Reactions with Hemagglutinin of Psittacosis Agent in Gel Diffusion[1] (35102)

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Hemagglutinin production has been described for a number of chlamydial agents (1–3). The hemagglutinin is a separate particle from the infectious organism. While the hemagglutination inhibition test has been used for the detection of chlamydial group antibodies it has not been as widely used as the complement fixation test (4, 5). Studies of antigens obtained from chlamydial organisms using gel diffusion have been reported (6–8). The present paper describes reactions obtained with hemagglutinin preparations and sera in gel diffusion.

Material and Methods. Chlamydia psittaci, psittacosis, strain 6BC, and meningopneumonitis, strain Francis, were obtained from the American Type Culture Collection. The agents were propagated in the allantoic cavity or yolk sac of the developing chick embryo.

Preparation of concentrated psittacosis hemagglutinin. Hemagglutinin was prepared from infected allantoic fluids. Procedures for inoculation, incubation, harvesting, and titration of hemagglutinating activity using either mouse or chicken erythrocytes have been previously described (3, 5). The fluids were centrifuged at 10,000 rpm for 30 min in a Sorvall RC2-B centrifuge, to remove the elementary bodies. The supernatant which contained the hemagglutinin was then centrifuged at 30,000 (120 min) or 40,000 (60 min) rpm in a Model L Spinco ultracentrifuge. The sediment was resuspended in 1/100 of the volume centrifuged using glass distilled water, and was designated CPH.

Absorption of allantoic fluid. Elementary bodies were removed from infected fluid as described above. The supernatant was treated as follows: One vol of 400 ml of 1:4 dilution in McIlvaine diluent (5) was absorbed for 1 hr at room temperature (25°) with 2.0 ml of packed chicken$^+$ erythrocytes and another volume was absorbed with chicken$^-$ erythrocytes as a control (3). When the allantoic fluid absorbed with chicken$^+$ erythrocytes was negative for hemagglutinating activity, both fluids were centrifuged following the procedure for preparation of CPH.

Deoxycholate extract. Extract containing chlamydial group antigen was prepared from meningopneumonitis agent following a modification of the procedure previously reported (7, 8). Purified particle suspension was extracted twice with 1.0% sodium deoxycholate in 0.01 *M* phosphate buffered saline. Extracted particles were removed by centrifugation and the second extract, which was a clear solution, was used as antigen.

Antisera. We are grateful to the following investigators for sera collected from patients suffering from various chlamydial infections: Dr. J. M. Joseph, Baltimore; Dr. C. H. Mordhorst, Copenhagen; Dr. E. S. Murray, Boston; Dr. E. Nabli, Tunis; Dr. J. Schachter, San Francisco; and Dr. M. L. Tarizzo, Geneva. Dr. D. T. Karzon, Nashville donated sera from cases of cat scratch fever. Sera from rheumatoid arthritis cases were kindly supplied by Dr. F. Milgrom, Buffalo. Sera were obtained from the Erie County Virology Laboratory collected from individuals with suspected viral infections. Sera from healthy blood donors were collected from laboratory personnel, Department of Microbiology, State University of New York at Buffalo and the Buffalo General Hospital.

Rabbit antiserum (no. 226) against *C. psittaci,* 6BC strain was produced by immunization with infected allantoic fluid inac-

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tivated by ultraviolet light. As part of the immunization schedule the allantoic fluid was mixed with complete Freund's adjuvant (Difco).

Hemagglutination inhibition (HI) and complement fixation (CF) tests. The HI test using human sera was performed as previously described (5). Chicken erythrocytes were used and sera were pre-absorbed with erythrocytes of this species prior to testing. The CF tests reported in this paper were performed at New York State Laboratories, Albany.

Gel diffusion. The micromethod for gel diffusion employed in this laboratory was used (7, 8). Microscope slides were coated with 0.8% agarose (Seakem, Bausch and Lomb) dissolved in 0.15 *M* saline. Serum wells were 4.0 mm in diameter, antigen wells were 5.0 mm. The distance between serum and antigen wells was 3.0 mm. After the wells were charged with reagents, the slides were incubated at room temperature for 24 hr. Lines were observed after this time, but were improved on further incubation of the slides at 4° for an additional 24–48 hr.

Results. The hemagglutination titer of unconcentrated infected allantoic fluid was in the range of 32–64 when tested against either mouse or chicken erythrocytes. CPH yielded hemagglutination titers which were higher than unconcentrated material, 128–256, but the titers were not at the level which might have been expected since the CPH was concentrated approximately 100-fold. At present no explanation is available for the relatively low hemagglutinating activity of the concentrated preparations.

