PRELIMINARY NOTES

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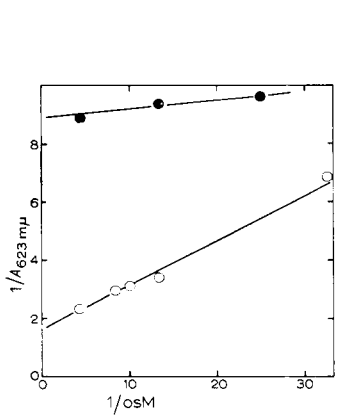
On the permeability of isolated bovine retinal outer segment fragments

The availability of a large scale preparation[1] of isolated bovine retinal outer segment fragments makes possible direct tests of the permeability properties of these organelles. Additional information on the permeability of these photoreceptor membranes would seem particularly desirable in view of recent interest in ionic hypotheses for the visual process[2]. In addition, comparison of the permeability and compartmentation of this organelle with the corresponding properties in mitochondria and microsomes may permit certain generalizations concerning the properties of intracellular membranes. The present communication describes a preliminary survey of the osmotic response of retinal outer segment fragments to various solutes and the modifications in permeability and morphology which can be induced by the addition of valinomycin and uncouplers of oxidative phosphorylation.

Retinal outer segment fragments, like isolated mitochondria, respond as osmometers when suspended in solutions of KCl (Fig. 1). The increase in volume with decreasing osmolarity of the suspending medium is readily detected by absorbance measurements as shown, by increased tritiated water content, and by packed volume



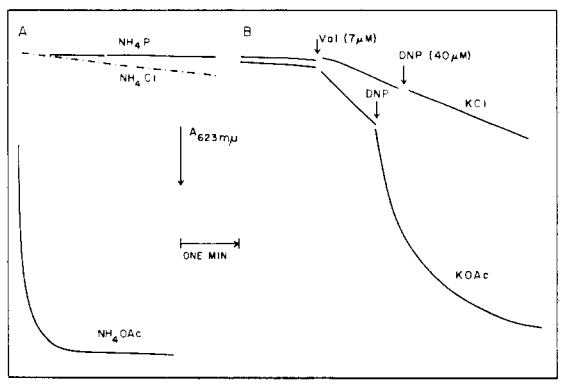


Fig. 1. Osmotic response of retinal outer segment fragments to KCl (○—○) and ammonium acetate (●—●). Retinal outer segment fragments (5 mg of protein) were stirred into 3 ml of the indicated salt solution in a cuvette and the absorbance at 623 mμ was read after 2 min using an Eppendorf photometer.

Fig. 2. Osmotic swelling of retinal outer segment fragments in various salt solutions. Retinal outer segment fragments (5 mg of protein) were added to 3 ml of a 0.12 M solution of the indicated salt and the absorbance at 623 mμ was recorded using an Eppendorf photometer. During the incubation shown in Fig. 2B valinomycin (Val; 7 μM) and 2,4-dinitrophenol (DNP; 40 μM) were added at the indicated points.

Abbreviation: CCCP, *m*-chlorocarbonylcyanidephenylhydrazone.

studies. The data indicate that a compartment is present which is enclosed by a semi-permeable membrane which responds to KCl. The studies of CHAPPELL AND CROFTS[3] have established that mitochondria also exclude K^+ and Cl^- but are readily permeable to NH_4^+ and acetate (presumably by virtue of the equilibrium between these ions and the unionized forms). The data of Fig. 1 establish that retinal outer segment fragments show little osmotic response when suspended in solutions of ammonium acetate. It can therefore be concluded that both of these ions also pass the semi-permeable membrane of the retinal outer segment fragments with ease. Osmotic swelling of retinal outer segment fragments in 0.12 M ammonium acetate is a rapid reaction, but a corresponding permeability is not observed in NH_4Cl or ammonium phosphate (Fig. 2A). Therefore, mitochondria and retinal outer segment fragments share a limited permeability to Cl^-. It has been postulated that the mitochondrion is permeable to P_i by virtue of a carrier for this anion in the membrane[3,4]. The impermeability of the retinal outer segment fragments to P_i would indicate that such a carrier is not present in the membrane of this organelle. The retinal outer segment fragment membrane also appears to be readily penetrated by propionate, formate, and lactate by these criteria but not by malate, succinate, and other Krebs cycle intermediates.

