mediated by insulin. Holt and Oliver (*21*) have suggested that different hormones may induce different forms of TAT, and Wicks (*1, 3*) has presented evidence that the enzyme induction involves cyclic AMP. In whole animal experiments we have obtained TAT induction that closely followed AIB intake when glucagon and theophylline were injected, possibly implicating cyclic AMP in both processes. It appears that the mechanisms underlying the changes in AIB transport can be separated from those underlying TAT induction by hydrocortisone, but complete concordance among cells in culture, in perfused liver, and in whole animals remains to be achieved.

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Charge-Mosaic Membranes: Dialytic Separation of Electrolytes from Nonelectrolytes and Amino Acids

Abstract. *Charge-mosaic membranes were used for dialytic separations of potassium chloride from low-molecular-weight nonelectrolytes and neutral amino acids. The permeability ratio (potassium chloride to uncharged species) ranged from about 6 in the case of methanol to about 86 in that of mannitol. A theoretical model predicts that optimum rates of dialysis should be achieved by dialyzing against salt concentrations other than zero; this prediction was confirmed by experiment. These observations suggest potential applications of mosaics in laboratory separations, industrial processing, and hemodialysis.*

We have described the observation of negative reflection coefficients and high electrolyte permeabilities in charge-mosaic membranes (*1*). The former property suggested the possible use of such membranes for desalination of seawater or brackish water by pressure dialysis (piezodialysis); the latter suggested their application to the separation of electrolytes from other low-molecular-weight species. In this paper we report dialytic separations of KCl from the uncharged organic species methanol, urea, glycine, L-phenylalanine, and D-mannitol.

A charge-mosaic membrane consists of a set of cation and anion exchange elements arranged in parallel, each element constituting a continuous pathway from one bathing solution to another. The high electrolyte permeability attainable with charge-mosaics, as compared with other membranes of tight structure, arises from two properties: (i) the high concentration, typically several molar, of cation in the cation exchanger and anion in the anion exchanger, and (ii) the ease with which the two ions can flow in parallel through their respective pathways, the two flows neutralizing each other electrically. These flows are equivalent to circulating electric currents, as predicted and later verified in model systems by Sollner and co-workers (*2*).

The membranes used in our studies and the method of their preparation have been described (*1*), A single layer of cation and anion exchange beads was embedded in an inert silicone matrix in such a way that each bead communicated with both surfaces of the membrane. The beads were of 8 percent cross-linked polystyrene-based resins.

For dialysis the membrane was mounted in a small Lucite cell; the "dialysand" compartment was filled, after several washings, with 1.3 ml of an aqueous solution containing KCl and labeled organic species. The initial concentration of KCl in all experiments was 0.2 mole/liter; the concentration of the organic species varied as indicated in Table 1. A solution of 0.01*M* KCl flowed from a large reservoir into the "dialysate" compartment; the effluent from this compartment was collected in flasks for analysis. A simple gravity-flow device maintained a constant flow of approximately 5 ml/hour, sufficient to ensure that the solution in the dialysate compartment remained within 10 percent of its nominal KCl concentration (0.01 mole/liter) and practically devoid of bulk and tracer organic solute. Teflon-coated magnetic stirrers provided vigorous stirring near each face of the membrane, and the temperature was controlled at 25.0° ± 0.2°C in an air thermostat. Concentrations of KCl in both compartments were determined from conductance measurements, whereas concentrations of organic solutes were obtained by scintillation counting of the tritium or carbon-14 label. Each experiment was continued for 35 to 50 hours (by which time the KCl concentration in the dialysand had been reduced to roughly one-third of its initial value). Figure 1 shows the time-course of a separation of urea and KCl.

The permeability ω^*_s of each solute, defined as

$$\omega^*_s = \left(\frac{J_s}{\Delta\pi_s}\right)_{\Delta p = 0} \quad (1)$$

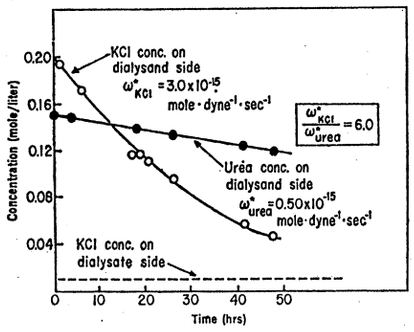

Fig. 1. Dialytic separation of KCl from urea by means of a charge-mosaic membrane. The permeability ω^*_{KCl} is that obtaining with 0.1*M* KCl in the dialysand.

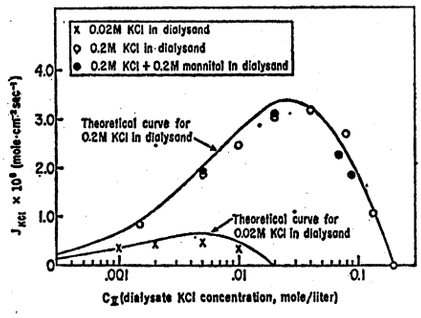

Fig. 2. Flow of KCl as a function of dialysate KCl concentration. The curves are calculated for dialysand concentrations of 0.2 and 0.02*M* KCl, with $(R_c + R_a)$ assumed to be 13.1 ohm-cm². The points are experimental.

was calculated from the measured values of solute flow J_s. Flows are considered positive if they take place from the dialysand (I) to the dialysate (II). $\Delta p = p^I - p^{II}$ is the difference in hydrostatic pressure, and $\Delta\pi_s = \pi_s{}^I - \pi_s{}^{II}$ is the difference in osmotic pressure due to the solute s (*3*).