A serum obtained from a case of lymphogranuloma venereum (LGV) was diffused against unconcentrated hemagglutinin and CPH. No reaction lines were obtained with the unconcentrated hemagglutinin but a line of reaction was observed with CPH. Several preparations of CPH did not form lines of reaction. It was found that sonication in a Bronwill Biosonic Vibrator for 15–30 min, while not affecting the hemagglutination titer, resulted in improvement of the reactions in gel. CPH was usually not active when diluted beyond 1:4.

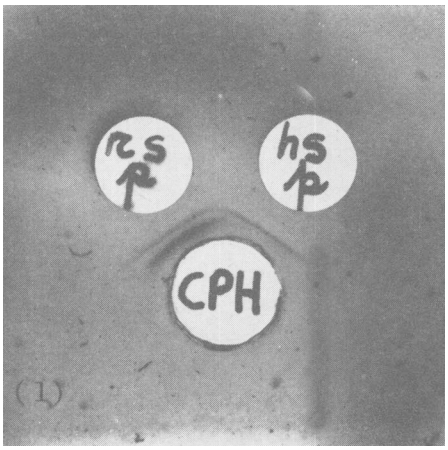

FIG. 1. Double diffusion gel precipitation: Upper wells contain antiserum: rsp = rabbit antipsittacosis (6BC) no. 226 pre-absorbed with lyophilized normal allantoic fluid; hsp = human psittacosis, no. 4, Dr. E. S. Murray. Lower well contains antigen: CPH = concentrated psittacosis hemagglutinin.

CPH was diffused against a serum from a patient with psittacosis and rabbit antiserum against psittacosis agent. The rabbit antiserum was pre-absorbed with lyophilized normal allantoic fluid. Two lines were formed with the rabbit antiserum (Fig. 1). One line which was heavy appeared closer to the antigen well. The second line which formed nearer to the serum well reacted in identity with the line formed by the human serum. Diffusion of concentrated uninfected allantoic fluid, prepared as described for CPH in *Material and Methods,* gave negative results with human or absorbed rabbit antiserum.

Experiments were also performed in which deoxycholate extract of meningopneumonitis agent and CPH were simultaneously diffused against the rabbit antipsittacosis serum. A single line of reaction was obtained with the extract which appeared to fuse with the line formed nearer the serum well by CPH.

Sera obtained from patients suffering from inclusion conjunctivitis, psittacosis and LGV were simultaneously diffused against CPH. As shown in Fig. 2, a line was obtained with each serum and these lines fused in a reaction of identity. While the psittacosis serum tested in Fig. 2 formed a line of reaction at a dilution of 1:8 the sera from the other pa-

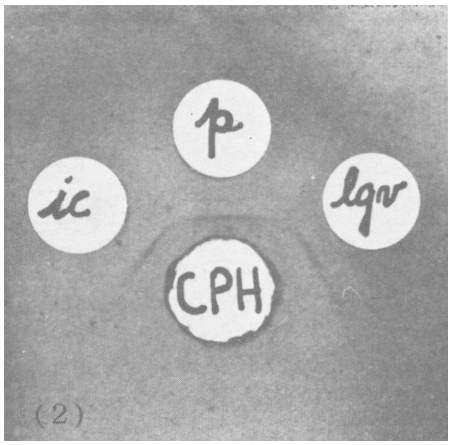

FIG. 2. Double diffusion gel precipitation: Upper wells contain human sera: ic = inclusion conjunctivitis, no. 6, Dr. J. Schachter; p = psittacosis, no. 4, Dr. E. S. Murray; lgv = lymphogranuloma venereum, no. 402, Dr. Dr. E. S. Murray. Lower well contains antigen: CPH = concentrated psittacosis hemagglutinin.

tients were active only when tested undiluted.

A case of LGV from whom multiple serum specimens were collected was studied. The results are summarized in Table I. No line of reaction was obtained with the first bleeding but the second serum specimen, collected 21 days later, was positive. All subsequent bleedings were positive in the gel diffusion test and the lines fused in reactions of identity. A rise in CF antibody titer was obtained which was considered diagnostic. Although antibody titer was found in the HI test, a definitive diagnosis could not be made by this method. At the time of the first bleeding the patient had a negative Frei test. The test was repeated following the second bleeding and the result was positive.

Sera collected from different individuals have been examined by gel diffusion with CPH: LGV, 9/11 positive; other chlamydial infections (TRIC (trachoma, inclusion conjuctivitis), [psittacosis] 24/71 positive; Reiter's syndrome, cat scratch fever, rheumatoid arthritis, 1/38 positive; suspected viral infections, 2/58 positive; blood donors, 0/57 positive.

