CRUDE TISSUE CULTURE ANTIGEN FOR DETERMINATION OF VARICELLA-ZOSTER COMPLEMENT FIXING ANTIBODY

Philip A. Brunell, M.D., and Helen L. Casey, Ph.D.

ALTHOUGH the antibody response to varicella-zoster (V-Z) infection may be measured by complement fixation (CF), this method has not been employed routinely because of the difficulty in preparing suitable antigen. Weller and Witton (*1*), Taylor-Robinson and Downie (*2*), and Caunt and associates (*3*) prepared antigen for the CF test by concentrating the media of V-Z-infected tissue cultures. The procedures described in these reports were tedious and frequently yielded antigens containing significant anticomplementary activity. In our hands only 4 of 19 antigens prepared from tissue culture fluids were satisfactory.

In the report of Weller and Witton (*1*), a single experiment was described in which CF antigen was prepared from monolayers rather than media from V-Z-infected tissue cultures. Subsequently, Caunt and associates (*3*) were unable to prepare antigen successfully by the same method.

Our procedure for the preparation of a crude tissue culture CF antigen, based on the single experiment of Weller and Witton (*1*), is relatively simple, and we show the results of testing human serums for V-Z complement fixation antibody with this antigen. The usefulness of this antigen is demonstrated in detecting individuals immune to chickenpox and in the laboratory confirmation of clinical cases of chickenpox and zoster.

Materials and Methods

Tissue culture. Monolayers were prepared with cells derived from the EL–1 strain of human fetal fibroblasts. This strain was isolated and subcultured as described by Hayflick and Moorhead (*4*). Eagle's medium as modified by Kissling (*5*), supplemented with 10 percent calf serum, was used for propagation of cells. This modified medium (*5*), containing only 2 percent calf serum, was used to replace the growth medium just before inoculation of cultures.

Virus. The Ellen strain was derived from varicella virus isolated from the vesicular fluid of a child with chickenpox (*6*). Serial passage of the virus was accomplished by trypsinizing an infected monolayer and then seeding a portion of the trypsinized cells in an established uninfected culture.

It was possible to store the trypsinized infected cells for 6 months at −63° C. without detectable loss of infectivity. For storage, approximately 10^6 cells per ml. were mixed in a final concentration of 40 percent calf serum and 10 percent glycerol.

Antigen preparation. Tissue cultures, propagated in 32-ounce prescription bottles, were inoculated with cells infected with the Ellen strain of virus. Cytopathic changes were confluent 5 to 7 days after inoculation. At this time veronal buffer (VB) was used to replace the tissue culture fluid (*7*); then the cell monolayers were scraped from the glass surface and ruptured in the VB by quick-freezing and thawing three times. The resulting mixture was centrifuged for 15 minutes at 480 g. The supernatant fluid was then removed and used as antigen. Control antigen was prepared simultaneously from uninoculated cultures. Each lot of antigen was tested in checkerboard fashion

Dr. Brunell is senior surgeon (R) of the Respirovirus Unit, and Dr. Casey is chief of the Immuno-Serology Unit, Virology Section, Laboratory Branch, Communicable Disease Center, Atlanta, Ga.

against a standard serum obtained from a patient during his convalescence from chickenpox. The optimal dilution of antigen determined in this way (*8*) was chosen to test serum specimens. Optimal dilutions for each lot of antigen are given in the table.

Serums. Paired serum specimens were obtained from 21 patients who had been clinically diagnosed as having chickenpox. Subsequent specimens were obtained from four of these patients. Serial serum specimens were taken from 13 additional patients at intervals up to 17 months after the onset of chickenpox; however, acute serums were not obtained from the 13 patients.

Serums were also collected from 23 normal adults, 11 patients clinically diagnosed as having zoster, and 11 susceptible children. The 11 children were either infants between 6 months and 2 years of age who were not known to have had chickenpox or children who became ill with chickenpox 2 weeks or more after the serums were obtained.

