Reports and Letters

Production of Pharyngoconjunctival Fever in Human Volunteers Inoculated with APC Viruses

In a previous communication (*1*) it was stated that intranasal inoculation of human volunteers with adenoidal-pharyngeal-conjunctival (APC) viruses (*2-5*) had failed to produce objectively recognizable illness. Similar results were reported for APC 4 (RI-67) virus by Hilleman *et al.* (*6*). However, successful transmission experiments in volunteers (*7*) with bacteria-free nasopharyngeal secretions from a case of ARD were shown by Ginsberg *et al.* (*8*) in retrospect to be associated with specific antibody responses to the RI-67 strain. Subsequently, as reported in this article (*9*), combined conjunctival and pharyngeal inoculation of type 3 and type 4 APC viruses produced easily recognizable definitive disease in susceptible human volunteers.

Twenty volunteers received type 3 virus (study G2); seven received a mixture of approximately equal parts of (i) a virus-containing throat washing from a patient with fever and sore throat and (ii) fluid from the sixth monkey kidney tissue culture passage of another strain of type 3 virus that was recovered from a case of pharyngoconjunctival fever (*5*); 13 received fluid from a pool of 40 virus-containing specimens taken during the same outbreak. In another study (H2) type 4 virus was used, the inoculum consisting of fluid from the third monkey kidney tissue culture passage, following isolation in human embryo trachea tissue culture of strain R.N. (*4*) that was recovered from a recruit at the Great Lakes Naval Training Center. The control inoculum in both studies was the maintenance medium used for monkey kidney tissue cultures; all inocula contained penicillin (250 units/ml) and streptomycin (250 μg/ml). All virus-containing inocula were cultured in thioglycolate broth and inoculated into suckling and adult mice, intraperitoneally and intracerebrally, and rabbits, intracutaneously into multiple sites and on the scarified cornea; the cultures were negative and the animals showed no evidence of illness.

A cotton applicator soaked in the test inoculum was gently swabbed on the lower tarsal conjunctiva of one eye and on the posterior pharynx; the other eye of the volunteer was similarly swabbed with the control inoculum. The observers did not know which eye had received the virus. The volunteers were examined at daily intervals for the first week and on alternate days during the second week. Bloods were collected prior to inoculation, 3 weeks after inoculation (study G2), and 4 weeks after inoculation (study H2). Specimens from each eye and the throat were collected by means of swabs on the fifth day after inoculation. Half of the volunteers in each study were white and half were Negro; their ages varied from 21 to 25 years.

Laboratory studies on the volunteers were carried out by personnel who had no knowledge of their clinical responses. Neutralization tests were done with the procedure designated as "procedure 2" (*4*); virus isolation attempts from swab specimens were made in HeLa cell tissue cultures, a specimen being considered positive if typical APC cytopathogenic effects occurred during the 12 days of observation.

The most pronounced and objectively obvious effect of the virus inoculum was an acute catarrhal conjunctivitis; for the purposes of this report, a volunteer was considered to have developed illness if conjunctivitis was noted on 2 successive days by the examining physicians and if the volunteer offered specific complaints referrable to the eye. Twenty-six of the 40 volunteers developed such illnesses. The incubation periods ranged from 2 to 7 days; 21 of the 26 ill persons had onset on or before the fourth day after inoculation.

The complaints in the 26 ill individuals were conjunctival symptoms (itching, burning, tearing, and foreign body sensation) in 26, sore throat in 20, cough in 15, headache in 13, and nasal symptoms in 12. Generalized aching and stiff neck, hoarseness, chest pain, chilliness, and malaise were noted in a few cases.

