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A new observation on *Halobacterium halobium;* light-induced volume flow through the whole organism

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Summary. A new observation on the *H. halobium* cells is reported. It has been observed that when the cells are exposed to light a volume flow is observed through them. The magnitude of the light-induced volume flow depends on the intensity and wavelength of the exciting light and is also influenced by temperature. The phenomenon appears to be relevant to the physiology of the organism.

The extreme halophile *Halobacterium halobium* has attracted attention in recent years, from the points of view of solar energy conversion and of bioenergetics[2,3]. In the present communication we report a new observation on *H. halobium* cells. It has been shown that when the cells are exposed to light, a volume flow is observed through them. Since under natural conditions the organism occurs in situations where it is invariably exposed to bright sunshine, the light-induced volume flow through the cells observed here may have a role to play in the physiology of the organism. The light-induced volume flow was found to increase with increase in the intensity of light and was found to depend on temperature and also on the wavelength of the incident light.

The experimental set-up designed to demonstrate the phenomenon of light-induced volume flow is depicted in figure 1, which has been labelled to make it self-explanatory. The U-tube (fig. 1) was partly filled with a 3% agar-agar (BDH) solution, prepared in a 4M aqueous sodium chloride (BDH analytical reagent) solution, containing 5 ml of an actively growing culture of *H. halobium* (cell mass 8.5×10^{-3} g on dry weight basis). After a few hours, when the solution in the U-tube had solidified, the rest of the glass cell (fig. 1) was filled with a 4M sodium chloride solution. At the beginning of the experiment the condition of no net pressure difference, $\Delta P = O$, was imposed on the system by adjusting the pressure head (fig. 1) – the pressure head was so adjusted that the liquid meniscus in the capillary L_1 L_2 remained stationary. The bulb B was then switched on and the consequent movement of the liquid meniscus in the capillary L_1 L_2 was noted with time using a cathetometer reading upto 0.001 cm and a stop watch reading upto 0.1 sec.

Table 1. Variation of the light-induced volume flow Jv with temperature

	Temperature (°C)		
	30	35	40
Jv × 10^{11} ($m^3 sec^{-1}$) (H. halobium cells entrapped in Agar-agar)	50.130	26.040	Not observable
Jv × 10^{11} ($m^3 sec^{-1}$)	4.098	2.306	Not observable

During the measurement of light-induced volume flow a constant and stabilised voltage of 220 V from A.C. mains was fed to the bulb B and the distance between the transport cell and the bulb B was kept fixed. To study the variation of the light-induced volume flow with intensity of the incident light, various voltages were fed to the bulb B to alter the intensity of the light. All measurements were made at constant temperature

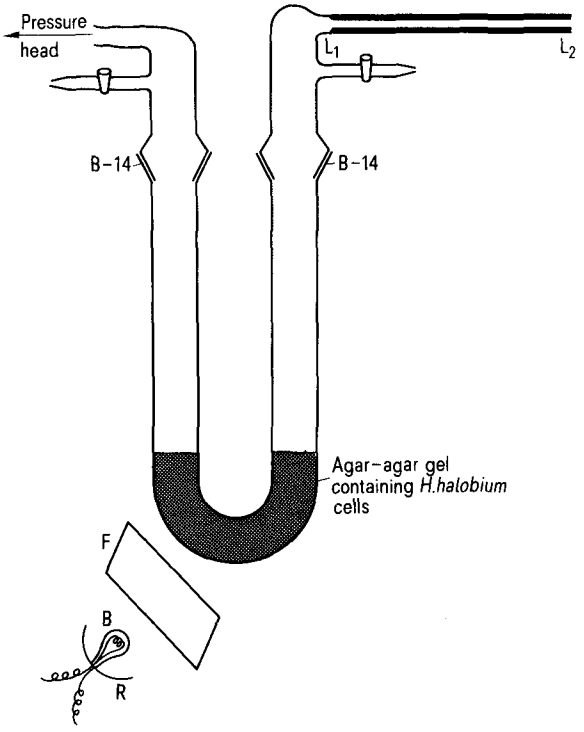

Figure 1. Experimental set-up for measurements of light-induced volume flow through *H. halobium* cells entrapped in agar-agar gel: R, reflector; B, 100 W bulb; F, Filter.