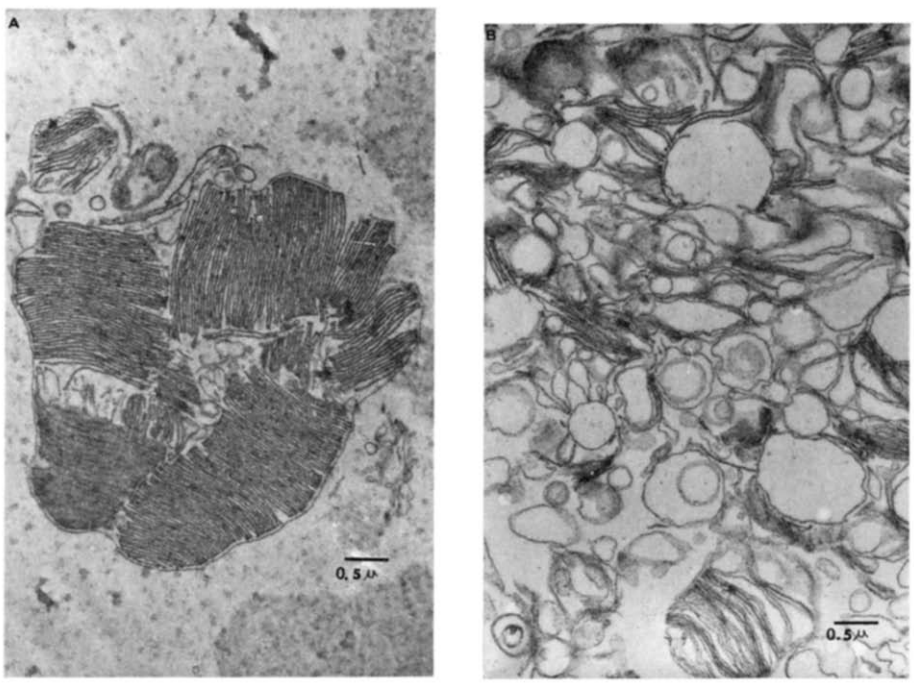


Fig. 3. Electron micrographs (× 12000) of retinal outer segment fragments isolated from isotonic potassium acetate. The preparations were incubated simultaneously with the isotope labeled experiment reported in Table I (*cf.* legend for Table I for the experimental conditions). After centrifugation the particles were fixed in 1% OsO_4 in buffered 0.12 M potassium acetate, dehydrated, embedded in Maraglas and sectioned at 150 Å. After double staining in lead citrate and uranyl acetate, the sections were examined in the electron microscope. Fig. 3A shows the appearance of typical retinal outer segment fragments isolated in the absence of gramicidin and CCCP; 3B shows a typical field from the retinal outer segment fragments incubated with gramicidin and CCCP.

Retinal outer segment fragments appear relatively impermeable to cations other than NH_4^+ (and to a limited degree to $Tris^+$). There is little tendency for K^+, Na^+, or Li^+ to support osmotic swelling, even in the presence of the permeant acetate anion (Fig. 2B). Permeability to K^+ can be increased markedly by the addition of low concentrations of gramicidin or valinomycin, and the increased osmotic swelling supported by K^+ in the presence of the antibiotic is dramatically increased by the addition of dinitrophenol or *m*-chlorocarbonylcyanidephenylhydrazone (CCCP) (Fig. 2B). Uncouplers in the absence of valinomycin do not induce osmotic swelling. The retinal outer segment fragment membrane can, therefore, be added to the growing number of natural and artificial membranes which respond to the "ionophoric" antibiotics and to combinations of these antibiotics with uncouplers[4,5]. Preliminary experiments indicate little change in permeability to Na^+ or K^+ by these criteria as a function of illumination of the retinal outer segment fragments.