In addition, paired serum specimens were obtained from five patients with smallpox, five patients with herpes simplex, and four patients with Rocky Mountain spotted fever. Serums were tested from patients with Rocky Mountain spotted fever because patients with rickettsial pox were not available. Since the organisms causing the two diseases are antigenically related (*9*), this substitution is considered valid.

Complement fixation technique. The CF technique used is a micro adaptation of the diagnostic complement fixation method (*8*). The volumes of reagents utilized were: 0.025 ml. serum dilutions (*10*), 0.025 ml. antigen, 0.025 ml. 2.8 percent sensitized cells, and 0.05 ml. complement containing five 50 percent units.

Results. The results of antigen titrations against a standard serum are presented in the table. Only a single variation was noted in replicate titrations of antigen from the same lot. In this instance the stability of antigen lot No. 64–126 at 4° for 1 month was determined. As a result of the observed decline in titer, all antigens were subsequently stored at −20° C.

Before September 1963, antigen was prepared by disrupting the monolayer from a 32-ounce prescription bottle in 7 to 11 ml. of VB. These antigens had to be used undiluted. More concentrated antigens produced in 1964 were prepared with smaller amounts of VB. It is recommended that the cells from a 32-ounce culture be disrupted in no more than 4 ml. of VB. Only once in the last nine attempts to prepare the antigen lots listed in the table was the product unsatisfactory. In this case the condition of the cell monolayers was poor.

Replicate assays of the standard serum using the same or different lots of antigen showed identical antibody titers (table).

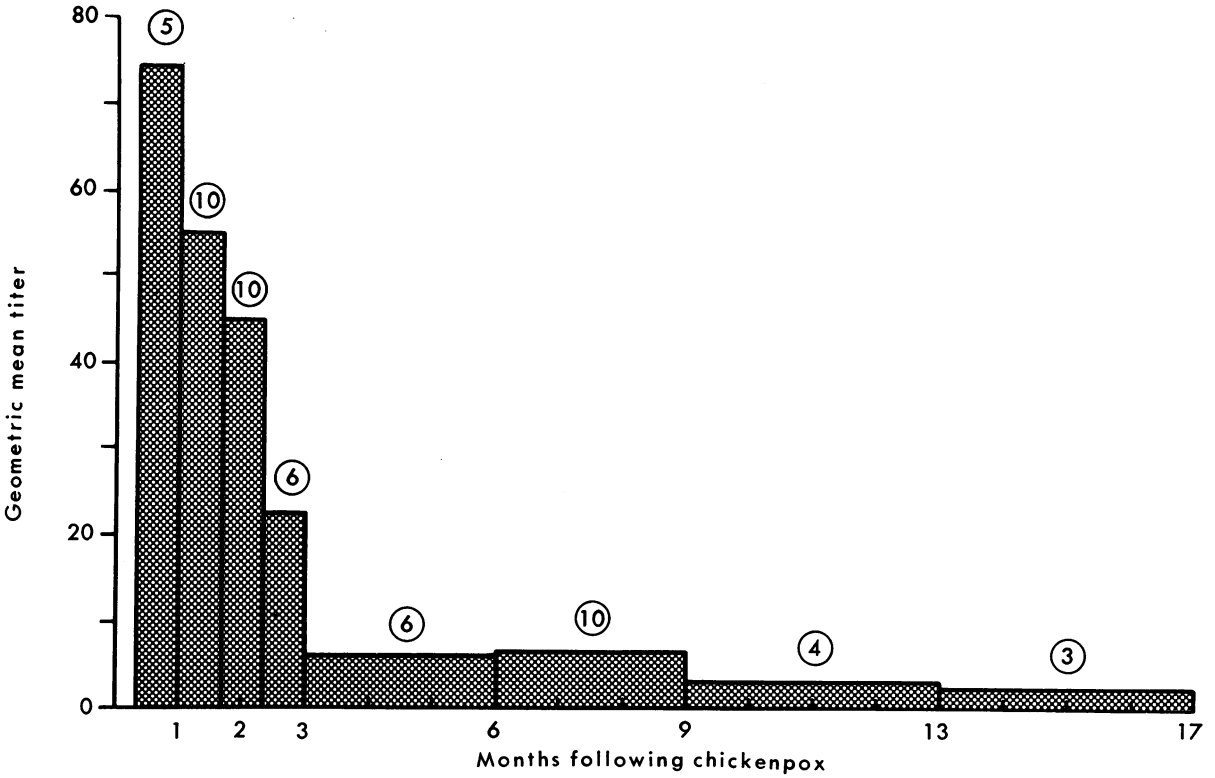
A fourfold or greater rise in V-Z antibody titer was detected in each of the 21 paired specimens of serums obtained from patients with cases of chickenpox. Antibody could not be detected at the 1:2 or 1:4 dilution in any of the serums obtained within 1 week of the appearance of the varicelliform rash. Antibody titers determined for the 17 patients from whom serial serum specimens were obtained are shown in the figure, with the decline in geometric mean titer during convalescence. Antibody could be detected at the 1:4 dilution of serums obtained from 16 to 23 normal adults. Most adult serums tested at this dilution fixed complement in the presence of control antigen. These reactions were not intense enough to interfere with

