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.

THOMAS G. WARD
Department of Microbiology, Johns Hopkins University, Baltimore, Maryland
ROBERT J. HUEBNER
WALLACE P. ROWE
RALPH W. RYAN
JOSEPH A. BELL
National Microbiological Institute and *National Institute of Neurological Diseases and Blindness, Bethesda, Maryland*

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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.

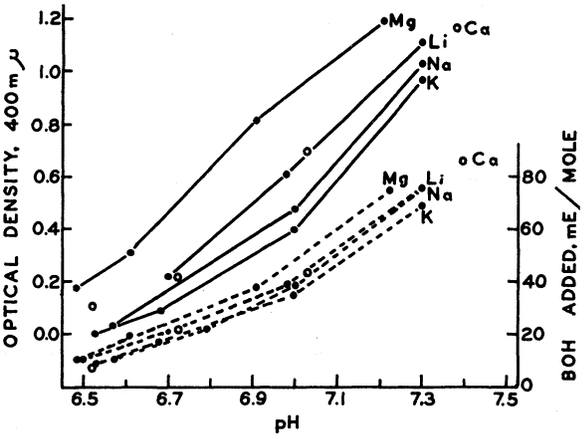
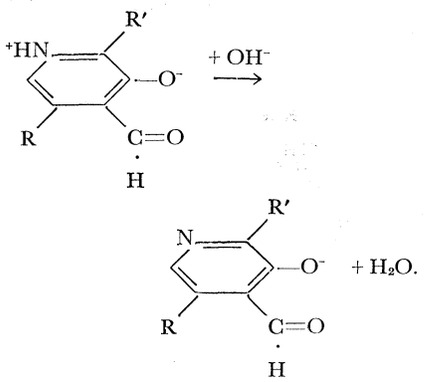


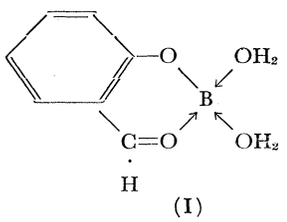
Fig. 1. Absorption and titration changes due to alkali and alkaline-earth cations. The solid lines show the optical density at 400 mμ and refer to the scale at the left; the dashed lines are titration curves, referring to the scale at the right.

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



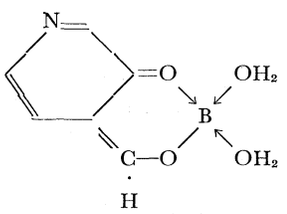
A *p*Ka of 8.70 has been obtained for this reaction, titrating, however, in 0.15*N* NaCl (*6*). The yellow color has been attributed to a quinoid tautomer (*7*). Our results indicate that a second reaction involving the cation occurs to produce proton displacement and new absorption.

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)

Pyridoxal contains groupings that make plausible chelation of the alkali metals, either in aqueous or lipoid phases. Furthermore, the reaction probably produces a neutral molecule that might carry the metal ion through cellular boundaries. Concentration gradients of the metal ion might be established if the molecule undergoes a secondary modification (for example, phosphorylation or displacement of the metal ion by an amino compound) on one side of the phase boundary.

Significantly, the metals that chelate more strongly with pyridoxal (Mg^{++}, Li^{+}, and Na^{+}) are the ones that tend to cause the apotryptophanase-pyridoxal phosphate system to dissociate, whereas K^{+} and Rb^{+} tend to stabilize the holoenzyme (*10*).

HALVOR N. CHRISTENSEN*
Department of Biochemistry and Nutrition, Tufts University School of Medicine, Boston, Massachusetts.

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* Present address: Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor.

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Electrophoresis of Serum Proteins in a Viscous Film

In the study of proteins by the technique of filter paper electrophoresis, convection or diffusion and adsorption of proteins to the paper with resultant trailing are among the limiting factors in the resolution that can be obtained (*1*). With a protein solution containing 98 percent albumin and 2 percent γ-globulin, the densitometer curves obtained from filter paper electrophoresis in our laboratory indicate that 10 to 15 percent of the albumin is lost in trailing. The work of Brakke (*2*) with graded density columns of sucrose, and of Philpot (*3*) who conducted electrophoresis employing a multilayered solution, suggested the possibility of electrophoresis in a viscous film. We felt that the viscosity and surface forces might be related to the ability of such a film to serve as an anticonvection medium and that the use of a fluid rather than a solid electrophoresis medium would tend to decrease trailing.

