5. Cannon, P. J., Ames, R. P., Laragh, J. H., *J.A.M.A.*, 1963, v185, 854.

6. Jick, H., Snyder, J. G., Finkelstein, E. M., Cohen, J. L., Moore, E. W., Morrison, R. S., *J. Clin. Invest.*, 1963, v42, 1561.

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Relationship of the Adenovirus Erythrocyte-Receptor-Modifying Factor to the Type-Specific Complement-Fixing Antigen. (29145)

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Infection of tissue cultures with adenovirus types 1, 2, 4, 15 and possibly type 5 results in formation of a factor demonstrable by its capacity to reduce the agglutinability of human O erythrocytes for 3 of the adenovirus serotypes which agglutinate these cells (1,2). This substance, termed the "erythrocyte-receptor-modifying factor," was distinguishable physically, serologically, or biologically from the infectious particles, the soluble group complement-fixing antigen(3, 4,5) and the cell-detaching factor(6). Since the erythrocyte-receptor-modifying factor was neutralized type-specifically, experiments were done to determine its possible relationship to the soluble type-specific complement-fixing antigen(4,5). This report describes results of these experiments.

Materials and methods. Virus preparations. Stock virus pools were prepared from prototype strains of adenovirus types 1 and 2. These strains, which had been previously propagated in cultures with medium containing 5% chicken serum, were passaged an additional 5 times (0.1 ml of 1:100 dilution) in HeLa tube cultures* with Eagle's basal medium(7). Following the final passage, cultures were frozen and thawed 5 times, pooled, centrifuged for 10 minutes at 2000 rpm and the supernatant fluid stored at —70°C until used.

Virus suspensions were reduced 100-fold in volume with polyvinylpyrrolidone (PVP)† by the method described by Kohn(8). This procedure was carried out at room temperature. Prior to use, suspensions were dialyzed overnight at 4°C against 0.01 M phosphate buffer solution pH 7.2.

Antisera preparation. Serum-free adenovirus suspensions prepared with either primary rabbit kidney or hamster kidney cultures were used to prepare antisera in rabbits as previously described(3).

Chromatography procedure. Fractionation of adenovirus suspensions was done by column chromatography with DEAE-cellulose‡ at room temperature, essentially using the procedure described by Wilcox and Ginsberg (5). Following addition of a suitable volume of concentrated and dialyzed adenovirus suspension to a DEAE column which had been previously equilibrated with 0.01 M phosphate buffer pH 7.2, fractions were collected by stepwise elution. Twenty-five ml of 0.01 M phosphate buffer solution pH 7.2 (0.0 M NaCl) and buffer solutions containing increasing concentrations of NaCl were added to the column and the effluents collected separately under conditions of free-flow. Each fraction was reduced in volume 10-fold by dialysis against PVP.

Assay of eluates. Each eluate was assayed for biologic activity, and presence of complement-fixing antigens against rabbit antiserum prepared with a crude type 1 suspension and human antiserum from volunteers experimentally infected with adenovirus type 26. The tests for demonstrating erythrocyte-receptor-modifying (ERM) activity and inhibition

* Tissue cultures were obtained from Microbiological Associates, Inc., Bethesda, Md., and Flow Laboratories, Rockville, Md.

† General Aniline and Film Corp., Linden, N. J.

‡ Eastman Organic Chemicals, Rochester, N. Y.

were done as previously described(2). The test for determining cell-detaching (CD) activity was done as previously reported(6).

Titrations for infectivity were done by inoculating 1 ml of serial 10-fold virus dilutions into each of 2 primary human embryonic kidney cultures. The maintenance medium used was that described previously(2); cultures were refed once weekly with 1.5 ml of medium. After a 28-day incubation period at 36.5°C in a horizontal stationary position, the endpoints of the titrations were determined by the method of Reed and Muench (9).

Complement-fixation (CF) tests were done as described by Rowe *et al*(3). Eluates were tested against 8 units of antibody.

The agar-gel diffusion test used was a modification of the double diffusion technique of Ouchterlony(10). A 2-inch square plate was overlaid with 1.2% Noble agar in physiologic saline. Wells were 3 mm wide and 4 mm deep with 7 mm spacing between wells. Plates were kept at room temperature and read after 48 hours.

Results. Since the results of repeated fractionations of adenovirus type 1 and type 2 suspensions were similar, data will be presented only for a representative type 1 chromatography experiment.

The elution characteristics of the soluble antigens and infectious virus are presented in Fig. 1. The biologic analyses of the eluates (Fig. 1a) demonstrated the distinctness of the ERM factor from the CD factor and infectious virus. In all experiments the bulk of the ERM factor was always recovered in the first effluent, whereas the CD factor was maximally eluted at either 0.075 or 0.1 M NaCl. The eluate in which the bulk of the ERM factor was detected also contained a CF antigen which was demonstrable in tests with type 1 rabbit antiserum but not in tests with human serum (Fig. 1b). The results of CF tests (not shown) with type 2 rabbit antiserum were similar to those shown for human serum. It should be noted that material recovered after loading of the column with the virus sample, *i.e.*, prior to addition of the first buffer, was completely negative in all assays.

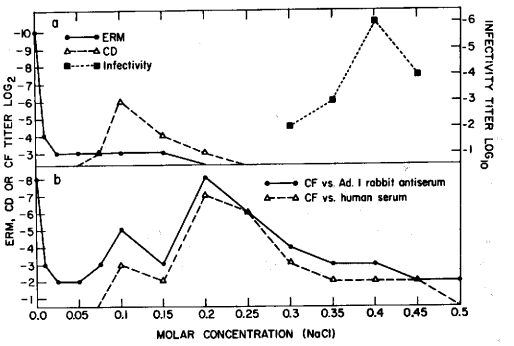

FIG. 1. Biologic and serologic analyses of eluates obtained from fractionation of adenovirus type 1 suspension by DEAE-cellulose chromatography. Fig. 1a shows results of biologic assays of collected eluates; Fig. 1b, results of serologic analyses of these same effluents.

