analyses ranged from 0.40 to 0.60 part per million; the median was 0.48 and the average 0.49. Comparison with the bismuthiol II method (*2,7*) was favorable. On native sulfur from Little Sitkin Island, Alaska (*6*), a 10-mg sample, in 16 analyses by the new method, showed from 3.0 to 7.0 parts of Te per million (median, 3.5; mean, 4.2). Six analyses of a 5-g sample by the bismuthiol II method showed 3.7 to 5.0 ppm (median, 4.8; average 4.5).

Although in its present state of development the method is not as precise as desired, its extreme sensitivity permits the determination of Te in a variety of geologic materials and especially in minute samples of pure minerals. The method is rapid, simple, and sufficiently precise to yield useful data for the study of the geochemistry of tellurium.

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6 May 1963

Thin Membranes of Parlodion

Abstract. *Parlodion membranes 50 to 1000 Å thick have been prepared by deposition of isoamyl acetate solutions on the air-water interface under conditions of controlled evaporation. Capacitance and resistance measurements have established the integrity of large areas of these membranes. The former also provides corroboration of the thickness estimated from the amounts of parlodion deposited.*

In a recent review of the preparation of membranes, Carnell and Cassidy described a method for the preparation of uniform and thin membranes of pyroxylin on glass surfaces (*1*). They also pointed out that Gershfeld (*2*) cast thin membranes (300Å or less) on aqueous surfaces with solutions of parlodion in isoamyl acetate solvent.

We encountered difficulty in obtaining uniformly intact membranes by both techniques. It was very difficult to float thin membranes of parlodian (200 Å or less) off the glass plate by the method of Carnell and Cassidy. There was the additional drawback that we were uncertain about the thickness of the membranes. The casting technique (*2*) proved unsatisfactory without proper precautions (*1*). In our hands, air currents removed the solvent so violently, that most of the aqueous surface was not covered with the membrane. As a result, repeated trials to cast completely intact membranes ended in failure.

We have developed a method for producing uniform membranes of definite thickness. It retains the simplicity of the Gershfeld technique but observes the precaution of the Carnell-Cassidy procedure of controlling solvent evaporation.

A known weight of parlodion (purified pyroxylin, Mallinckrodt), usually not less than 5×10^{-3} g/ml of solution, was dissolved in isoamyl acetate. Stronger solutions could be used to prepare thick membranes. A cell of the type shown in Fig. 1 was used for forming membranes. It had a glass ring (inside diameter, 4.6 cm; height, 1.6 cm), one end of which was closed by a tight-fitting rubber stopper that had been cleaned by boiling with strong alkali, and subsequently with distilled water. A platinized platinum electrode, or one end of a salt bridge, or both, could be introduced into the cell through holes bored in the rubber stopper in order to make electrical measurements. The volume of the cell was about 13 ml.

Since the quality of the membrane was governed by the water used, ordinary distilled water, redistilled with acid permanganate in a two-stage quartz still was always used in the preparation of solutions. The cell was filled to the brim with aqueous solution (for example, 0.1*N* KCl), and the surface was repeatedly swept with a clean rectangular piece of Teflon 0.5 cm thick. The cell was covered by a close-fitting beaker (150 ml) with a pin hole at the top through which a calculated quantity of the solution of parlodion in isoamyl acetate was transferred gently on to the aqueous surface with a Hamilton microsyringe. Interference colors develop and slowly disappear, leaving an intact membrane covering the entire aqueous surface. The thickness was estimated from the known

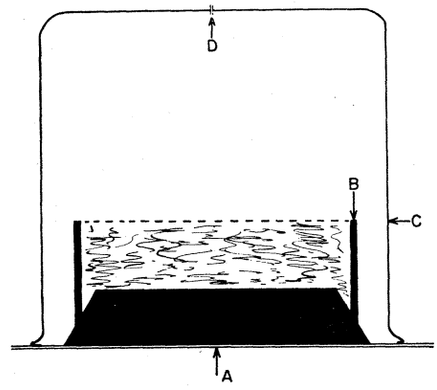

Fig. 1. Assembly for preparation of thin parlodion membranes. *D*, opening in inverted beaker, *C*, through which the fine needle of a Hamilton syringe is inserted to apply a measured parlodion-isoamyl acetate drop on the surface of the solution in glass ring, *B*. *A*, rubber stopper seal for *B*, through which electrodes are inserted.

quantity of parlodion deposited in the known surface area. This was confirmed by calculations from the measured capacitance and the known dielectric constant (6.4) of parlodion (*3*).