Isolated mitochondria appear to contain two compartments, one readily permeable to sucrose and to other solutes of low molecular weight, and the other which responds as an osmometer to changes in the concentration of these solutes[6]. Isolated retinal outer segment fragments show marked changes in absorbance at 623 mμ when suspended in solutions of mannitol or sucrose of various osmolarities. The response does not agree quantitatively with that seen in KCl and other non-penetrating salts, however. The basis for this discrepancy is under investigation by techniques employed in the study presented in Table I. This study establishes that a large portion (about 50 %) of the water of a packed pellet of retinal outer segment fragments which has been sedimented from 0.12 M potassium acetate is permeable to dextran. This water

TABLE I

DEXTRAN, MANNITOL, AND K^+ SPACES OF ISOLATED RETINAL ROD OUTER SEGMENT FRAGMENTS

Isolated retinal outer segment fragments (9.3 mg of protein) were incubated for 15 min at 4° in 6 ml of a medium of potassium acetate (0.12 M), sucrose (17 mM), and Tris acetate (3.3 mM, pH 7.0) containing tritiated water ($4 \cdot 10^6$ disint./min) and ^{14}C-labeled mannitol (50 μmoles, $2.2 \cdot 10^5$ disint./min). Parallel incubations contained the identical number of disint./min of ^{14}C-labeled dextran (mol. wt., 60000–90000; New England Nuclear) in place of the labeled mannitol. A third set of incubations contained no label and was fixed for electron microscopy (*cf.* Fig. 3). Where indicated gramicidin (3 μM) and CCCP (3 μM) were added immediately after the rod outer segments. The segments were isolated by centrifugation (7 min at 20000 rev./min in a Sorvall SE 12 rotor), extracted with acid, and the ^3H and ^{14}C radioactivity of the extracts determined by liquid scintillation spectrometry in BRAY'S[7] medium. K^+ was determined by atomic absorption spectrometry of appropriate dilutions of the acid extracts. The solute-permeable spaces were calculated by comparing the specific activity (disint./min per μl or μmoles K^+ per μl) of the supernatants with the disint./min found in the centrifuged pellets (essentially the procedure of MALAMED AND RECKNAGLE[6] as modified in this laboratory[8]).

	μl/mg protein	
	No addition	*Gramicidin + CCCP*
Total water	8.16	11.23
Dextran permeable water	4.33	4.92
Dextran impermeable	3.83	6.31
Mannitol permeable	7.14	7.65
Mannitol impermeable	1.02	3.58
Dextran impermeable and mannitol permeable	2.81	2.73
K^+ permeable	9.33	15.00

is therefore presumed to be extra-particulate suspending medium which is trapped in the pellet. Of the water which is not penetrated by dextran, about 75 % is permeable to mannitol and 25 % is not. Analysis of the K^+ present in the pellets reveals that more K^+ is present that can be accounted for by 8.16 μl of water containing 0.12 M K^+ and that therefore binding of K^+ must take place in addition to penetration. The apparent penetration by dextran, mannitol, and K^+ is qualitatively similar to the compartmentation seen in isolated mitochondria. When gramicidin and CCCP are added to these suspensions, a marked increase in water and K^+ content is evident (Table I) but little change in the mannitol and dextran penetration occurs. Electron micrographs of the preparations examined for solute penetration in the study of Table I are presented in Fig. 3. The retinal outer segment fragments isolated from 0.12 M K^+ acetate show large numbers of reasonably intact stacks of discs (the predominant and most typical form is presented in Fig. 3A), whereas the gramicidin and CCCP-treated preparations appear to contain mostly vesicles derived from the stacked discs (Fig. 3B). It appears possible that the membranes of the discs exclude both mannitol and K^+. Addition of gramicidin increases the permeability of these membranes to K^+ and causes extensive uptake of K^- and acetate in the direction of the concentration gradient. This uptake of salt results in osmotic swelling of the discs into vesicles which retain the capacity to exclude mannitol.

A more complete account of this work will be presented for publication elsewhere.

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