Titrations of V-Z antigen against a standard serum

Date of test	Antigen lot No.	Antigen optimal dilution used in test	Titer of serum
9–20–63	63–137	1:2	1:64
10–3–64	63–137	1:2	1:64
9–18–63	63–143	1:2	1:64
10–22–63	63–143	1:2	1:64
10–22–63	63–150	1:2	1:64
10–31–63	63–150	1:2	1:64
1–27–63	63–150	1:2	1:64
12–3–63	63–158	1:2	1:64
12–13–63	63–158	1:2	1:64
12–20–63	63–158	1:2	1:64
1–6–64	64–104	[1]	1:64
3–6–64	64–126	1:8	1:64
4–7–64	64–126	1:4	1:64
4–16–64	64–37	1:32	1:64
3–31–64	64–40	1:16	1:64
3–31–64	64–42	1:2	1:64
4–10–64	64–42	1:2	1:64

[1] Not detectable.

Serum antibody titers determined for 17 patients following cases of chickenpox



NOTE: Circled figures indicate number of observations during each time interval.

interpretation of the results with V-Z antigen but contraindicated testing at the 1:2 dilution.

Ten of the 11 convalescent serum specimens obtained from patients with zoster were found to contain V-Z antibody. The geometric mean titer of the 11 specimens was 112, which was higher than that obtained for convalescent serums of patients with chickenpox (see chart). The serum in which antibody could not be detected was obtained from a patient suffering from chronic lymphatic leukemia and receiving antimetabolite therapy when his zoster occurred.

No significant rises in antibody titer were observed when acute and convalescent serums from patients with herpes simplex, Rocky Mountain spotted fever, or smallpox were tested with V-Z antigen.

Discussion

CF antigen was prepared from cell monolayers of cultures infected with V-Z virus. Media from these cultures were frequently found to be anticomplementary. By removing the media and disrupting the infected cell monolayers in VB, the anticomplementary activity in the resulting crude antigen was virtually eliminated. The volume of VB in which the cells from each culture were disrupted determined the concentration of antigen. This method of preparation was less complicated and yielded satisfactory CF antigen more frequently than methods in which tissue culture fluids were concentrated to produce antigen (*1–3*).

Heterologous reactions were not observed when testing serums from patients with illnesses which occasionally are confused clinically with chickenpox or zoster. Serologic confirmation of V-Z infection was possible in all cases of chickenpox and in all but one case of zoster. Failure to detect antibody in the serum of this one case might have been predicted since it is recognized that patients with leukemia or lymphomas receiving antimetabolite therapy may have a deficient immune response to V-Z infection (*11*).

