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Demonstration of Rubella Complement-Fixing Antigens of Two Distinct Particle Sizes by Gel Filtration on Sephadex G-200.* (31596)

NATHALIE J. SCHMIDT, EDWIN H. LENNETTE, AND PINKIE S. GEE
Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley

Previous studies on rubella complement-fixing (CF) antigens derived from the fluid phases of infected RK-13 rabbit kidney cells (1) and the GMK-AH 1 line of green monkey kidney cells(2) have shown virtually all of the CF activity to be "soluble" in nature, *i.e.*, separable from the infectious viral particle by high-speed centrifugation(1) or by filtration(2).

Recently we have found(3) that high-titered rubella CF antigens can be prepared from the fluid phase of infected BHK-21 hamster kidney cultures, and using such higher-titered preparations it has been possible to demonstrate, by gel filtration on Sephadex G-200, the occurrence of two distinct CF antigens. One of the antigens is associated with the infectious viral particle and is sedimentable by centrifugation at 80,000 $\times$ *g* for 3 hours, and the other is smaller than the infectious particle and is not sedimented under these conditions of centrifugation. This report describes the separation of the two antigens.

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Materials and methods. Rubella virus preparations. Rubella virus (RV strain†) was propagated in cultures of the BHK-21 line of hamster kidney cells as recently described(3). Fluids from infected cultures were clarified by centrifugation at 1500 rpm for 15 minutes and then concentrated 100-fold by dialysis against polyethylene glycol (Carbowax 20 M‡). For certain experiments the fluid concentrates were treated with either(3) prior to Sephadex gel filtration.

Complement fixation tests. Materials were assayed for rubella CF antigen activity in block titrations against known positive human sera using our standard procedure adapted to use in the microtiter system(4).

Infectivity titrations. Infectivity titrations were performed in tube cultures of the BS-C-1 line of grivet monkey kidney cells. After 7 days incubation at 36°C inoculated cultures were challenged with 100 TCD_{50} of echovirus type 11, and results were read 2 to 3 days later. The presence of rubella virus in the

† Obtained from Drs. J. L. Sever and G. M. Schiff, Nat. Inst. for Neurological Diseases and Blindness, NIH, Bethesda, Md.

‡ Carbide and Carbon Chemicals Co., Union Carbide & Carbon Corp., New York.

cultures was evidenced by their resistance to the cytopathic effect of the challenge echovirus, *i.e.*, by interference. Titers were expressed in terms of the number of 50% interfering doses (InD_{50}) of rubella virus in 0.1 ml of test material.

Gel filtration on Sephadex G-200. A 2.5 × 40 cm column of Sephadex G-200 was prepared and equilibrated with 0.2 M NaCl in 0.1 M tris (hydroxymethyl) aminomethane buffer, pH 8.0. A 3 ml sample was applied to the column and eluted with the tris buffer. The flow rate was 15 to 20 ml per hour, and fractions were collected in 5 ml volumes.

Estimation of protein concentration. The protein concentration of the various fractions eluted from the Sephadex G-200 columns was estimated by measuring absorbence at 280 mμ in a Beckman DK-2 spectrophotometer.

Results. Elution patterns of rubella CF activity and infectivity from Sephadex G-200. Fig. 1 shows the elution patterns in representative experiments in which fluid concentrates from rubella-infected BHK-21 cultures were subjected to gel filtration on Sephadex G-200. The first preparation was an untreated, infectious concentrate, and the second was an ether-treated preparation.

Protein elution patterns for both of the preparations were the same, but less protein was present in the ether-treated preparation. It is likely that the serum present in the concentrates accounted for much of the protein; cultures were maintained on 2 percent fetal bovine serum, and the fluids were concentrated 100-fold.

In both preparations CF antigen was demonstrable in several fractions coming off the column just after the void volume was eluted (in the region of fractions 11-16) as well as in several fractions coming off later (in the region of fractions 21-28), indicating that CF activity was attributable to particles of 2 different sizes. With the first preparation recovery of CF activity was complete; a total of 120 units of CF antigen was applied to the column and 120 units were recovered—50 in the early fractions containing larger sized particles and 70 in the later fractions containing smaller sized particles. In the case of the ether-treated preparation, 60 units of CF antigen were put onto the column and 45 were recovered, 25 in the first fractions and 20 in the later fractions.