CPH was re-centrifuged at 40,000 rpm and the supernatant fluid was tested for hemagglutinating and gel diffusion activities. No lines of reaction were observed and no hemagglutinating activity was detected. On the other hand, when the sediment was again resuspended, the material was positive by hemagglutination and gel diffusion.

Infected allantoic fluid was absorbed with chicken erythrocytes as described in *Material and Methods* to remove hemagglutinating activity. In one experiment all hemagglutinating activity was removed after four absorptions with chicken$^+$ erythrocytes. After concentration of this fluid no hemagglutinating activity was detected. However, the material was still active in gel diffusion.

Discussion. It has been shown by the HI test that hemagglutinins produced by different chlamydial strains contain chlamydial group antigen. Thus, hemagglutinin produced by psittacosis agent could be used to detect antibodies in sera from trachoma cases (4). Diffusion of CPH against sera from patients suffering from psittacosis, LGV, and inclusion conjunctivitis resulted in the formation of lines of reaction which fused in identity. Diffusion of CPH against sera collected from various chlamydial infections resulted in a high incidence of positive reactions. The incidence of positive reactions obtained with sera from healthy blood donors, individuals suffering from known or suspected viral infections, and other diseases, was low. Positive results by gel diffusion using CPH could thus be considered as presumptive evidence for chlamydial infection.

The formation of two lines observed with

TABLE I. Comparison of Results Obtained with Sera from LGV Patient by Gel Diffusion, Hemagglutination Inhibition, and Complement Fixation.

Date	Gel dif	HI	CF
Patient G. M. (67-364)			
12-18-67[a]	Neg	20	8
1- 8-68[b]	Pos	40	16
1-16-68	Pos	40	32
1-30-68	Pos	40	ND[c]
2-13-68	Pos	40	ND

[a] Frei test negative.
[b] Frei test positive.
[c] ND = not done.

CPH and rabbit antipsittacosis serum would lead to the conclusion that at least two antigenically different particles were present in CPH. The antigen which formed the line nearer the serum well was apparently related to chlamydial group antigen since reactions of identity were obtained with sera from different chlamydial inefctions. This line also fused with the line obtained with deoxycholate extract of meningopneumonitis agent (8). The second antigen in CPH was apparently not reactive with human sera. The presence of this antigen in psittacosis (6BC) hemagglutinin preparations would not have been detected in conventional HI tests because this method does not readily permit detection of different antigens. In addition, it is not known if the antigen possesses hemagglutinating acting. It may have group or species specificity and will require further elaboration.

The data have shown that reactions obtained by gel diffusion and hemagglutination are associated with particulate material, since centrifugation resulted in sedimentation of the components active in both tests. The failure to remove these antigens by absorption with sensitive erythrocytes can be explained on the grounds that the particles which react in gel diffusion are unrelated to those with hemagglutinating activity or alternatively that the unabsorbed particles have lost hemagglutinating ability as a result of inactivation and are not removed by absorption. The latter explanation is compatible with the observation that concentrated preparations did not have a high hemagglutinating titer.

Summary. Concentrated hemagglutinin of psittacosis agent was reactive in gel diffusion with sera collected from cases of human chlamydial infections and animal immune serum. The reactions obtained with human sera appeared to be associated with chlamydial group antigen. A second antigen was detected in the hemagglutinin preparations when they were diffused against rabbit antipsittacosis serum.

1. Hilleman, M. R., Haig, D. A., and Helmold, R. J., J. Immunol. **66**, 115 (1951).

2. Gogolak, F. M., J. Infec. Dis. **95**, 220 (1954).

3. Barron, A. L., and Riera, M. C., Proc. Soc. Exp. Biol. Med. **131**, 1087 (1969)

4. Goto, T., Matumoto, M., Nakamura, H., Naito, H., Matsushima, S., and Morita, S., Jap. J. Exp. Med. **31**, 237 (1961).

5. Barron, A. L., Zakay-Roness, Z., and Bernkopf, H., Proc. Soc. Exp. Biol. Med. **119**, 377 (1965).

6. Katzenelson, E., and Bernkopf, H., Amer. J. Ophthalmol. **63**, 1483 (1967).

7. Barron, A. L., and Collins, A. R., Amer. J. Ophthalmol. **63**, 1487 (1967).

8. Collins, A. R., and Barron, A. L., J. Infec. Dis. **121**, 1 (1970).

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