Table 2. Values of the light-induced volume flow Jv at different wavelength ranges

	Filters used White light	330–430 nm (Filter No. B-Hg-1)[a]	465–565 nm (Filter No. B-505)[a]	560–660 nm (Filter No. B-610)[a]	600–660 nm (Filter No. N-630)[a]	(IF 950)[b]	(IF 1100)[b]
$Jv^c \times 10^{11}$ ($m^3 sec^{-1}$) (Bacteriorhodopsin)	4.098 (3.326)[e]	0.198	1.115	1.350	0.825	Not detectable	Not detectable
$Jv^d \times 10^{11}$ ($m^3 sec^{-1}$) (*H. halobium* cells entrapped in agar-agar)	26.040	1.940	12.550	10.840	10.090	Not detectable	Not detectable

[a] Obtained from Photovolt Corporation, New York; [b] Infra-red filters obtained from VEB Carl Zeiss JENA, Germany; [c] Experiments performed at 30 °C; [d] Experiments performed at 35 °C; [e] Value of Jv on short-circuiting the electrodes E_1 and E_2 (fig. 3).

using a thermostat set at the desired temperature. In all experiments, whether on the whole organism or on bacteriorhodopsin (to be described later) a time gap of around 30 min was allowed between 2 successive observations. This was found to be necessary for getting reproducible results.

The data on light-induced volume flow through the cells of *H. halobium*, entrapped in agar-agar gel, are recorded in tables 1 and 2 and in figure 2. In the control experiments, i.e. without *H. halobium* cells, no light-induced volume flow was observed. The significant observations can be summarized as follows: 1) When the light was switched on, the volume flow in the capillary L_1 L_2 was not noticed instantaneously – an induction period whose magnitude was never more than 10 sec was always observed. 2) The flow in the capillary L_1 L_2 continued as long as light remained incident on the organisms trapped in the U-tube. 3) The magnitude of the light-induced volume flow increased with an increase in the intensity of the incident light (fig. 2). 4) The magnitude of the light-induced volume flow decreased with increasing temperature (table 1). 5) The magnitude of the light-induced volume flow varied with the wavelength of the incident light. Amongst all the filters used the magnitude of the volume flow was found to be maximal with the filter corresponding to the wavelength range 465–565 nm, indicating that the absorption of light by bacteriorhodopsin[4] (λmax 560 nm) may be responsible for the observed volume flow.

Experiments on the light-induced volume flow were also conducted on the *H. halobium* cells entrapped on a Sartorius cellulose acetate microfiltration membrane. The experimental set-up designed for these experiments is depicted in figure 3 (The bright platinum electrodes E_1 and E_2 in figure 3 were, infact, for use in the subsequent supporting experiments on bacteriorhodopsin). A Sartorius cellulose acetate microfiltration membrane M (Cat. No. 11107, average pore size 0.2 µm) of area 5.150×10^{-5} m^2 separated the cell (fig. 3) into 2 compartments C and D. The compartment D containing the microfiltration membrane consisted of 2 parts which could be joined to or detached from each other through a B-24 joint. The upper portion of the compartment D containing the capillary L_1 L_2 was detached and 5 ml of the actively growing *H. halobium* culture was filtered through the microfiltration membrane M (fig. 3). Since the diameter[4] of the cells of *H. halobium* is 0.5 µm and the average pore size of the microfiltration membrane is 0.2 µm only, the organism cannot pass through the membrane and is trapped on it. The compartments C and D were then assembled as shown in figure 3 and the 2 compartments filled with a 4 M solution of sodium chloride. The condition $\Delta P = O$ was imposed on the system and the data on light-induced volume flow through the organism were obtained. In these experiments also, an induction period of about 10 sec was noticed. The data obtained showed the same trends as those in the experiments with *H. halobium* cells entrapped in agar-agar gel; the value of the light-induced volume flow, which continued as

