Comparison of Neutralization and Hemagglutination-Inhibition Techniques for Measuring Mumps Antibody. (32278)

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It is often of considerable importance to employ the most sensitive test possible to measure antibody following natural infection or vaccination against viruses. Norrby(1) showed that treatment of measles virus with Tween 80 and ether effected a 4- to 8-fold increase in titer of hemagglutinin, presumably due to release of hemagglutinating subunits of the virus. Use of such treated viral antigen in the hemagglutination-inhibition test effected a 2-fold increase in titer of antibody compared with the values obtained when whole virus was employed. Stewart and Douglas(2) applied the tween-ether treatment to mumps virus hemagglutinin. The development, in our laboratories, of the Jeryl Lynn strain (3-5) live attenuated mumps virus vaccine led to conduct of large-scale trials in man (6-9) and necessitated evaluation of procedures for measuring antibody responses to the vaccine. The present report describes the preparation of tween-ether treated mumps hemagglutinin and compares the findings in tests for antibody measured by the hemagglutination-inhibition test using untreated or tween-ether treated mumps hemagglutinating antigen with those obtained by the serum neutralization method.

Materials and methods. Patients' sera. Paired sera obtained prior to and one month following vaccination with B level (chick embryo passage 17) Jeryl Lynn strain (3-5) live attenuated mumps virus vaccine were selected at random from the collections of a previous study(6,7) carried out during 1965-66 in the Havertown-Springfield suburb of Philadelphia. The sera from patients with mumps were from the same study. All sera were stored frozen at —20°C in a deepfreeze from the time of collection until tested. *Hemagglutination-inhibition (HI) test.* The Barnes (B82) strain of mumps virus used in the HI tests was obtained from Dr. Klaus Hummeler and was passed in this laboratory in embryonated hens' eggs *via* the allantoic route. The HI tests were carried out by usual procedures employing as antigen either untreated allantoic fluid from embryonated hens' eggs infected with Barnes strain mumps virus or such material which had been treated further with Tween 80 and ether. To prepare the latter antigen, the mumps virus in the freshly harvested allantoic fluid was sedimented by centrifuging one hour at 18,000 rpm in the No. 21 rotor of the Model L Spinco centrifuge. The virus pellet was resuspended to 1/10 initial volume in phosphate buffered saline solution (pH 7.2) and transferred to a flask with magnetic stirrer. Tween 80 in 1.0% solution was slowly added to give a final concentration of 0.1% and stirred for 5 minutes after which time 1/2 volume of Merck anesthetic ether was added. After 5 hours continuous stirring at room temperature the aqueous phase was recovered and residual ether was removed *in vacuo*. Adult chicken erythrocytes were employed routinely in the hemagglutination and HI tests. All sera were inactivated at 56°C for 30 minutes prior to test and were absorbed with kaolin and chick erythrocytes to remove nonspecific inhibitors of hemagglutination. The hemagglutination titer was the highest initial dilution of antigen which effected hemagglutination of all red cells, and the HI titer was the greatest initial dilution of serum which caused total suppression of hemagglutination by 4 units of hemagglutinin in the tests. *Serum neutralization tests* were performed in chick embryo cell cultures employing Jeryl Lynn strain virus which had been passed a total of 7 times in embryonated hens' eggs and in chick embryo cell culture. The sera were inactivated at 56°C for 30 minutes prior to test and 30 to 100 $TCID_{50}$ of virus were employed as test dose. The tests were read for hemadsorption

TABLE I. Effect of Tween-Ether Treatment on Hemagglutination Titer of Concentrated* Barnes Strain Mumps Virus.

Antigen lot	Reciprocal of hemagglutination titer: Untreated	Tween-ether treated	Fold increase in titer
R1	320	640	2
C1	512	1024	2
C2	512	2048	4
C3	256	512	2

* Virus concentrated 10-fold, by centrifugation.

TABLE II. Stability of Tween-Ether Treated Mumps Virus Hemagglutinin on Storage.

Storage: Approximate period of storage	Storage: Temperature	Reciprocal of hemagglutinin titer: Lot No. R1	C1	C2
0*	—	640	1280	640
3 days	—20°C	320	640	640
3 "	—70°C	640	640	640
2 wk	4°C	640	—	—
1 mo	"	320	640	—
2 "	"	640	640	—
3 "	"	320	640	—
5 "	"	—	320	—
6.5 "	"	160	—	—

* Initial titer.

on the fifth day of incubation at 32°C and the titer was the highest initial dilution of serum in the tests in which there was total absence of hemadsorption.

