2. The solid lines are values of J_{KCl} calculated from Eq. 3 for a range of dialysate concentrations with 0.2*M* and 0.02*M* KCl in the dialysand. The values of R_I and R_{II} were calculated as described by Weinstein *et al.* (*4*). The value of the combined membrane resistances used, $(R_c + R_a)$, was that which best fitted both curves simultaneously to the experimental points (*5*). The calculation thus contained one free parameter.

The experimental points were measurements of KCl permeability conducted in essentially the same manner as the dialysis experiments but in glass cells (*1*) fitted with conductance electrodes. The dialysand concentration was allowed to change by only about 5 percent during the course of an experiment. Addition of 0.2*M* mannitol to the dialysand had no discernible effect on KCl permeability (Fig. 2).

During dialysis the optimum dialysate salt concentration clearly shifts downward as the dialysand becomes more dilute. In the experiments summarized in Table 1 we chose as a compromise a dialysate concentration of 0.01*M* KCl, somewhat lower than that appropriate to the initial 0.2*M* dialysand. In practice the dialysate concentration might be programmed to vary with time as is the eluent concentration in gradient elution chromatography.

As emphasized by the electrical model, dialysis with a mosaic is similar in mechanism to electrodialysis but without external electrodes. The electromotive force driving the ionic currents is supplied by concentration potentials rather than by an external source. Electrodialysis simultaneously desalts a solution and concentrates the uncharged species present. Dialysis with appropriately chosen charge-mosaic membranes might perform similar functions with a significant simplification of apparatus. Desalination and concentration might also be achieved in the pressure dialysis mode of operation, in which hydrostatic pressure is applied to develop streaming potentials and circulating current.

The separatory properties of mosaic membranes may find application in industry, in the laboratory, and also in medical technology (for example, in altering the concentrations of ionic species in the blood). One specific example is suggested by a recently proposed design for a portable artificial kidney (*6*). In such a system a charge-mosaic might perform salt-reabsorption analogous to that of the mammalian proximal tubule.

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3. In the absence of a second solute, the permeability ω^*_s differs from the more usual coefficient ω_s only by a coupling term due to volume flow (*1*). Using data reported in (*1*), one can show this term to amount to a correction of no more than a few percent in our mosaic membranes (volume flow in these experiments is calculated to be approximately 0.01 ml cm^{-2} hr^{-1}). In the presence of a second solute additional coupling effects may appear.
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Rapid Screening Test for Detecting Hepatitis-Associated Antigen

Abstract. *Hepatitis-associated antigen can be detected within 2 hours by using an electrophoretic technique and cellulose acetate membranes saturated with antibody. The speed of the technique now allows testing of blood intended for transfusion on the day of collection, and the sensitivity of the method compares favorably with standard immunodiffusion.*

The lack of specificity of traditional tests for detecting serum hepatitis bloods before transfusion appears to have been partly overcome recently by the identification of an antigenic lipoprotein associated with viruslike particles in the serum of some hepatitis patients and chronic carriers of the infection. Current methods for detecting the hepatitis-associated antigen (HAA) (*1*), originally called Australia Antigen (*2*) or S.H. antigen (*3*), are not sensitive enough to detect all infectious bloods (*4*). However, immunological tests using antiserums from the rare patients found to be hyperimmunized via repeated transfusions are being carried out in an increasing number of transfusion centers. Unfortunately it is often necessary to transfuse fresh blood before there is time to screen the blood for HAA using tests such as Ouchterlony immunodiffusion (*2, 3*) or complement fixation (*5*) which usually require incubation overnight. This report describes an electrophoretic method which can be completed within 2 hours and is at least as sensitive as the Ouchterlony technique while avoiding the necessity for multiple dilutions and reagent standardizations required by the complement-fixation method (*5*). The current technique is based upon Laurell's modification of crossed electrophoresis (*6*) but substitutes a preformed microporous cellulose acetate membrane (*7*) for agar.

The cellulose acetate membrane (95 by 75 mm, by 0.01 mm thick, Millipore Filter Corporation or Gelman Instrument Company), saturated by 1 ml of a suitable dilution of antiserum (1:16 was used in the present study), was placed on a Parafilm-covered neoprene rubber base which served to compress the membrane snugly against a top Lu-

Table 1. Comparison of electrophoretic and Ouchterlony methods in quantitating hepatitis-associated antigen.

