the cell. While marginal folds may occur in addition, the structures which we describe here and which we have compared by scanning and transmission electron microscopy are not folds but rather projections with a circular profile in transverse section. It is unlikely that these fingerlike projections function in conjunction with the process of pinocytosis by engulfing plasma as suggested for the marginal folds (*1*). Although the projections may reflect onto and fuse with the main body of the cell, their cylindrical shape would make them no more efficient than an apposed thumb and forefinger for holding water. On the other hand, the projections could possibly enmesh small chylomicra.

The functions of the endothelial surface projections are not known. Clearly, the projections vastly increase the surface area of endothelial cells and cannot fail to affect fluid dynamics. Their density and the irregular meshwork which they form are such that they could have the effect of producing an eddying flow of cell-free plasma along the surface of the cell body. This possibility is of special interest in relation to large vessels with a high flow such as the pulmonary artery, where nutrient capillaries enter the adentitia and outer media but do not penetrate to the endothelium. A retarded flow of plasma along the surface of the cell could provide conditions of flow and pressure favorable for the exchange of metabolites and, possibly, for the metabolism of circulating hormones.

UNA SMITH
JAMES W. RYAN
Department of Medicine, University of Miami, and *Howard Hughes Medical Institute, Miami, Florida 33152*
DAVID D. MICHIE
Division of Thoracic and Cardiovascular Surgery, University of Miami School of Medicine, Miami, Florida 33152
DAVID S. SMITH
Department of Medicine, University of Miami, and *Papanicolaou Cancer Research Institute, Miami, Florida 33152*

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3. Dogs were anesthetized with sodium pentobarbital (25 mg per kilogram of body weight) administered intravenously. A portion of the pulmonary artery near the pulmonary valve was removed, immediately dropped into 0.25*M* glutaraldehyde in 0.05*M* cacodylate buffer (pH 7.4) containing 0.17*M* sucrose, and prepared for transmission electron microscopy. For scanning electron microscopy, material was transferred from the cacodylate buffer to 1 percent OsO_4 in the same buffer. The small tissue blocks were frozen in liquid Freon 12 cooled by liquid nitrogen and dehydrated under vacuum. The specimens were then attached to glass cover slips with the luminal surface of the endothelium uppermost. The material was shadowed by evaporation of a gold and platinum alloy on the surface and examined in a Philips AMR 900 microscope.
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8. U. Smith and J. W. Ryan, unpublished micrographs.
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Plasmalemma: The Seat of Dual Mechanisms of Ion Absorption in Chlorella pyrenoidosa

Abstract. *Dual mechanisms of absorption of rubidium were demonstrated in a nonvacuolate unicellular alga,* Chlorella pyrenoidosa, *in both the light and the dark. The two mechanisms were sensitive to metabolic inhibitors. At high concentrations rubidium enhanced the respiration of* Chlorella *cells. The findings support the conclusion that the mechanisms of rubidium absorption in both the low and high concentration ranges are active processes and reside in the plasmalemma.*

Epstein *et al.* (*1*) discovered that the rate of ion absorption by plant cells followed two distinct patterns possibly due to two different mechanisms, one operating over a concentration range of the ion up to 1 m*M*, and the other extending from 1 m*M* upward. Later, a theory proposed by Laties and his coworkers (*2, 3*) suggested that the first mechanism is located at the plasmalemma and the second at the tonoplast. It is further held that ions in the high concentration range enter the plasmalemma by diffusion. However, this theory has been questioned by others (*4–6*) who hold that both mechanisms are located in the plasmalemma and operate in parallel. Welch and Epstein (*6*) consider that the Laties theory contradicts the classical view of the semipermeability and ion selectivity properties of the plasma membrane.

Dual patterns of absorption have been recorded for a number of ions in tissues of several higher plants (*4, 7*) and for carbohydrate in microorganisms (*8*). Studies made so far with *Chlorella pyrenoidosa* relate to the membrane potentials and the kinetics of potassium influx (*9, 10*). Dual patterns of rubidium absorption in *C. pyrenoidosa* are reported here. It is further demonstrated that both mechanisms reside in the plasmalemma and that absorption of rubidium in the high concentration range (1 to 50 m*M*) is metabolically mediated.