The catarrhal conjunctivitis, which in many cases later became follicular, began in the area of inoculation, and progressed rapidly to involve the palpebral and bulbar surfaces. Edema of the eyelids was a frequent finding, and a few cases showed some hemorrhage into the conjunctiva. Slit lamp examination during the acute stage of illness and several weeks after recovery revealed no corneal or uveal tract involvement. Other physical signs in the 26 cases included inflamed pharyngeal lymphoid patches in 24, inflamed nasal mucosa in 20, nasal discharge in 17, and preauricular lymph node enlargement in 16. Fever of 100°F or more occurred in eight persons. Several cases showed distinct enlargement of the tonsil on the same side as the virus-inoculated eye. The usual duration of illness was 7 to 8 days, and in some cases signs and symptoms continued for more than 10 days.

Conjunctivitis was seen in the eye inoculated with control material in only two individuals, and in these the eye became irritated on the seventh and eighth days after the virus-inoculated eye had shown definite conjunctivitis. One volunteer, inoculated with type 3 virus, on the 17th day after inoculation developed a temperature of 104.8°F and physical and x-ray signs of pneumonitis in the lower left lobe; he had had severe conjunctivitis during the first week after inoculation. Virus was not recovered during the period of pneumonitis.

The clinical manifestations, which closely resembled those of pharyngoconjunctival fever (*5*), produced by the two different virus types appeared indistinguishable, as were the illnesses produced by the two type 3 inocula. Racial differ-

Table 1. Occurrence of illness in relationship to preexisting antibody level, neutralizing antibody response, and recovery of virus. Figures given show number of ill per number of volunteers.

Type of APC virus given	Preinoculation antibody titer			Neutralizing antibody response (fourfold or more)*		Recovery of virus from eye or throat swabs	
	< 4†	4–8	> 8	Rise	No rise	Positive	Negative
3	12/12	2/3	1/5	14/15	1/4	14/16	1/4
4	8/ 8	2/8	1/4	11/14	0/5	11/14	0/6

* One person in each study not available for convalescent serum. † Reciprocal of dilution.

ences did not appear to influence the clinical response.

Table 1 gives the relationship of the occurrence of illness to the laboratory findings. Although the numbers are small, the differences in each category appear to be significant. These data show that the frequency of illness was inversely related to the level of preexisting neutralizing antibody to the virus type inoculated and directly related to the development of a neutralizing antibody response and to recovery of virus from the inoculated sites. In summary, conjunctival and pharyngeal inoculation of type 3 and type 4 APC viruses in volunteers having little or no preexisting neutralizing antibody produced illnesses indistinguishable from pharyngoconjunctival fever.

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References and Notes

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9. We are indebted to the inmates at the Maryland State Reformatory for Males, to the authorities of the Department of Correction, State of Maryland, and particularly to Robert L. Clopper, assistant superintendent of the Maryland State Reformatory for Males, without whose assistance these studies would not have been conducted. The work at Johns Hopkins University was supported in part by a grant from the Research Grants Division, National Microbiological Institute, and by the Common Cold Foundation.

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Possible Role of Chelation between Alkali Metals and Pyridoxal in Biological Transport

The cellular transfer processes for the amino acids and the alkali metals are closely connected. Heavy loading of either process appears to inhibit the other. On the one hand, the concentrative transfer of amino acids falls off rapidly when the potassium ion level is raised (*1*). Conversely, a large transfer of amino acid into the cells causes potassium loss and sodium gain (*1, 2*).

Furthermore, pyridoxal and related aromatic *o*-hydroxyaldehydes not only stimulate amino acid accumulations by ascites tumor cells but also cause profound shifts of the alkali metal ions. At low pyridoxal levels, potassium loss is more conspicuous and the cells shrink; at higher levels, sodium entrance predominates and the cells swell (*3*).

Whereas pyridoxal is known to form metabolically active derivatives with the amino acids, evidence for its combination with the alkali metals apparently has not been previously reported. In the present study, spectrophotometric and titrimetric evidence for differential chelation of the alkali metals with pyridoxal in water solution has been obtained (*4*).