Results. Preparation and storage stability of tween-ether treated antigen. Table I shows the effect of tween-ether treatment on the concentrated Barnes strain mumps virus. It is seen that the hemagglutination titer was increased 2- to 4-fold by tween-ether treatment in a series of 4 different preparations of antigen. The treated antigen, as seen in Table II, was stable on freezing at —20° or —70°C and showed only minor decline in titer when stored at 4°C in the ordinary refrigerator for periods up to 3 months*.

Test of sera from children who received live mumps virus vaccine. Table III presents the HI and serum neutralization test results with sera collected following vaccination from 12 children who were initially seronegative and who were selected to represent the total group. It is seen that the serum neutralization test was the most sensitive of the three, detecting mumps antibody in all sera in a titer of at least 1:2. The HI test employing tween-ether treated antigen was more sensitive than that using whole viral antigen. More sera gave positive HI results with the former antigen and the titers were always greater. This finding was confirmed in the tests shown in Table IV of sera from 55 children collected following vaccination and from 18 children convalescent from natural mumps.

A comparison of the post-vaccination serum neutralization and HI titers (using tween-ether treated antigen) of sera from 55 initially seronegative children is shown in Fig. 1. It is seen that the titers obtained by neutralization were usually greater than by HI and the difference in geometric mean titer was about 1.4-fold. The neutralization technique also gave higher titers than did the HI test in assays of convalescent sera from cases of mumps acquired in nature. As seen in Fig. 2, the neutralization titers were nearly always higher than obtained by the HI method and the mean-fold difference in titer was 1.7. The higher antibody titers found in the children following convalescence from natural mumps compared with vaccination were consistent with the findings reported earlier(6).

TABLE III. Selected Examples Showing Results Obtained When Various Procedures Were Used to Assay for Antibody Response to Mumps Virus Vaccine in Initially Seronegative Children.

Patient No.	Reciprocal of post-vaccination* antibody according to test procedure: Hemagglutination-inhibition: Whole virus antigen	Tween-ether antigen	Serum neutralization
259	<5	<5	2
236	<5	<5	4
141	<5	<5	8
373	<5	5	2
418	<5	5	4
448	<5	10	8
315	<5	5	16
378	5	10	4
4043	5	10	8
4095	5	10	16
4100	5	40	16
121	5	10	32

* 28 days following administration of vaccine.

* The Cl, 2 and 3 antigen lots were prepared and tested by Mr. G. Erie, Biologics Control Laboratories, Merck Sharp & Dohme.

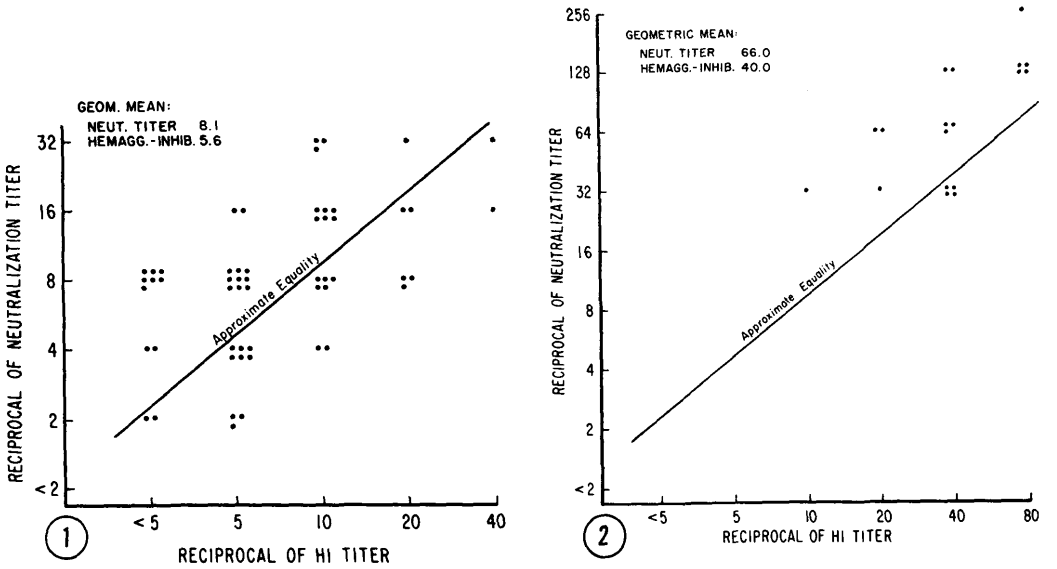

FIG. 1. Comparison of neutralizing and hemagglutination-inhibiting (tween-ether treated antigen) antibody titers in 55 initally seronegative children following live attenuated mumps virus vaccine.
FIG. 2. Comparison of neutralizing and hemagglutination-inhibiting (tween-ether treated antigen) antibody titers in 18 childern convalescent from natural mumps infection.