Demonstration of V-Z antibody in the serum of an individual may be accepted as evidence of his immunity to chickenpox since antibody was not found in the serums of susceptible children. On the other hand, failure to demonstrate antibody in serum does not prove susceptibility to chickenpox since the percentage of adults in which antibody could be detected was considerably less than the percentage of adults estimated by epidemiologic means to be immune (*12*). Moreover, antibody could not be detected in some serums obtained from children within a year following the onset of chickenpox.

REFERENCES

(*1*) Weller, T. H., and Witton, H. M.: The etiologic agents of varicella and herpes zoster: Serologic studies with the viruses as propagated in vitro. J Exp Med 108: 869–890, Dec. 1, 1958.

(*2*) Taylor-Robinson, D., and Downie, A. W.: Chickenpox and herpes zoster. I. Complement fixation studies. Brit J Exp Path 40: 398–409, August 1959.

(*3*) Caunt, A. E., Rondle, C. J. M., and Downie, A. W.: The soluble antigens of varicella-zoster virus produced in tissue culture. J Hyg (London) 59: 249–258, June 1961.

(*4*) Hayflick, L., and Moorhead, P. S.: The serial cultivation of human diploid cell strains. Exp Cell Res 25(3): 585–621 (1961).

(*5*) Kissling, R. E., and Reese, D. R.: Anti-rabies vaccine of tissue culture origin. J Immun 91: 362–368, September 1963.

(*6*) Brunell, P.: Transmission of chickenpox prior to the observed exanthem. To be published.

(*7*) Kabat, E. A., and Mayer, M. M.: Experimental immuno-chemistry. Ed. 2. Charles C Thomas, Springfield, Ill., 1961, p. 149.

(*8*) U.S. Public Health Service: Diagnostic complement fixation method (LBCF). Laboratory Branch, Communicable Disease Center, Atlanta, Ga., September 1962.

(*9*) Smadel. J. E.: Rickettsial diseases. *In* Diagnostic procedures for virus and rickettsial diseases. Ed. 2. American Public Health Association, New York City, 1956, pp. 513–552.

(*10*) Sever, J. L.: Application of a microtechnique to viral serological investigations. J Immun 88: 320–329, March 1962.

(*11*) Pinkel, D.: Chickenpox and leukemia. J Pediat 58: 729–737, May 1961.

(*12*) Ross, A. H.: Modification of chickenpox in family contacts by administration of gamma globulin. New Eng J Med 267: 369–376, Aug. 23, 1962.

Fish Killed by Water Pollution

Water pollution killed more than an estimated 7.8 million fish during 1963 in the United States, an increase of 750,000 over the estimate reported by the Public Health Service in 1962. More than 2,200 miles of river and more than 5,600 acres of lakes were involved. Industrial operations, the largest identified cause, accounted for almost 3.2 million dead fish; municipal sewage, for more than 1 million; and agricultural operations, for more than 760,000.

Data on three massive fish kills are included in the 1963 estimate ("Pollution-Caused Fish Kills 1963," PHS Publication No. 847, 1963 edition). In the Wahiawa Reservoir on Oahu Island in Hawaii, an estimated 2 million fish were killed; they were found dying in the vicinity of the sewage treatment plant, but exact cause of death was not proved. An accidental spill of lethal quantities of resin acid soaps from a paper company near Weldon, N.C., killed about 100,000 fish in a 100-mile stretch of the Roanoke River. Near Kelso, Mich., an accidental break in a hose dumped 4,000 gallons of diesel oil into the Coweeman River, killing an estimated total of 59,000 fish.

The number of fish that died in another heavy fish kill, on the lower Mississippi River in Louisiana, is not specified in the report. This kill destroyed more than 5 million fish between October 1963 and February 1964.

Eight States did not submit reports on fish kills; three States reported no known kills.