Fresh 0.01*M* pyridoxal solutions were prepared that included a metal chloride and small quantities of a metal hydroxide (added as a 0.02*N* solution) to give *p*H values increasing in steps from 6.5 to 7.5. In the experiments of Fig. 1, the following combinations were used: (i) no chloride and KOH; (ii) 0.15*M* KCl and KOH; (iii) 0.15*M* NaCl and NaOH; (iv) 0.15*M* LiCl and LiOH; (v) 0.05 *M* $CaCl_2$ and $Ca(OH)_2$; and (vi) 0.05*M* $MgCl_2$ and KOH. Preparations of NaCl and LiCl from two different sources were tested. After 1 hour at 25° to 27°C, the optical densities at 400 mμ were determined, using silica cells and the Beckman spectrophotometer. The *p*H of the remaining portion of each solution was then determined at once with the Beckman laboratory model *p*H meter.

With magnesium the extra yellow coloration was very obvious to the eye. The optical densities decreased in the order Mg, Ca, Li, Na, and K (Fig. 1). The amounts of alkali required to produce a given *p*H were in the same order. The effect of Ca^{++} at 0.05*M* was only moderately larger than that of Na^+ at 0.15*M*; accordingly the reaction with Na^+ should predominate at typical extracellular levels of the two ions. The extra density produced by the various cations increased gradually for 1 hour, and then gradually decreased.

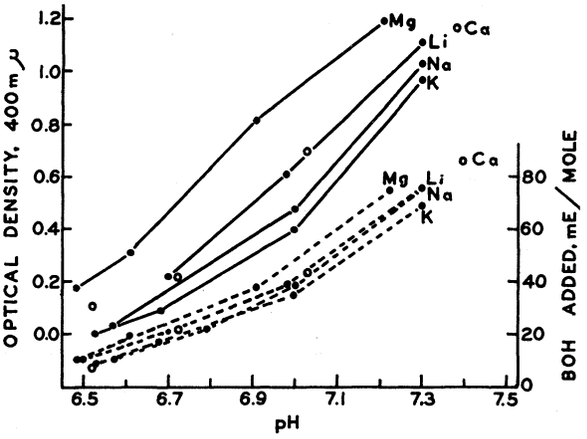
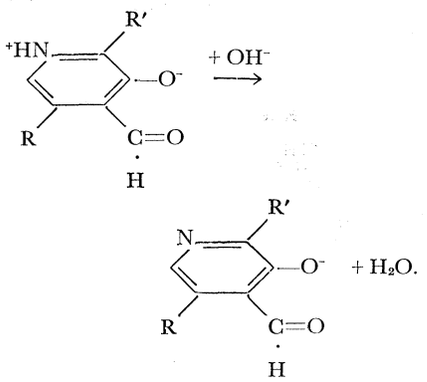


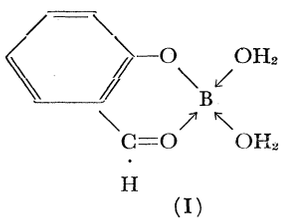
Fig. 1. Absorption and titration changes due to alkali and alkaline-earth cations. The solid lines show the optical density at 400 mμ and refer to the scale at the left; the dashed lines are titration curves, referring to the scale at the right.

The reaction with the hydroxyl ion alone may be represented as follows (*5*):



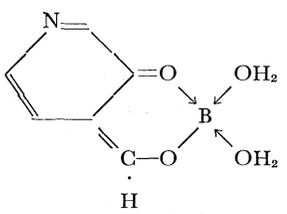
A *p*Ka of 8.70 has been obtained for this reaction, titrating, however, in 0.15*N* NaCl (*6*). The yellow color has been attributed to a quinoid tautomer (*7*). Our results indicate that a second reaction involving the cation occurs to produce proton displacement and new absorption.

Alkali metal chelates of *o*-salicylaldehyde and its derivatives were obtained in nonaqueous systems by Sidgwick and Brewer (*8*) and Brady and Bodger (*9*). Structure (I) was proposed.



(I)

With pyridoxal, the quinoid form (II) should be considered.



(II)