Serological Relationship of Trachoma, Psittacosis and Lymphogranuloma Venereum Viruses.* (25815)

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The agents of psittacosis-lymphogranuloma venereum and trachoma-inclusion conjunctivitis have been classified together in the family *Chlamydiaceae* on morphological grounds, but placed in separate genera because of the assumption that their cultural characteristics differed(1). Rake *et al.*(2), employing crude lymphogranuloma venereum antigen, reported presence of complement fixing antibodies to the group antigen of psittacosis-lymphogranuloma venereum (P-LV) in a small number of sera from trachoma patients. Kornblueth and co-workers(3) tested sera from 104 patients suffering from various stages of trachoma with similar antigen and found that 24% gave a positive serological reaction. Other workers employing trachoma antigens prepared from epithelial scrapings of patients with active infection reported complement fixing antibodies in sera of patients with trachoma, lymphogranuloma venereum and ornithosis(4). Recent isolation of trachoma virus in yolk sac of embryonated eggs(5-10) provided the opportunity for more definitive studies of the relationship of trachoma to the P-LV group. Most trachoma virus strains isolated in different parts of the world contained the P-LV group antigen(6,7,9,10). Use of trachoma virus prepared as group antigen for diagnosis of trachoma proved disappointing, and we recently reported preliminary results with specific "purified" elementary body (PEB) trachoma virus antigen, which measures complement fixing antibodies in 33 to 62% of persons on Taiwan with clinical diagnosis of trachoma(11). The work reported here makes use of this specific purified antigen along with group antigens to study the relationship of trachoma virus to psittacosis and lymphogranuloma venereum viruses in the complement fixation test.

Materials and methods. Trachoma virus, strain TW-29(11), in 16th egg yolk sac passage was used. Psittacosis virus, strain 6BC, has been maintained in the laboratory of one of the authors since 1947(12). Lymphogranuloma venereum (LGV) virus, strain JH, was obtained from American Type Culture Collection by Dr. F. B. Gordon who made 2 passages. Psittacosis and LGV viruses were brought to NAMRU-2 as frozen 20% yolk sac suspensions. *Antisera.* Human sera were used throughout, and were stored in frozen state. Trachoma sera with relatively high CF titer when tested with PEB antigen were chosen for study. Trachoma sera from Egypt were obtained in cooperation with Dr. Abdel-Fattah M. Mohamed at Memorial Inst. for Ophthalmic Research, Giza, Cairo. The 2 LGV sera pairs were obtained from Virus Dept., Walter Reed Army Inst. of Research. These sera were lyophilized for transport to NAMRU-2. Two fresh sera specimens were obtained on Taiwan from active cases of LGV. Psittacosis antisera from 3 cases diagnosed during 1959 were obtained from Dr. Helen L. Casey, Communicable Disease Center, U. S. PHS. A fourth pair of psittacosis sera was available from a person who contacted laboratory infection with the 6BC strain in 1947. *Preparation of antigens.* Six- to 8-day-old chick embryos were inoculated into the yolk sac with predetermined virus inoculum to produce a maximum yield of virus. Yolk sacs from all embryos surviving 3 days or longer were harvested just before or after death, smeared, cultured and stored at −65°C. Only sterile membranes, heavily infected with virus by microscopic examination using Macchia-