In the first preparation infectious virus was seen to elute shortly after the void volume, along with larger-sized CF antigen. The bulk of the second CF antigen was present in fractions showing no infectivity. Approximately 60 per cent of the infectious virus applied to the column was recovered in the eluates.

Sedimentation properties of rubella CF antigens eluted from Sephadex G-200 columns. Fraction 12 and fraction 25 from gel filtration of the infectious preparation, representing large-particle and small-particle CF antigens respectively, were subjected to high-speed centrifugation at 80,000 × *g* for 3 hours, after which the supernatant fluids and pellets (resuspended to the original volume) were examined for CF activity and infectivity.

Since the CF titers of eluates from the ether-treated preparation were low, fractions 13, 14 and 15 were pooled, as were fractions 25, 26 and 27. Both pools were concentrated 5-fold by dialysis against Aquacide I,§ and the concentrates were subjected to high-speed centrifugation as described above.

Table I shows the results of these centrifugation experiments, and it is seen that with fraction 12 of the infectious preparation all of the CF activity recovered was in the pellet, and almost all of the infectivity was recovered in the pellet. On the other hand, in fraction 25, which had no demonstrable infectivity, the CF activity remained in the supernatant fluid after high-speed centrifugation. Similar results were obtained with the ether-treated preparation; the CF antigen in the early fractions was sedimentable, while that in the later fractions was not.

Sedimentation of rubella CF antigen in fluid concentrates. Sephadex gel filtration was found to be a more efficient method for separation of the large- and small-particle antigens than was direct high-speed centrifugation of fluid concentrates (Table II). The first preparation (Lot 239) was a portion of the same infectious fluid concentrate for which gel

§ Calbiochem, Los Angeles, Calif.

filtration elution patterns are shown in Fig. 1, and it is seen that while a fairly large proportion of the CF activity was recovered in the early Sephadex eluates and was sedimentable by centrifugation at 80,000 × *g* for 3 hours, only a small portion of the total CF activity of the concentrate was recovered in the pellet upon direct high-speed centrifugation. In another infectious preparation only a trace of CF activity was recovered in the pellet, and in the ether-treated concentrate no sedimentable CF activity was demonstrable. Density gradient centrifugation should yield information as to the homogeneity of large- and small-particle rubella CF antigens.