Antigen	Electrophoresis		Ouchterlony	
	Positive cases	Cumulative total	Positive cases	Cumulative total
4+	7	7	9	9
3+	15	22	2	11
2+	12	34	6	17
1+	4	38	21	38
Negative		62		62
Series total	38	100	38	100

cite template secured by bolts tightened evenly by a torque wrench preset at 5×10^6 dyne/cm. The template was perforated by the 13 conical wells used to align and apply 2-μl serum samples to the membrane below. Barbital buffer, 0.05*M*, *p*H 8.2, found to optimize antigen migration toward the anode, was used for antibody dilutions and for soaking the sponges used to establish continuity of electrodes with the ends of the membrane. A current of 6 ma (approximately 250 volts) was applied for up to 2 hours, although as little as 30 minutes would bring out the characteristic rocket-shaped immunoprecipitates in serums containing high antigen titers. The unprecipitated antibody, antigen, and normal serum proteins were removed by washing the membrane for 10 to 15 minutes in normal saline, and the precipitates were revealed by staining (Ponceau-S dye with acetic acid clearance was used in this study). The dried membrane was retained as a permanent record. Antigen was quantitated by measuring the migration distance of the cone of the rocket-shaped immunoprecipitate (*6*), or, more accurately, as a function of its planar area (*8*).

The appearance of the immunoprecipitates is illustrated in Fig. 1. The antiserum was obtained from a much-transfused donor with hemophilia and had been shown to interact with serums from patients with viral hepatitis (as confirmed by liver biopsies) which were HAA positive by other well-characterized antiserums. Specificity of the antiserum for an antigen in the serums of viral hepatitis patients is suggested in Fig. 1 by the lack of immunoprecipitation with serum samples from normal individuals and patients with other illnesses.

Positive reactions were obtained in the electrophoretic technique with purified hepatitis-associated antigen derived by ultracentrifugation of infected serum, which in the Ouchterlony technique gave a single immunoprecipitin line of identity with whole HAA-positive serums. Confirmation of specificity was found in six independent laboratories.

Investigation of the sensitivity of the electrophoretic method was made by comparison to the standard microimmunodiffusion method (*2*, *3*) by using serums obtained from 100 acute viral hepatitis patients within 1 month of onset of the disease. A definitive epidemiological classification was not possible in 40 percent of the series tested. The same antibody (undiluted) was used in the immunodiffusion method, and a known antigen-positive serum was used in wells spaced appropriately to confirm identity reactions when antigen was detected among the 100 test samples. The antigen-positive control was arbitrarily assigned a value of 4+ in both methods, and positives among test samples were graded 1+ to 4+ in comparison to the control. The data were obtained by using coded specimens and were compared only after the entire series was completed. Table 1 shows that both methods indicated 38 percent of the samples to be positive. The electrophoretic technique detected 37 of the 38 positives by immunodiffusion, failed to pick up one weak reaction, but detected a weak reaction missed by immunodiffusion.

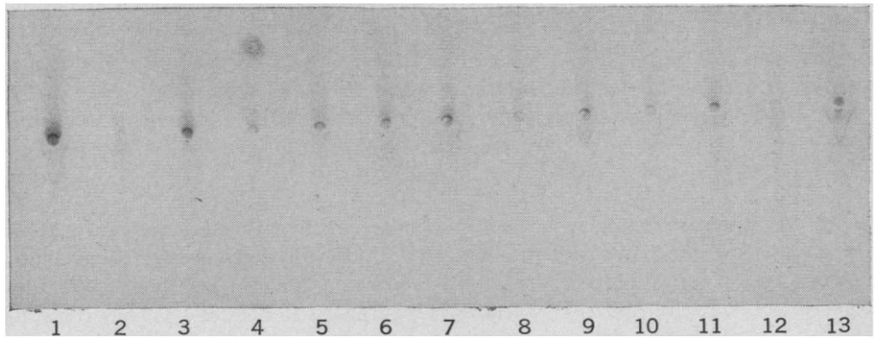

Fig. 1. Electrophoretic immunoprecipitates (or absence thereof) associated with acute viral hepatitis (samples 1, 5, 6, 9, 10, 11, and 13), resolving viral hepatitis (sample 7), idiopathic chronic active hepatitis (sample 8), normals (samples 2 and 12), and gallstones and biliary cirrhosis (samples 3 and 4). Positive samples are those with rocket-shaped precipitates pointed toward the anode at the bottom (samples 1, 5, 6, 7, 9, 10, 11, and 13)

In the comparative series using unconcentrated serums (Table 1), reactions detected by electrophoresis were graded as 2+ or greater more often than when immunodiffusion was used. Following concentration of the serums sixfold by using acrylamide gel chips prior to immunodiffusion, and threefold concentration by freeze-thaw prior to electrophoresis, both of the weak reactions were confirmed as positive, as well as ten other previously undetected positives.

Failure to detect hepatitis antigen in half of the samples, even after concentration, could mean that some of them contained stable titers of antigen below the threshold of sensitivity of the test, and in still other cases, the hepatitis may have been of the type not associated with circulating antigen. The known rapid disappearance of antigen from serums of acute cases suggests that in some it might have declined to exceedingly low levels. Thus, in spite of the limitations described, the electrophoretic technique with modifications as suggested appears to be simple, reliable, rapid, and sensitive enough to eliminate many infectious blood donors. It may be a prototype of the screening method ultimately selected for routine use in transfusion centers which have a rapid turnover of fresh blood.

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