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Elimination of False-Positive Cytomegalovirus Immunoglobulin M-Fluorescent-Antibody Reactions with Immunoglobulin M Serum Fractions

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The cytomegalovirus fluorescent-antibody test for immunoglobulin M (IgM) antibody was found positive in seven of nine infants with congenital rubella infection, in addition to eight of eight infants with confirmed cytomegalovirus infection. When the test was repeated on IgM fractions of the same sera freed from IgG by ultracentrifugation, only negative reactions were observed in those from the rubella-infected infants, whereas IgM fractions from the cytomegalovirus-infected infants remained positive.

In congenital cytomegalic inclusion disease, Hanshaw considered that the most convenient and sensitive serological method of establishing a diagnosis was the demonstration of a positive cytomegalovirus (CMV) immunoglobulin M (IgM)-fluorescent-antibody (FA) reaction (1). The apparent advantage of this technique is that whole serum may be used without prior separation of IgM, and it is therefore suitable for application to screening programs. However, false-positive reactions have been reported when whole serum was used in other IgM-FA techniques. For example, in the diagnosis of congenital syphilis, Kaufman et al. (2) found a lack of specificity in the IgM fluorescent-treponemal-antibody test (FTA), and recently this was confirmed by Reimer et al. (5), who reported false-positive IgM-FTA reactions in infants with other congenital infections.

The purpose of this brief communication is to report our findings with the CMV IgM-FA test in the course of a routine screening program for congenital viral infections in infants under 1 year of age. When the CMV IgM-FA test (1) was performed on serum from eight infants known to have congenital CMV infection (confirmed by virus isolation) all were found to be positive. However, serum from seven of nine infants with congenital rubella infection (confirmed by virus isolation and the presence of rubella-specific IgM antibody) also gave a positive CMV IgM-FA reaction. When the tests were repeated on IgM fractions of sera freed from IgG by sucrose density ultracentrifugation (6), all of the fractions from the CMV-infected infants remained positive, whereas all of those from rubella-infected infants were negative.

The false-positive CMV IgM-FA tests are in keeping with the observations of Reimer et al. (5), who found false-positive IgM-FTA tests for syphilis in infants with other congenital infections. These workers showed that IgM antibody directed against maternal IgG, recognized as rheumatoid factor (RF), was present in the serum of most infants with congenital infections and that this antibody reacted with maternal IgG bound to the antigen used in the FTA test, resulting in fluorescence indistinguishable from direct reactions between IgM-specific antibody and antigen.

Sera from six of the seven rubella-infected infants with false-positive CMV IgM-FA tests in this study also gave a positive latex agglutination test for RF when tested by the method of Reimer et al. (5). Although RF could not be detected in the remaining CMV IgM-FA-positive rubella serum, it is possible that it was present at a concentration sufficient to give rise to a positive IgM-FA test.

Although six of the seven false-positive sera from rubella-infected infants contained CMV IgG antibody, as detected by the complement fixation test (1), serum from an infant with congenital rubella infection, which gave a positive CMV IgM-FA test with whole serum, did not contain any specific CMV antibody but did contain RF. Thus, an alternative explanation is required for the false-positive fluorescence in this patient. Keller et al. (3) showed that CMV-infected human fibroblasts produce IgG-Fc receptors on their surfaces. This finding was confirmed by Westmoreland et al. (7), who further demonstrated that IgG could bind to CMV-infected cells both specifically and nonspecifically; i.e., not only may anti-CMV IgG anti-

body combine via the Fab antigen combining site, but any IgG may bind to the CMV-infected cells via the Fc portion of the molecule. This latter nonspecific binding of IgG to CMV-infected fibroblasts could result in false-positive IgM fluorescence even in the absence of CMV-specific antibody.

Recently, Knez et al. (4), using a radioimmunoassay technique, found false-positive CMV IgM reactions in sera containing RF. They found that this problem could be overcome by removing IgG and RF from sera by absorption with *Staphylococcus aureus* protein A and insoluble IgG. The results of the present study show that separation of IgM from serum by sucrose density ultracentrifugation is an alternative means whereby false-positive CMV IgM antibody reactions may be eliminated in sera containing IgM RF activity.

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