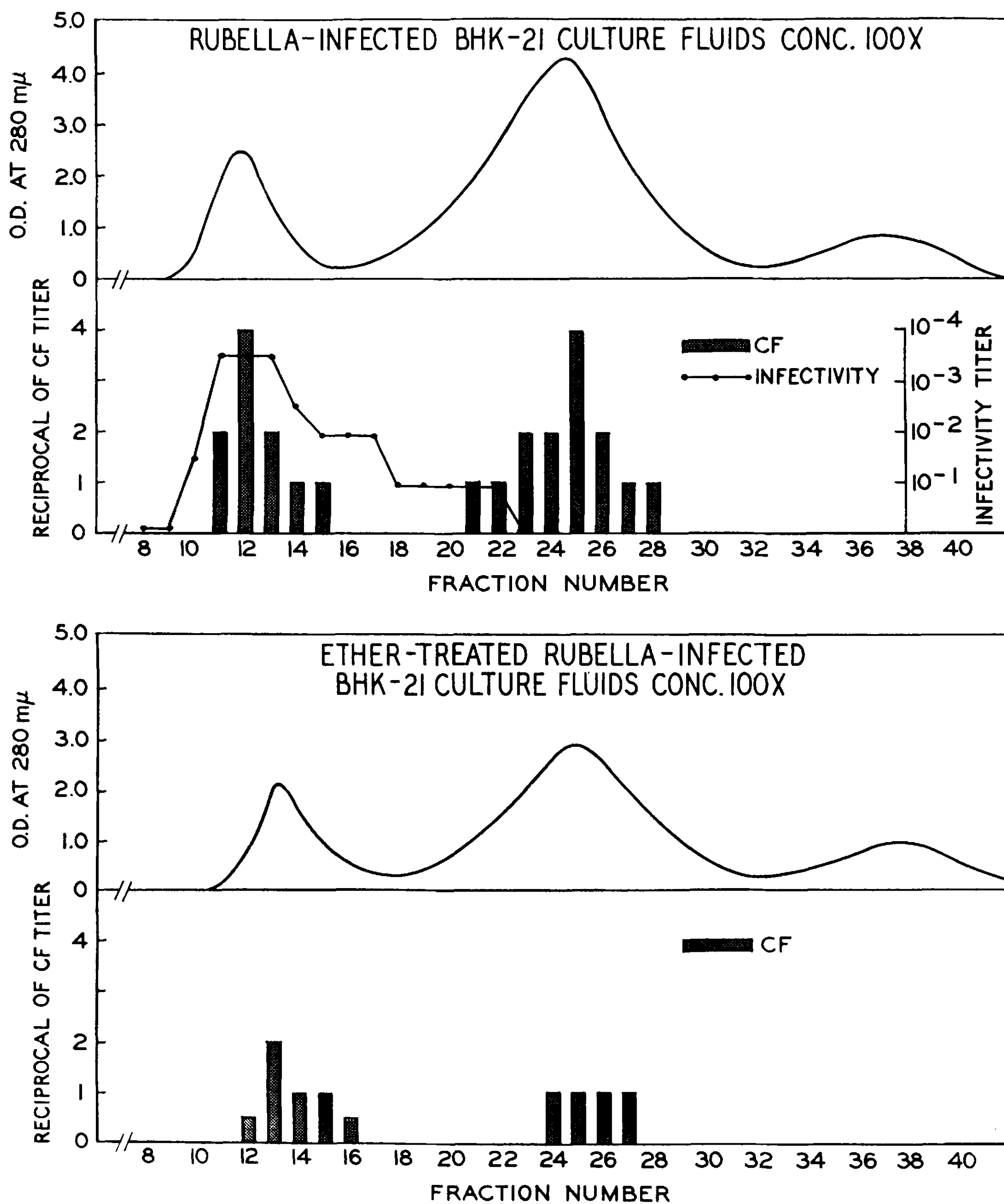

FIG. 1. Elution patterns of rubella CF antigen and infectivity on Sephadex G-200.

TABLE I. Sedimentation Properties of Rubella CF Antigens Obtained by Gel Filtration on Sephadex G-200.

Material tested	CF titer	Infectivity titer
Infectious concentrate		
Sephadex fraction 12	1:4	$10^{-3.5}$
Centrifuged 80,000 × g 3 hr		
Supernatant fluid	<1:1	$10^{-1.0}$
Resuspended pellet	1:2	$10^{-3.0}$
Sephadex fraction 25	1:4	$<10^{-1.0}$
Centrifuged 80,000 × g 3 hr		
Supernatant fluid	1:2	$<10^{-0}$
Resuspended pellet	<1:1	$<10^{-0}$
Ether-treated concentrate		
Sephadex fractions 13, 14, 15, conc 5×	1:4	—
Centrifuged 80,000 × g 3 hr		
Supernatant fluid	<1:1	—
Resuspended pellet	1:4	—
Sephadex fractions 25, 26, 27, conc 5×	1:4	—
Centrifuged 80,000 × g 3 hr		
Supernatant fluid	1:4	—
Resuspended pellet	<1:1	—

Discussion. These studies clearly demonstrate that rubella CF antigens of 2 particle sizes are produced in infected BHK-21 cell cultures. The larger particles elute from the Sephadex column along with infectious virus, and when these eluates are centrifuged at 80,000 × g for 3 hours, the CF activity is sedimented along with the infectivity. The second antigen elutes from the column after the infectious virus, and does not sediment under the same conditions of centrifugation. It is likely that the large-particle antigen is the viral particle, either infectious or non-infectious, while the smaller antigen may represent a subunit or an "early antigen." Studies on the temporal appearance of these 2 antigens in infected cell cultures would be of interest, as would studies on the antigenic properties of the two antigens.

Both large- and small-particle CF antigens have been demonstrated in ether-treated preparations, and in roughly the same proportion (although not at the same concentration) as are demonstrable in infectious preparations. This suggests that ether-treatment did not degrade large-particle antigen into small-particle antigen.

Veronelli and coworkers(2,5) have also found infectious rubella virus to elute from Sephadex G-200 columns shortly after the void volume, but all of the CF activity in their preparations came off the column later than the infectious virus(2). These investigators used antigens derived from continuous monkey kidney cell lines, and it is of interest that in this laboratory when antigen derived from the RK-13 line of rabbit kidney cells was subjected to gel filtration on Sephadex G-200, CF activity was demonstrable only in concentrates of fractions coming off the column in the region of small-particle antigens. The use of higher-titered preparations derived from infected BHK-21 cells may account for the ability to demonstrate large-particle size CF antigen, or possibly varying proportions of the 2 types of antigen may be produced in different host cell systems.