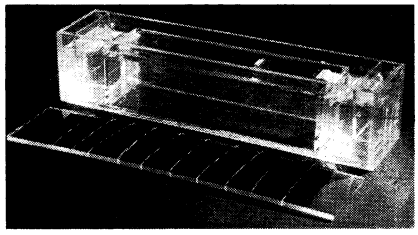

Fig. 1. Plexiglas apparatus set up for film electrophoresis.

An electrophoresis apparatus was constructed of Plexiglas for this purpose; it is shown in Fig. 1. It consists of a longitudinal platform between two electrode vessels containing platinum electrodes and labyrinthine dividers. The experiments were conducted by placing a glass plate 1⅞ by 10 inches on the shelf and a film on the glass plate. After investigating several substances, we used the following method to provide a viscous film of suitable properties. Agar-agar, 0.13 g, was added to 100 ml of barbiturate buffer of *p*H 8.6 and ionic strength 0.05. This was then heated and boiled for 1 minute, until the agar-agar dissolved. Methyl cellulose, 0.028 g, was then added to the solution and thoroughly mixed. The resultant solution is near the gel point, containing slightly less than the amount of agar-agar necessary for gel formation. While the solution was still warm, it was added with a pipette to the glass plate to form a film about 1/16 inch thick.

The film was then connected to the buffer in the electrode vessels by means of filter paper wicks, covered, and voltage applied for 10 minutes, after which more of the solution used to make the film was added if the thickness of the film had decreased. (The surface of the film should be smooth, and it should exhibit interference colors from reflected light. If the concentration of agar-agar is increased above the amount used, there is a decrease in the protein mobility and in the resolution, and the dried film tends to crack and peel off. If a smaller concentration is used, the resolution again appears to decrease.) The serum was then added by means of placing a small piece of filter paper, about 10 mm by 4 mm, which had been soaked in the serum, on the film. The cover of the apparatus, lined with wet blotting paper, was sealed with Saran wrap in order to prevent evaporation. If this precaution is observed, the film is still liquid at the end of the run, and it will run down the glass plate if the glass plate is significantly tilted. It was found that a suitable separation could be obtained by the application of 100 v for a period of 16 hours. The current obtained at 100 volts with this setup is about 3 ma.

When the electrophoresis has proceeded a sufficient length of time, the protein pattern can be obtained by two different procedures. A slightly damp strip of filter paper can be carefully placed over the film and the glass plate inserted into a drying oven at 120°C. As soon as the film is dry, the filter paper can be removed and developed in the usual manner (*4*). An alternative procedure is to dry the film as such, without any filter paper, insert the glass plate in an alcoholic solution of bromphenol blue, wash the film briefly with 1-percent acetic acid, and redry the film by placing the glass plate in the oven again. The glass plate can then be inserted directly into a densitometer, and the optical density curve can be obtained. The protein pattern can be accentuated by placing a blank strip of filter paper between the plate and another glass plate that is blank.

The mobility on the film is approximately the same as it is on filter paper. Albumin stained with bromphenol blue was found to migrate at about 0.65 cm/hr at 100 v. (This includes a velocity component in the opposite direction owing to electroosmosis.) The distance of migration is linear with time.

Figure 2 shows the results obtained

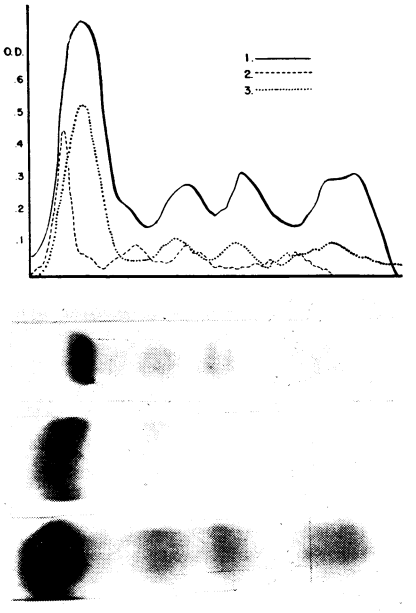

Fig. 2. Electrophoresis strips and corresponding densitometer patterns of the same sample of serum made by three different procedures: curve 3 and top strip show serum run on a film and developed on glass, curve 2 and middle strip show serum run on a film and picked up on paper, curve 1 and bottom strip show serum run on paper by conventional procedure.