The integrity of the membranes was checked by measuring both their electrical resistance and capacitance with a General Radio impedence-admittance bridge. A small fluid electrode (0.1*N* KCl in contact with platinized platinum wire, as in the subphase) of 0.28 cm² was brought gently into contact with the surface at a number of points in succession so that many determinations of capacitance and resistance were made on each membrane. The order

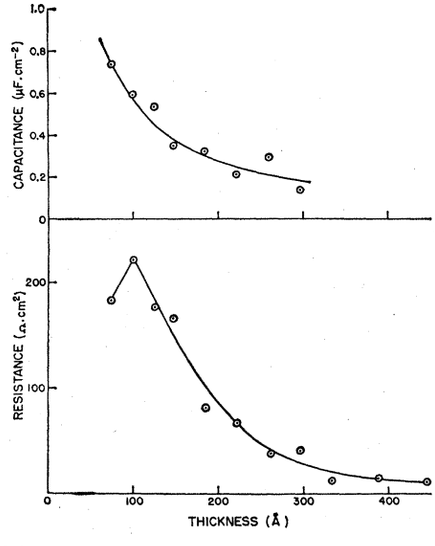

Fig. 2. Typical curves relating membrane resistance and capacitance to the thickness of parlodion membranes.

of variability of both electrical parameters for a given 100-Å membrane was about 10 to 15 percent and was less for thicker membranes. These parameters were approximately 250 ohm cm^2 and 0.57 μfarad/cm^2 for a 100-Å membrane.

These procedures, including diffusion potential measurements, have been applied to hundreds of membranes of thicknesses ranging from 50 to 1000 Å. In Fig. 2 are shown the typical increases in both resistance and capacitance with decreasing thickness of membrane formed from solution containing 5 mg of parlodion per milliliter of isoamyl acetate.

The resistivities vary inversely with thickness; they are about 2×10^8 ohm cm for 100–Å membranes and about 5×10^6 ohm cm for 400–Å membranes (*4*).

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3 May 1963

Erythrocytes: 5′-Adenylic Acid Deaminase Requirement for Ammonia or Monovalent Metal Ion

Abstract. *5′-Adenylic acid deaminase free from sodium and potassium ion is prepared from erythrocytes by a convenient method. Like adenosine triphosphatase and adenylic kinase from erythrocytes, the deaminase is activated by some monovalent cations, but unlike these enzymes it requires the presence of a monovalent cation. In all three instances the pattern of activation by ions is similar and suggests a common mechanism.*

The ability of most cells to accumulate K^+ in preference to Na^+ from a medium with higher concentrations of sodium than potassium is dependent on a process of active transport. The movement of ions against their electrochemical potential gradient requires energy supplied by cell metabolism. In recent years accumulated evidence from several laboratories has indicated a close connection between the Na^+-, K^+-activated adenosine triphosphatase of various membrane preparations and the active transport of monovalent metal ions (*1*, *2*). Evidence for such a relationship is strongest for erythrocytes, in which enzymic activities of the isolated membranes have been correlated with those of active transport in the intact cells (*2–4*). The enhancement of the activity of adenosine triphosphatase of the erythrocyte membrane requires both Na^+ and K^+, and it can be prevented by ouabain (*2*, *4*). When the membranes are broken by ultrasonic vibrations, this activity is enhanced with either Na^+ or K^+, and in this case enhancement is no longer affected by ouabain (*6*).

