SUMMARY

Adenovirus types 1-5,7 and 8 were grown in three cell lines of human epithelial origin (HeLa, KB and HEp2). Among these, HEp2 provided the best growth of the adenoviruses. Log titres with adenovirus types 3,5 and 8 were comparatively higher than the rest of four types.

The different adenoviral antigens, namely, the crude preparation, adsorbed viral preparation and the purified hexon antigen were resolved onto discontinuous SDS-PAGE and also tested by CIE using polyclonal antibodies to purified hexon antigen.

The hexon antigen could be successfully purified as SDS-PAGE revealed a single band at mol wt of 116 kDa in the purified preparation from among the five structural proteins as obtained in the adsorbed viral antigen preparation. There was no contamination of penton or fibre antigens. Thus as expected, the polyclonal antisera raised against this purified antigen preparation produced a single line of precipitin band when tested against purified, adsorbed or crude antigen preparations of adenoviruses. The group specificity of the hexon antigen appeared retained when tested by rabbit hyperimmune sera. The growth of three different enteroviruses in BGM was not neutralized by the rabbit hyperimmune sera, whereas all the adenoviruses were effectively neutralized. Therefore, this polyclonal antibody raised against the purified hexon preparation had its specificity restricted only to adenoviruses and this reagent could be utilized for identification of adenoviruses from CPE positive culture
supernatants of samples from ARD cases. This hyperimmune sera had a high titre, 1:1000 in NT and 1:64,000 in indirect ELISA. Similar to NT, this polyclonal antibody reacted in ELISA to purified hexon antigen and adsorbed viral antigen preparations but not to Polio, Coxsackie A 9 and ECHO type 4 viruses, thereby again reconfirming its specificity to adenoviruses.

Following immunization with the adsorbed adenovirus type 5 and selection of clones using purified hexon antigen, eight stable reactive clones were obtained. Of these monoclonal antibodies, ADV-1, ADV-3 and ADV-5 had high sensitivity for hexon antigen. There was a differential pattern in reactivity of these clones when tested with various adenovirus types. In dot ELISA as well as Western blotting, ADV-1 and ADV-3 reacted with all the adenoviruses tested. In Western blotting the reaction of both the clones was observed at the 116 kDa region (hexon antigen). ADV-3 in addition showed reactivity at 80 kDa region of the viruses, the region of pentone protein. Both the clones, therefore were group specific. ADV-5 showed a type specific activity, reacting only to adenovirus type 5 both in dot-ELISA and the Western blotting. It could have utility as a typing reagent for adenoviruses. The epitope for this monoclonal antibody might be in the Epsilon portion of Hexon antigen, the established region of type specificity. All the three clones did not react to any of the enteroviruses tested.

The rabbit polyclonal antisera to purified hexon antigen and the specific monoclonal antibodies to adenoviruses were utilized for the development of rapid and sensitive immunoassays for the detection of adenoviruses from clinical cases of ARD in children.
The coagglutination test with colored *Staphylococcus aureus* cells specifically detected all adenoviral isolates, standared adenovirus type 5 and remained negative with the control enteroviruses. Results with this test are obtained within five minutes. This test was applied on culture supernatant of 31 CPE positive samples from the patients of ARD and compared with conventional NT. The test had a 100 per cent specificity for adenoviral identification and a sensitivity of 75 per cent. The latex agglutination test showed identical results to those obtained with coagglutination test.

Detection of adenoviruses directly from clinical samples of throat swab was tried using coagglutination test. Results obtained were comparable to NT. This test, therefore, appeared reliable, rapid and simple. The stability of the sensitized coagglutination reagent was found to be over nine months at 4°C. The test holds promise as an appropriate system for clinical use.

In sandwich ELISA, 54 samples from the clinical cases were tested. A high degree of correlation of ELISA was observed with viral isolation and NT. The sandwich plate ELISA could be further simplified to a convenient dot ELISA modality with nearly identical results. Total time taken to perform the dot ELISA was three to four hrs.

Antibody detection from cases of ARD in children was undertaken by dot ELISA using tissue culture antigens of adenovirus types, 3, 4, 5, 7 and 8. When compared with virus NT, a good correlation was observed between the two test systems.
Demonstration of antibodies in children, therefore, appeared to be a reliable indicator of acute adenoviral respiratory syndrome. Antibody testing in adult cases with febrile respiratory illness revealed a high incidence of adenoviral infections.