increase in titer was obtained with virus material grown in a human embryonic cell line (Lu 106) and an 8- to 32-fold increase when virus was grown in dog kidney TC. Maximal HA titers obtained with unconcentrated material were respectively 1:1024 and 1:4096 per 0.4 ml with the two different materials. Serum titers greater by a factor of 2 were obtained when Tween and ether treated material was substituted for untreated material as antigen in HI tests.

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