The alga *C. pyrenoidosa* was grown in aerated nutrient culture (*11*) at 20°C, under 5000 lu/m^2 of fluorescent light. The cells were washed thrice with deionized distilled water before use. The procedures for incubation and measurement of ion absorption were those

Table 1. Effects of metabolic inhibitors on rubidium absorption by *Chlorella* cells from 0.2 and 10 m*M* RbCl in the light and the dark. The inhibitors (10^{-6}*M*) were presented along with RbCl. Values are means ± standard errors of the means.

Treatment	Rubidium absorption (nmole/mg per hour)			
	0.2 m*M* RbCl		10 m*M* RbCl	
	Light	Dark	Light	Dark
Control	25.5 ± 0.4	9.5 ± 0.1	37.0 ± 2.8	23.1 ± 0.9
Antimycin A	12.7 ± .8	2.5 ± .3	28.8 ± 1.2	8.0 ± .9
m-Cl–CCP	21.2 ± 1.2	7.6 ± .6	29.8 ± 1.1	15.3 ± .1
p-CF_3O–CCP	21.5 ± 0.6	7.9 ± .7	30.1 ± 1.8	14.5 ± .1

reported for isolated leaf cells (*12*). The absorption medium contained 50 mg dry weight equivalent of cells per 50 and 25 ml, in the low (0.01 to 0.5 m*M*) and high (1 to 50 m*M*) concentration ranges, respectively, and ^{86}RbCl. The flasks containing the experimental solution were shaken in a reciprocating water bath at 20° ± 2°C. At the end of the absorption period, the cells were rapidly centrifuged and washed thrice with equimolar unlabeled RbCl at 5°C, and air-dried triplicate samples were radioassayed. The *p*H of the experimental solution ranged from 6.5 to 7. All experimental solutions contained 0.1 m*M* $CaCl_2$. For experiments in which the effects of metabolic inhibitors were studied, the inhibitors were presented along with RbCl.

Efflux studies were carried out by first allowing the cells (500 mg dry weight equivalent) to absorb from 250 ml of 0.2 or 10 m*M* ^{86}RbCl for 4 hours under light. The cells were rapidly centrifuged and resuspended in unlabeled RbCl. Portions (10 ml) of the suspension were centrifuged, and the radioactivity retained in the cells was measured. The suspensions were vigorously aerated during the absorption and efflux.

Table 2. Effects of different concentrations of RbCl on the respiration of *Chlorella* cells. Respiration was measured manometrically in Warburg flasks, containing 50 mg dry weight equivalent of cells, over a period of 3 hours. Values are means ± standard errors of the means.

RbCl concentration (m*M*)	Respiration (μl of O_2 per 50 mg of cells)		
	1 hour	2 hours	3 hours
Control	43.4 ± 2.3	74.1 ± 5.8	105.8 ± 4.7
0.1	42.7 ± 0.6	73.1 ± 0.8	103.8 ± 1.3
0.5	44.8 ± 2.2	79.9 ± 1.9	113.0 ± 1.8
10.0	44.4 ± 2.2	87.7 ± 4.9	125.7 ± 5.8
30.0	45.6 ± 2.3	93.3 ± 1.7	130.3 ± 2.1

Respiration of *Chlorella* cells was measured manometrically in Warburg flasks, each of which contained 50 mg dry weight equivalent of cells, and RbCl as a variable, in a final volume of 4.4 ml.

The absorption of rubidium by *Chlorella* follows two patterns, one in the low and the other in the high range (Figs. 1 and 2). Furthermore, the dual uptake pattern is observed in both the light and the dark. The first mechanism has a high affinity for the ion [Michaelis constant (K_m) = 0.038 m*M* and maximum velocity (V_{max}) = 28.6 nmole/mg per hour in the light; and K_m = 0.182 m*M*, V_{max} = 11.11 nmole/mg per hour in the dark]. The V_{max} for uptake of rubidium in the dark, in the low concentration range, is very close to that reported for the uptake of potassium (*4*). The mechanism in the high concentration range, however, has a low affinity for rubidium (K_m = 6.45 m*M*, V_{max} = 86.9 nmole/mg per hour in the light; and K_m = 12.38 m*M*, V_{max} = 56.49 nmole/mg per hour in the dark). Absorption of rubidium from 0.2 and 10 m*M* RbCl is inhibited by antimycin A, carbonyl cyanide chlorophenyl hydrazone (*m*-Cl–CCP), and *p*-trifluoromethoxyphenyl hydrazone (*p*-CF_3O–CCP), especially in the dark (Table 1). Antimycin A is found to be most inhibitory among the inhibitors used. Absorption of rubidium from 10 m*M* RbCl in the dark is nearly equally inhibited by the two phenylhydrazones. The results of efflux studies show that there is very little loss of rubidium absorbed either from 0.2 or from 10 m*M*