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vello's stain, were used for antigen preparation. PEB antigens were prepared according to technic developed in this laboratory(11). Twenty % buffered saline suspensions of yolk sac tissue adjusted to pH 8.0 with 1% sodium carbonate were incubated with 0.1 ml of 0.1% trypsin/ml of suspension at 37°C for 3 hours. Trypsin was added and pH adjusted at end of first and second hours of digestion. Only the pH was adjusted at end of third hour of incubation. Then following 2 cycles of low speed centrifugation to remove floating fat and large particles, the virus was sedimented 3 times at 10,000 rpm for 30 minutes in No. 40 head of Spinco model L centrifuge. The resuspended elementary bodies were further purified by precipitation of foreign material with polymyxin B, 100 γ/ml, and elution of any accompanying virus from the precipitate. Formaldehyde USP was added to 0.02% concentration. PEB antigens were adjusted in volume to 40% original yolk sac suspension. These preparations had C. F. antigen titers of 1:12 to 1:24 in presence of 2 units of homologous antisera. Boiled phenolized antigens were prepared after method of Nigg, Hilleman and Bowser(13). Phenol to 0.5% concentration was added to 10% suspensions of infected yolk sacs, and incubated at 37°C for 4 weeks, then placed in boiling water for 20 minutes. After centrifugation at 1500 rpm for 10 minutes the straw colored supernatant was removed and used as group antigen. The complement fixation test used in the current work was the modified Kolmer technic employing optimal proportions method of Friedewald, as described by Rosenbaum and Woolridge(14). In all tests 2 units of antigen and 2 full units of complement were mixed with serial dilutions of serum and incubated overnight at 5° C. The following day after the tubes were warmed at 37°C for 10 minutes, 2 units of hemolysin and 2% sheep red blood cells were added. The materials were again incubated at 37°C for 30 minutes after which the tests were read. Total volume of each tube was 1.5 ml. The highest serum dilution in which 3+ fixation occurred (75%) was taken as endpoint and reported as reciprocal of original serum dilution.

Results. The Table records results of complement fixation tests. All convalescent sera from psittacosis reacted with each of the 3 group antigens and with PEB preparations of both psittacosis and LGV. Somewhat higher antibody titers were found with PEB antigens and trachoma virus group antigen than with psittacosis and LGV group antigens, but the differences in titer were small. LGV sera reacted more irregularly but followed in general the same pattern of reaction as psittacosis sera. No psittacosis or LGV sera fixed complement with trachoma PEB antigen except the Mitchell serum at 1:8 dilution. This serum had been stored for 9 years and contained a slight precipitate. On the other hand, many sera from trachoma patients reacted only with PEB trachoma virus antigen. None of these serums reacted with PEB antigens of psittacosis and LGV. The group antigens reacted only with trachoma sera having highest antibody titer and then in low titer. Trachoma and psittacosis virus group antigens reacted with trachoma sera more frequently than LGV antigen. Serum from Taiwan patient #7182 reacted with all antigens. This patient came to the clinic to be treated for lymphogranuloma venereum and had not only a large bubo but also clinical trachoma in active stage.

Discussion. Although many studies on antigens of the P-LV group have been undertaken to obtain a serologic procedure for identifying individual members, no simple test has been developed(15). In the present work, infections with trachoma virus could be clearly differentiated serologically from those caused by psittacosis and LGV viruses by use of PEB preparations as antigen in the complement fixation test. However, with similar purified antigens of psittacosis and LGV viruses no differentiation was possible between sera from patients with these 2 diseases. Therefore, the method of purifying elementary bodies does not provide a new tool to separate viruses of the P-LV group but shows that trachoma virus contains a specific antigen different from other human pathogens of the group.

Summary. Sera from patients with trachoma, psittacosis and lymphogranuloma ven-

TABLE I. Complement Fixation Antibody Titers of Serums from Patients with Psittacosis, Lymphogranuloma Venereum and Trachoma with Purified Elementary Body (PEB) and Boiled Phenolized Group Antigens.

Disease	Serum	Complement fixation antibody titer					
		PEB antigen			Group antigen		
		Trach	Psitt	LGV	Trach	Psitt.	LGV
Psittacosis	59-0661 Spec. 1	0	0	0	0	0	0
	Idem 2	0	64	32	32	16	16
	" 3	0	32	32	32	16	16
	59-0855 Spec. 1	0	32	32	32	8	16
	Idem 2	0	128	64	32	16	16
	" 3	0	128	128	32	16	16
	59-1124 Spec. 1	0	32	16	16	8	8
	Idem 2	0	128	64	32	32	16
	E. J. April 1947	0	0	0	0	0	0
	Sept. "	0	64	64	64	32	16
LGV	Mitchell 12- 8-50	0	0	0	0	0	0
	4-11-51	8	128	128	64	16	32
	McC. 6-18-53	0	0	0	0	0	0
	7-15-53	0	64	64	8	0	0
	Taiwan pt. #6183	0	64	128	32	128	128
Trachoma	Taiwan pt. # 363	16	0	0	0	0	0
	Idem #1081	16	0	0	0	0	0
	" #1167	64	0	0	16	8	8
	" #1079	64	0	0	16	8	0
	Egypt pt. #14	32	0	0	8	0	0
	Idem #17	32	0	0	16	16	0
	" #11	64	0	0	16	16	0
	" #15	16	0	0	0	0	0
LGV and trachoma	Taiwan pt. #7182	64	128	256	64	64	64