The elution characteristics of adenovirus soluble antigens and infectious virus as well as the quantitative recovery of these antigens were essentially in agreement to those previously reported(5). Recovery of the ERM factor never exceeded 35% of the starting material.

To further purify the ERM factor, the buffer eluate (0.0 M NaCl in Fig. 1) was rechromatographed under the same conditions. The ERM factor and type-specific CF antigen were again found in the initial eluate in titers essentially the same as those obtained in the first fractionation run.

The material which had been rechromatographed was used in ERM inhibition tests with rabbit antiserum prepared with types 1, 2, 4, and 15. The ERM activity was inhibited only with homologous antiserum.

The twice chromatographed eluate and unfractionated type 1 and type 2 suspensions were analyzed in agar-gel double diffusion tests with type 1 rabbit antiserum. The results of this test are illustrated in Fig. 2. The reaction of the fraction which contained the ERM factor and type-specific antigen (0.0 M eluate) with type 1 rabbit antiserum gave a single precipitation line which was continuous with one of the lines produced with the unfractionated type 1 suspension. The absence of this precipitation line in the reaction between unfractionated type 2 material and type 1 antiserum indicated the type-specificity of this antigen.

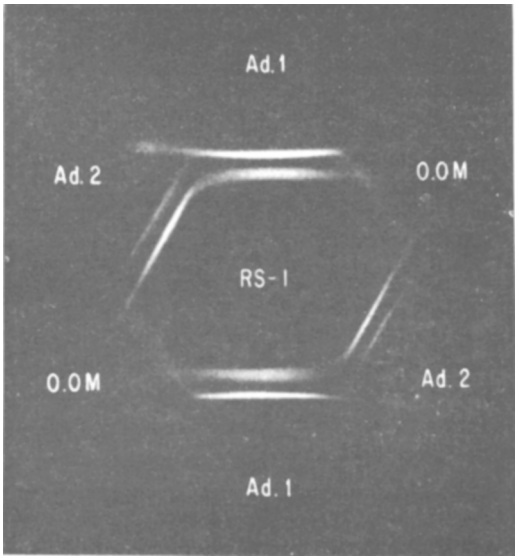

FIG. 2. Precipitation bands obtained by agar-gel double diffusion technique. Comparison of 0.0 M eluate with unfractionated type 1 and type 2 suspensions concentrated 50 times with PVP. Center well contains type 1 rabbit antiserum prepared with unfractionated type 1 suspension.

Discussion. Three lines of evidence indicate that the type-specific CF antigen of types 1 and 2 is the carrier of ERM activity: the similarity in elution characteristics of type-specific antigen and ERM factor by chromatography with DEAE-cellulose; the formation of a single precipitation line in agar-gel double diffusion tests when purified material possessing both activities is reacted with homologous antiserum; and the type-specific serologic reactions of both antigens.

Since type-specific CF antigens have been demonstrated with a number of adenovirus types, the question arises as to why ERM activity is demonstrable only with a few. It is possible that the ERM factor is a functional moiety of the type-specific antigen which is more labile with some types than others, or that other conditions are necessary to demonstrate its activity.

Although the type-specific antigen is a structural subunit which is incorporated in the virion(11), its role in the mechanism of virus infection is not known. It is tempting to speculate that the ERM enzymatic activity, which is active on human cells, may play a role in initiation of infection. A possible functional activity of the type-specific antigen may be of significance in view of the continued production of this antigen in adenovirus 12- and 18-induced tumors(12).

Summary. The similarity in elution characteristics of the ERM factor and type-specific CF antigen following fractionation of adenovirus type 1 and type 2 suspensions by chromatography with DEAE-cellulose; the formation of only a single precipitation line in agar-gel double diffusion tests with purified material containing both activities; and the type-specific serologic reaction of both antigens indicated that the type-specific CF antigen was the carrier of the ERM factor.

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1. Kasel, J. A., Rowe, W. P., Nemes, J. L., *Virology,* 1960, v10, 388.

2. ———, *J. Exp. Med.,* 1961, v114, 717.

3. Rowe, W. P., Huebner, R. J., Hartley, J. W., Ward, T. G., Parrot, R. H., *Am. J. Hyg.,* 1955, v61, 197.

4. Klemperer, H. G., Pereira, H. G., *Virology,* 1959, v9, 536.

5. Wilcox, W., Ginsberg, H. S., *Proc. Nat. Acad. Sci. U. S.,* 1961. v47, 512.

6. Rowe, W. P., Hartley, J. W., Roizman, B., Levy, H. B., *J. Exp. Med.,* 1958, v108, 713.

7. Eagle, H., *Science,* 1955, v122, 501.

8. Kohn, J., *Nature,* 1959, v183, 1055.

9. Reed, L. J., Muench, H., *Am. J. Hyg.,* 1938, v27, 493.

10. Ouchterlony, O., *Ark. Kemi., Mineral., Geol.,* 1949, 26B, 1.

11. Wilcox, W. C., Ginsberg, H. S., Anderson, T. F., *J. Exp. Med.,* 1963, v118, 307.

12. Huebner, R. J., Pereira, H. G., Allison, A. C., Hollinshead, A. C., Turner, H. C., *Proc. Nat. Acad. Sci. U. S.,* 1964, v51, 432.

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