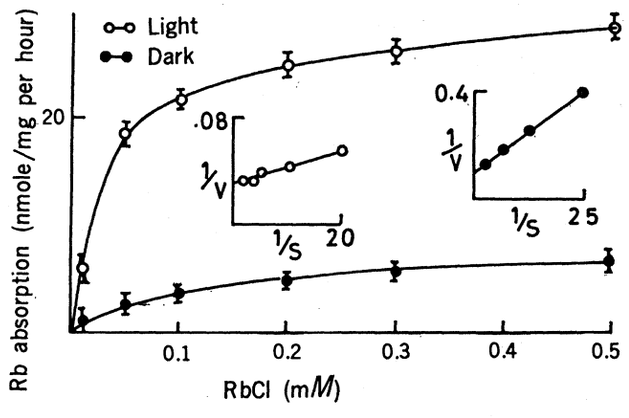

Fig. 1 (above). Absorption of rubidium by *Chlorella* in the low concentration range, in the light and the dark. Insets: Reciprocal plots of the rate of uptake *V* (in nanomoles per milligram per hour) versus substrate *S* (in millimoles per liter). Vertical bars represent standard deviations of the mean.

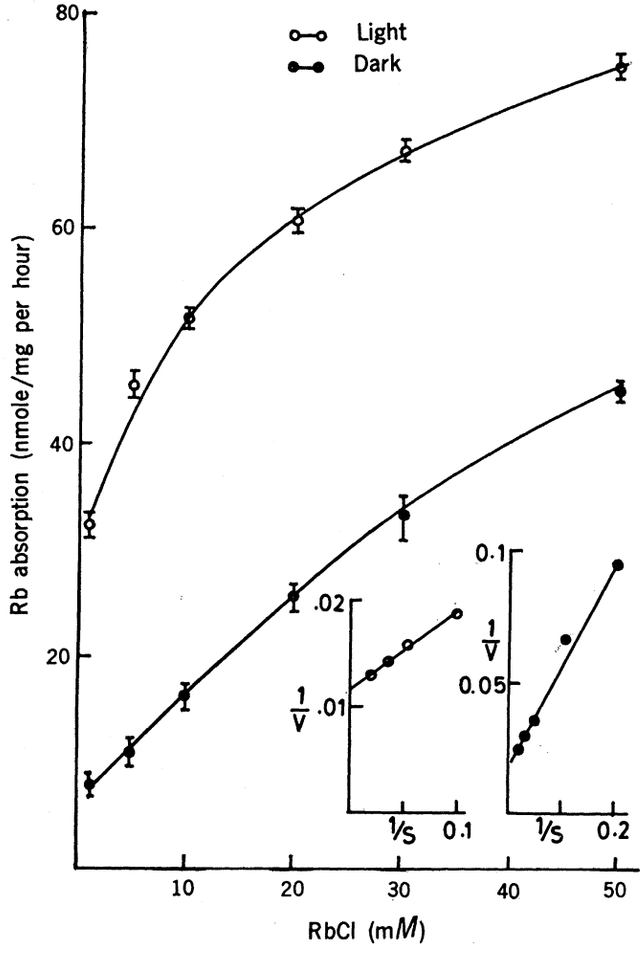

Fig. 2 (right). Absorption of rubidium by *Chlorella* in the high concentration range, in the light and the dark. Insets: Reciprocal plots of the rate of uptake *V* (in nanomoles per milligram per hour) versus substrate *S* (in millimoles per liter).

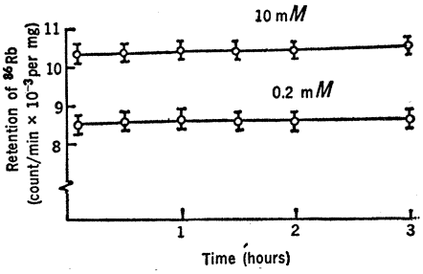

Fig. 3. Efflux of rubidium absorbed by *Chlorella* from 0.2 and 10 m*M* RbCl. *Chlorella* cells (500 mg dry weight equivalent) were allowed to absorb from ^{86}RbCl for 4 hours under light and were then transferred to 1 liter of unlabeled RbCl of equimolar concentrations. Samples were drawn at different times to measure the radioactivity retained in the cells.