Discussion. The data revealed quite clearly the superiority of the HI test using tween-ether treated antigen compared with whole virus both in proportion of sera shown to contain antibody and in mean height of antibody titer. Myxoviruses, of which mumps virus is an example, are composed of a nucleoid surrounded by an envelope in which the hemagglutinins are located. Treatment with Tween 80 and ether is presumed to dissolve the lipid envelope, releasing the individual hemagglutinating units. The mass of free hemagglutinin required to bind 2 red blood cells and causing hemagglutination is likely considerably less than contained in a single virus particle and hence, appears to require less antibody to be inhibited. This would explain the higher antibody levels when the tween-ether treated antigen was used. The antigen was simple to prepare and could be used following storage for at least 6.5 months at 4°C.

TABLE IV. Findings in Children Who Were Vaccinated or Who Recovered from Natural Disease Showing the Greater Sensitivity of the Hemagglutination-Inhibition Test Using Tween-Ether Treated Antigen Compared with Whole Viral Antigen.

	No. of children with post-vaccination or convalescent serum titers according to test antigen used			
	Post-vaccination (55 children)		Convalescent from natural mumps (18 children)	
Reciprocal of HI titer	Whole virus antigen	Tween-ether antigen	Whole virus antigen	Tween-ether antigen
<5	30	11	0	0
5	25	20	1	0
10	0	16	6	1
20	0	6	6	3
40	0	2	5	9
80	0	0	0	5
Geometric mean	2.1	5.6	17.8	40.0

The serum neutralization test was considerably more sensitive than the HI test, even when tween-ether treated antigen was used. Such difference was of little importance in tests of sera from patients convalescent from mumps in whom the antibody levels were high. In sera from patients following vaccination, however, the antibody titers were sometimes low and the neutralization test was necessary to detect all the serologic responses. Further, the neutralization test was required to detect antibody in the sera of all persons who displayed antibody prior to vaccination.

Summary. Mumps hemagglutinating antigen treated with Tween 80 and ether showed

2- to 4-fold enhancement of hemagglutinin titer. The hemagglutinating activity of the treated antigen was stable on freezing and on storage for at least 3 months at 4°C. Sera from children following vaccination with Jeryl Lynn strain live mumps virus vaccine or following natural mumps infection nearly always gave higher antibody titers when the tween-ether treated antigen was used in the HI test than when whole virus antigen was employed. The serum neutralization test, however, was more sensitive than the HI technique and was required to detect antibody in specimens with low level of antibody. Comparative titer values of sera obtained by the various test procedures are presented.

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Serum Complement: Changes After Skin Grafting in Guinea Pigs.* (32279)

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The role of complement (C′) in the rejection of tissue or organ transplants is unclear. However, this group of heat labile serum proteins, interacting with specific antibody in the presence of calcium and magnesium ions, regularly participates in other immunological cytocidal reactions such as immune hemolysis(1) and the lysis of bacteria, protozoa, and tumor cells(2,3,4). Sequential attachment of C′ components to cell-membrane surfaces under these circumstances results in membrane injury and cellular death (5). Changes in the level of serum C′ may result from variations in the rates of either its synthesis or utilization. Such changes have been sought in a variety of experimental and clinical settings. Injection of guinea pigs with antigen-antibody complexes results in nearly complete removal of whole C′ from their serum for a period of several hours(6). A decrease in serum C′ activity accompanies anaphylactic shock in the same animal(7). The level of serum C′ in patients with certain "autoimmune" diseases, such as systemic lupus erythematosus and glomerulonephritis, is nearly always depressed during the acute phase of these illnesses(8,9,10). Such observations are usually interpreted as a reflection of C′ consumption during acute-phase immunological reactions.

Evidence for the participation of C′ in the rejection of tissue and organ transplants is mounting. Winn(11) has shown that C′ participates in the destruction of transplants

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