In each experiment the permeability ω^*_{KCl} increased substantially as the concentration of KCl in the dialysand diminished. This behavior is predicted qualitatively by the simple model considered below. In contrast the permeability of the second species appeared to remain constant, as is to be expected for nonelectrolytes (and for amino acids insofar as they cross the membrane as zwitterions). The solutions were not buffered, but *p*H values measured both before and after the experiments were always in the range of 6 to 7. Thus glycine and phenylalanine were predominantly in the zwitterionic form, at least in the two bathing solutions.

Results for a series of solutes are collected in Table 1. The values of ω^*_{KCl} reported are those which obtained when the concentration of KCl in the dialysand had decreased to 0.1 mole/liter. The membrane used had an active area (*1*) of 0.077 cm², approximately equally divided between cation and anion exchange regions. Control measurements with plain silicone disks without ion exchange beads indicated that KCl, urea, mannitol, glycine, and phenylalanine do not pass through the silicone at significant rates. Methanol passed at 7 percent of its rate through the mosaic, an indication that the separation ratio for KCl and methanol for the ion exchange beads themselves was several percent higher than that tabulated.

In dialysis the composition of the dialysand is generally determined by the problem at hand, whereas that of the dialysate can be adjusted for optimum results. With a nonmosaic membrane removal of a given species always proceeds most rapidly if its concentration in the dialysate is kept as low as possible, ideally at zero. In contrast, a dialysate salt concentration other than zero is optimum for desalting with a mosaic, as can be shown by consideration of a simple electrical model for the circulation of current between two ion exchange regions. Suppose $\Delta\psi_c$ and $\Delta\psi_a$ are the potential differences which would be measured with salt bridges across the cation and anion exchange regions, respectively, if no volume flow occurred and no current were permitted to circulate (that is, if each region were open-circuited). Let R_c and R_a be the electrical resistances of the cation and anion exchange regions for unit total active membrane area (ohm-cm²); let R_I and R_{II} be the resistances encountered by the circulating current in the two solution compartments (again for unit total active area). The circuit thus consists of a single loop containing four resistances and two electromotive forces.

It is convenient to introduce a loop current density (amperes per square centimeter of the total active area) defined by

$$I_{loop} = \gamma_c I_c = -\gamma_a I_a \qquad (2)$$

where γ_c and γ_a are the fractions of total active area occupied by cation and anion exchange regions, respectively ($\gamma_c + \gamma_a = 1$), and I_c and I_a are the corresponding circulating current densities in each region. Assume $\Delta\psi_a$ and $\Delta\psi_c$ to be given by their values for ideally permselective materials, neglecting activity coefficients. Remembering that approximately 1 mole of salt crosses the membrane for each Faraday passing around the loop, we have by Kirchhoff's voltage law

$$J_{KCl} \approx \frac{I_{loop}}{F} \approx \frac{1}{(R_c + R_a + R_I + R_{II})} \cdot \frac{2RT}{F^2} \ln\left(\frac{c_I}{c_{II}}\right) \qquad (3)$$

where c represents KCl concentration and F the Faraday. This expression should apply equally well to a mosaic with numerous anion and cation exchange regions, that is, with many loops of current circulating in parallel (*4*).

We have found the membrane resistances of our mosaics, R_c and R_a, to be essentially independent of external salt concentration (at least in the range 0.05 to 0.4*M* KCl). For a given membrane, R_I and c_I are specified by the composition of the dialysand. It follows, from Eq. 3, that an appropriate choice of the dialysate KCl concentration c_{II} (and hence of R_{II}) will maximize J_{KCl}. This effect is shown in Fig.

Table 1. Dialytic separation of KCl from five uncharged solutes (u); experimentally determined values of the KCl permeability ω^*_{KCl}, the permeability of the uncharged species ω^*_u, and the permeability ratio. The results of two separate experiments are tabulated for each uncharged solute; the measured permeabilities generally agreed to within 15 percent. The initial concentrations of uncharged species were chosen arbitrarily and should not appreciably influence the values of ω^*_{KCl} and ω^*_u obtained.

Uncharged solute (u)	Molecular weight	Solute u in dialysand (mole/liter)	$\omega^*_{KCl} \times 10^{15}$ (mole dyne^{-1} sec^{-1})	$\omega^*_u \times 10^{15}$ (mole dyne^{-1} sec^{-1})	$\omega^*_{KCl}/\omega^*_u$
Methanol	32.0	0.01	3.2	0.47	6.8
		.01	2.7	.49	5.5
Urea	60.1	.15	3.0	.50	6.0
		.15	2.7	.49	5.5
Glycine	75.1	.01	2.8	.18	16.
		.01	3.1	.21	15.
L-Phenylalanine	165.2	.05	3.1	.13	24.
		.05	3.0	.15	20.
D-Mannitol	182.2	.20	4.3	.043	100.
		.20	3.3	.046	72.

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