Preparations of erythrocyte membrane also contain an adenylic kinase which is activated by either Na^+ or K^+ (*6*). In my studies of adenosine triphosphatase and adenylic kinase, a 5′-adenylic acid deaminase was discovered in the membrane preparations. This enzyme, in contrast to the adenosine triphosphatase which is tightly bound to the membrane, can be obtained in soluble form. While adenosine triphosphatase and the adenylic kinase exhibit a basic activity even in the absence of Na^+ and K^+, the deaminase absolutely requires a monovalent ion if activity is to occur. However, the pattern of activation of the three enzymes by Na^+ and K^+ is quite similar. Potassium ion is a better activator for all, and the concentration of each ion for half-maximal activation (*Km*) is the same for the three enzymes. This similarity suggests either a close relationship between the three enzymic activities or a common mechanism of activation of three separate enzymes. In any case, studies on the properties and the mechanism of ion activation of the soluble deaminase should contribute to our understanding of the molecular basis of cation selectivity in erythrocytes.

Partially purified 5′-adenylic acid deaminase was prepared as follows: erythrocytes were obtained by centrifugation from blood-bank blood 3 to 5 weeks old and washed in saline. Packed cells were hemolyzed in ten volumes of ice-cold distilled water. The hemolyzate was allowed to stand at 4°C for 2 hours. The precipitate formed was separated from the supernatant by centrifugation at 2000*g* and washed six times with five volumes of cold distilled water. The fluffy precipitate contained the membranes of erythrocytes and some hemoglobin as was evident from its pink color. It was practically free from Na^+ and K^+, as ascertained by flame photometry tests. The precipitate was suspended in one volume of 0.08*M* tris-HCl buffer, *p*H 7.4, and stirred in cold for 1 hour. The suspension was then centrifuged at 18,000*g* for ½ hour. The supernatant was decanted. Table 1 shows the 5′-adenylic acid deaminase activity of this extract in the presence of various ions.

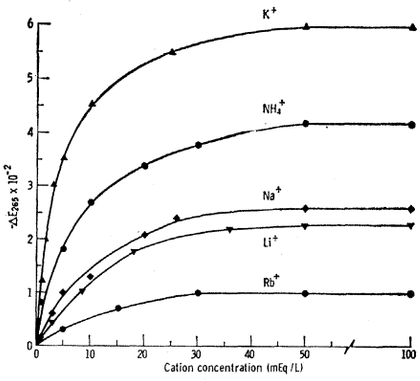

Fig. 1. Effect of varying concentrations of ions on the 5′-adenylic acid deaminase activity of human erythrocytes. The ordinate represents the decrease in the extinction of 1:30 dilutions of the deproteinized reaction mixtures.

The total deaminase activity of eryth-

Table 1. Effect of cations on 5′-adenylic acid deaminase of erythrocytes. The reaction mixture contained 5 μmoles of 5′-adenosine monophosphate, 0.1 mmole of tris-HCl, *p*H 7.1, 0.125 meq of the cations, and 0.5 ml of enzyme solution. Total volume 2.5 ml. Chloride salts were used in all cases. Histidine and imidazole were added as buffer solutions at *p*H 7.1. The solutions were incubated at 37°C for 90 minutes and deproteinized by the addition of 1.5 ml of 8 percent $HClO_4$. Extinctions of appropriate dilutions were measured at 265 mμ and compared with those of the control. A decrease of 60 percent in *E* indicates complete deamination (*8*). Ammonia was determined colorimetrically after the solution had been treated with Nessler's reagent.

Cation	Decrease of *E* at 265mμ (%)	NH_3(μmole)
$Tris^+$	0	0
K^+	58	5
Na^+	56	5
NH_4^+	56	—*
Li^+	57	4.9
Rb^+	49	4.1
Cs^+	2	0
$Imidazole^+$	3	0
$Histidine^+$	0	0
$N(CH_3)_4^+$	1	0
Mg^{++}	0	0
Ca^{++}	1	0

* The added NH_3 was much larger than that produced in the reaction so that no measurement could be made.