RbCl, even at 3 hours (Fig. 3). Respiration of *Chlorella* is significantly enhanced by 10 and 30 m*M* RbCl (Table 2).

A dual isotherm for the absorption of a given ion points to two transport systems and raises the question of the location of each system, whether they operate in parallel at the same membrane or function consecutively across the plasmalemma and tonoplast. The evidence in support of the sequential transport through the two membranes is derived mainly from the absorption isotherms of nonvacuolate root tips and vacuolated segments of corn roots (*13*). Nonetheless, it has been admitted that the distinctions made on this basis cannot be absolute since the subapical cells differ from the apical ones for reasons other than vacuolation. Furthermore, the conclusion that the compartments into which the ions move are subcellular in origin is drawn from the kinetic analysis of ion absorption by multicellular systems (*3, 13*), which consist of cells differing morphologically and physiologically between themselves. The unicellular alga. *C. pyrenoidosa* is nonvacuolate (*9*) and hence devoid of an inner tonoplast membrane. The mechanisms for the dual pattern of rubidium absorption observed in this system therefore must reside in the plasmalemma. Chloroplasts are known to accumulate ions. However, these are functionally and anatomically different from the vacuoles. The occurrence of a dual pattern of absorption by *Chlorella* cells in the dark is evidence that the chloroplast is not implicated in the two ion-absorption mechanisms.

It has been contended that ions at high concentrations readily negotiate the plasmalemma by diffusion (*2*). However, sensitivity to metabolic inhibitors and nonexchangeability of the ion clearly support the view that the uptake, in the low and high concentration ranges, is energy-dependent. The energy for the operation of dual mechanisms in both the light and the dark (Figs. 1 and 2) is presumably derived from photosynthesis and respiration. The implication of respiration with rubidium absorption in the high concentration range (Table 2), which has been observed in higher plants (salt respiration) (*14, 15*), suggests that ion absorption by the second mechanism is not diffusive.

SESHADRI KANNAN
Biology Division, Bhabha Atomic Research Centre, Bombay 85, India

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Receptive Field Organization of Units in the First Optic Ganglion of Diptera

Abstract. *Centripetal spike potentials were recorded from two classes of units (transient and sustained) in the intermediate chiasma of flies. On-off units were characterized by a transient discharge after the onset and cessation of a light spot presented within its elliptical receptive field. Receptive fields of sustaining units were composed of three roughly circular regions arranged adjacently along a line; stimulation of the center region elicited a sustained discharge, whereas stimulation of either adjacent region elicited an off discharge. Adjacent regions antagonized the central region, for stimulation of either inhibited the discharge resulting from stimulation of the central region.*

The retina and first optic ganglion of flies have highly ordered structures. The retina is comprised of an array of several thousand ommatidia, each consisting of eight retinular cells; the first optic ganglion is composed of a corresponding array of synaptic compartments, called cartridges, each containing endings of six retinular cell axons and several second-order neurons (*1, 2*). Within each cartridge numerous synaptic contacts are made between the six retinular cell axons and two second-order neurons (type I monopolar cells) which send their axons to the second optic ganglion (*2*). Furthermore, the presence of elements conceivably capable of supporting interaction between neighboring cartridge elements (*2–4*) suggests that more than simple summation may occur within the first optic ganglion. Although slow potentials (*5, 6*) have been recorded from the cartridge region of flies, the fact that no spike potential has ever been observed raises the question of how neural signals are transmitted to the second optic ganglion. Experiments were performed which show that, indeed, spike potentials are carried by fibers en route to the second optic ganglion. Two types of fibers were identified whose spatial and temporal characteristics of discharge indicated complex integration mechanisms.

Several species of flies, principally *Phaenicia sericata*, were studied, and no species differences were observed. A specimen was prepared for an experiment by removal of a small triangular flap of exoskeleton from the posterior surface of the head capsule, which exposed one optic lobe. The preparation was placed at the center of a reflecting sphere (2 m in diameter) upon which stimulus patterns were projected. Tungsten microelectrodes (noise level 20 μv, peak-to-peak) were placed in the intermediate chiasma, and centripetal discharges representing two distinctly different classes of units were recorded (*7*).

One type is an on-off unit which responds to a diffuse light pulse with a transient discharge following the onset and cessation of stimulation. The other type is a sustaining unit which is quiescent in the dark, as is the on-off unit, but a diffuse light pulse elicits