Results are expressed as reciprocals of original serum dilution. 0 = <8.

ereum were tested in the complement fixation test with both "purified" elementary body antigens and boiled phenolized group antigens made with each of the 3 viruses. Group antigen prepared with trachoma virus reacted similarly to group antigens made with psittacosis and lymphogranuloma venereum viruses. The trachoma elementary body antigen reacted only with sera from trachoma patients. Psittacosis and lymphogranuloma venereum elementary body antigens failed to react with trachoma serum but did react about equally with sera from both psittacosis and lymphogranuloma venereum patients. It is concluded that trachoma virus contains the group antigen of the P-LV group and in addition a specific antigen which permits its serologic differentiation from the agents of psittacosis and lymphogranuloma venereum.

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Infection of Hamsters with Para Influenza 3 Virus. (25816)

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A property shared by many myxoviruses is their capacity for multiplication in lungs of certain rodents(1,2). Many of the genetic factors and quantitative aspects of experimentally induced infections with influenza viruses have been described(3). With extensive use of tissue culture for virus isolation, a group of myxoviruses with common properties has recently been recognized(4,5). These viruses were designated as para influenza viruses 1, 2, and 3 to indicate their distinctness from influenza viruses. Because of their importance in the etiology of childhood respiratory illness, and of their potential importance in adult respiratory disease, experimental animal hosts were sought in which *in vivo* virus-host relationships could be studied(6). The present study is concerned with occurrence and quantitative aspects of pulmonary infection in hamsters with the type 3 para influenza virus.

Methods and materials. Virus. The C 243 strain of para influenza 3 virus, isolated in 1957 from a child with pneumonia(5), was passaged 7 times in rhesus monkey kidney cultures and twice in Salk monkey heart cultures. A stock pool of virus, prepared by combining equal volumes of infected monkey heart culture fluid and skimmed milk, was stored in glass sealed ampules in a CO_2 chest. *Inoculation of hamsters.* One-month-old, recently weaned, golden hamsters of both sexes, weighing 60 to 70 g were used. One-tenth ml of varying dilutions of tissue culture fluid or lung suspension was inoculated intranasally under ether anaesthesia. Inoculated animals were housed in groups of 2 or 3 in shoebox-sized containers in semi dark room. Control animals were inoculated with sterile normal lung suspension and housed similarly. At various intervals following inoculation, hamsters were lightly anaesthetized with ether and exsanguinated. Lungs were removed aseptically, washed twice in Hanks' balanced salt solution, and a 10% suspension prepared in 50% skimmed milk in Hanks' solution by grinding in mortar with alundum. Lung suspensions were stored at -60°C until titered. *Titration.* Decimal dilutions were prepared in Hanks' solution and 0.2 ml was inoculated into each of 2 rhesus monkey kidney cultures. These cultures were maintained in Eagle's basal medium without serum. Seventy-two hours following inoculation, cultures were examined for evidence of infection by the hemadsorption technic. Cultures were washed twice with Hanks' solution and 1 ml of 0.1% suspension of guinea pig red cells was added. Following 20 minutes incubation at 4°C they were examined for presence of erythrocytes adsorbed to the tissue culture sheet. TCD_{50} (50% tissue culture dose) was calculated by the method of Reed and Muench(7). *Serology.* Complement fixation tests were performed by modification of the method of Bengston(5). Complement fixing antigens for various myxoviruses were prepared as described previously (8).

Results. Para influenza 3 virus was carried through 15 consecutive intranasal passages in hamsters. The inoculum of tissue culture virus which initiated the passage series con-

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