Summary. By means of gel filtration on Sephadex G-200, rubella complement-fixing antigens of two distinct particle sizes have been demonstrated in concentrates of fluids from infected BHK-21 cultures. The large-particle antigen elutes from Sephadex columns together with infectious virus, shortly after the void volume is eluted, and is sedimented by centrifugation at 80,000 × g for 3 hours

TABLE II. Attempts to Sediment Rubella CF Antigen by Direct High-Speed Centrifugation of Fluid Concentrates.

Material tested	CF titer	Infectivity titer
Lot 239, infectious fluids concentrated 100×, clarified at 1500 rpm 15 min	1:40	$10^{-4.5}$
Centrifuged 80,000 × g 3 hr		
Supernatant fluid	1:32	$10^{-1.0}$
Resuspended pellet	1:8	$10^{-3.5}$
Lot 233, infectious fluids concentrated 100×, clarified at 1500 rpm 15 min	1:32	$10^{-4.0}$
Centrifuged 80,000 × g 3 hr		
Supernatant fluid	1:20	$10^{-1.0}$
Resuspended pellet	1:2	$10^{-3.5}$
Lot 225, ether-treated fluids concentrated 100×, clarified at 1500 rpm 15 min	1:20	—
Supernatant fluid	1:16	—
Resuspended pellet	<1:2	—

together with the infectious virus. The small-particle antigen elutes after the infectious virus and is not sedimented under conditions of centrifugation which sediment the infectious virus. Sephadex gel filtration was shown to be more effective than direct centrifugation of rubella-infected fluids in separating the two different antigens.

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Some Central Nervous System Actions of Beta-Mercaptoethylamine. (31597)

A. J. East, R. L. Mundy,* M. H. Heiffer, G. E. Demaree, E. H. Herman, and D. P. Jacobus

School of Pharmacy, Northeast Louisiana State College, Monroe, La. and Department of Pharmacology, Walter Reed Army Institute of Research, Washington, D.C.

The cardiovascular actions of β-mercaptoethylamine (MEA), a radio-protectant compound, have received considerable attention (1-4). However, little is known concerning its action on the central nervous system (CNS), although investigators have noted convulsions and tremors in animals treated with it. Recently Benigo and Palazzoadrino have reported that MEA synergizes with barbiturates in a specific manner to prolong their central action without depressing vital centers (5). On the other hand, Yam and co-workers have reported that pentobarbital is an excellent antidote against the early, excitant toxicity of MEA(6).

The purpose of this preliminary report is to explore the pattern of CNS activity of MEA by combining a CNS stimulant or a depressant with it. Specifically, interactions of MEA with pentylenetetrazole and pentobarbital are reported.

Methods. Walter Reed, Bagg-Swiss, male mice[†] weighing between 21-30 g were used. Animals had access to food and water at all times prior to the experiment. Ten mice were selected randomly from colony cages for each experiment group. Each group was maintained in a separate cage during the experiment.

β-mercaptoethylamine HCl was dissolved in distilled water (23.4 mg/ml) and injected intraperitoneally (150 mg/kg of the base). Pentylenetetrazole solution (60 mg/kg) and pentobarbital sodium solution (130 mg/kg) were prepared in a dilution such that the volume for each injection was 9.6 ml/kg, and injected intraperitoneally. Pentylenetetrazole was injected at 60, 30, and 15 minutes before MEA, simultaneously with MEA, and at 15, 30, and 60 minutes after MEA.[‡] Pentobarbital was injected according to the same schedule. In control experiments 0.9% sodium chloride solution was substituted for MEA. Mortality was recorded at 24 hours after the injections.

Results. Table I shows results obtained with combinations of pentylenetetrazole and MEA and each agent with saline. The MEA and pentylenetetrazole doses were non-lethal. When the pentylenetetrazole was adminis-

* Present address: Dept. of Pharmacology, University of Alabama Medical Center, Birmingham.

† The mice were supplied by Walter Reed Army Inst. of Research, shipped by air express to our laboratory and allowed to become accustomed to their new surroundings 5-7 days before use in an experiment.

‡ Additional observations made on pentylenetetrazole administered 120 min after MEA.