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Structural Proteins of Human Cytomegalovirus[1]

RUDY L. EDWARDS

Community Blood Center of Greater Kansas City
4040 Main Street, Kansas City, Missouri 64111

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At the Community Blood Center our interests have not only been in studying the epidemiology of cytomegalovirus transfusion-associated infection, but we have also involved ourselves in the biochemical characterization of CMV proteins. I will present some of our laboratory's preliminary findings. We worked with extracellular virus isolated in essentially the same manner as Huang *et al.* (1) except that 20 to 60% sucrose gradients were used. Tissue culture fluid concentrated by Amicon ultrafiltration and run in 20 to 60% sucrose gradients resolved three bands designated top, middle, and bottom. These three bands have densities of 1.175, 1.190, and 1.246 g/cm^3, respectively.

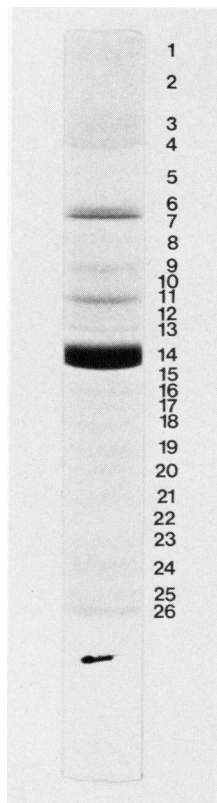

FIG. 1. Photograph of a 9% acrylamide gel stained with Coomassie Brilliant blue after electrophoresis of the dissociated proteins of sucrose-purified cytomegalovirus strain AD169.

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Electron microscopy (EM) of the top band revealed that it was largely contaminated with membrane vesicles, some homogeneous dense bodies, and viral particles of different states of maturity. The middle band showed a preponderance of completely enveloped viral particles and the presence of some dense bodies. The bottom band, when examined by EM, was comprised primarily of homogeneous dense bodies. It is of interest that Craighead *et al.* (2), using immunoelectronmicroscopy, showed serological cross-reaction between dense bodies and enveloped virions.

When the middle component from sucrose gradient was centrifuged to equilibrium in CsCl for 18 hr it was found to have a density of 1.210 g/cm^3, also in agreement with the findings of Huang *et al.* (1). On the basis of the electron microscopy and ultracentrifugation results we chose to use the middle band material for subsequent studies. Polyacrylamide gel electrophoresis (PAGE) of the proteins was carried out in a discontinuous system as described by Maizel *et al.* (3). Briefly, the middle band material was dissociated with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (2-ME) and subjected to PAGE in various acrylamide concentrations. Figure 1 is a 9% acrylamide gel and, as you can see, we have been able to detect 26 viral proteins. Viral protein 14 appears to be a major protein having an average molecular weight of 82,000 daltons. We have determined the molecular weights for the 26 proteins, and they range from 26,000 to 230,000 daltons.

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