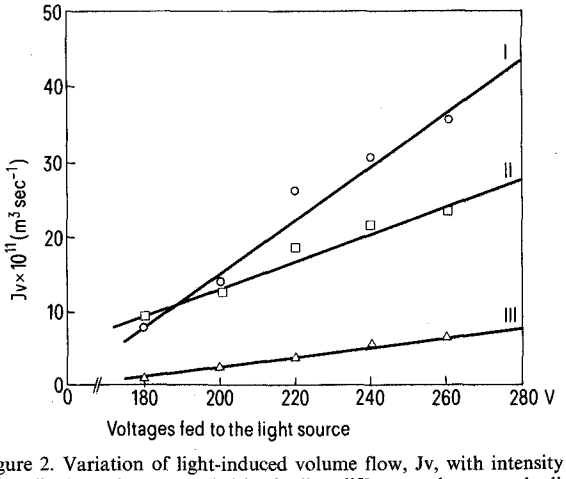

Figure 2. Variation of light-induced volume flow, Jv, with intensity of light. The intensity was varied by feeding different voltages to the light source. Curves I and II are for *H. halobium* cells, entrapped in agar-agar gel and on the microfiltration membrane respectively. Curve III is for bacteriorhodopsin.

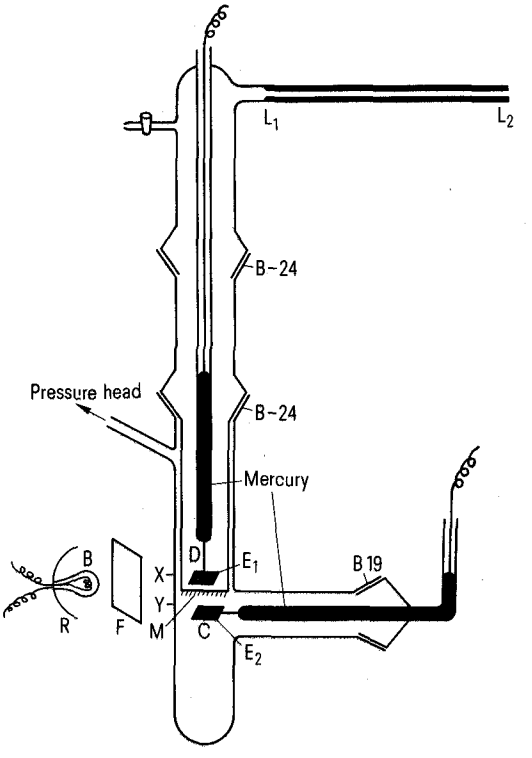

Figure 3. Experimental set-up for measurement of light induced volume flow in the case of bacteriorhodopsin. R, reflector; B, 100 W bulb; F, filter, E_1 and E_2, platinum electrodes; M, cellulose acetate micro-filtration membrane.

long as the organisms remained exposed to light, increased with increase in the intensity of the incident light, decreased with the increase in temperature etc. The data on the variation of volume flow with the intensity of light is plotted in figure 2 as an example. The control experiments, i.e. without *H. halobium* cells, did not show any light-induced volume flow in this case either. In order to ascertain whether the *H. halobium* cells remained alive during the experiment or not, the membrane filter M (fig. 3) on which the organisms were entrapped was put into sterile medium after the experiments. After a few days growth of the organism was observed, confirming that the cells remained alive during the experiments. The medium containing the organisms thus grown was exposed to light and its pH was monitored. The pH of the medium first increased and then decreased, which is in agreement with earlier observations[5,6] on living cells of *H. halobium*.

In order to gain information on the role of bacteriorhodopsin in the observed phenomenon of light-induced volume flow, experiments were also conducted on bacteriorhodopsin. For these experiments, the external surface of both compartments C and D of the transport cell (fig. 3) was painted black except for a window XY in the outer compartment C such that the solution in the compartment C in the vicinity of the membrane filter M could be illuminated while the solution in compartment D was kept dark. A 0.02 ppm solution of bacteriorhodopsin (Sigma, Cat. No. B3636; lyophilized powder from aqueous solution) was filled into the 2 compartments of the transport cell and the condition $\Delta P = O$ was imposed on the system by adjusting the pressure head. Bacteriorhodopsin is a membrane protein of the *H. halobium* cells; it is surface-active in nature and has both hydrophobic and hydrophilic moieties in its structure. It is expected, therefore, that in both compartments C and D a monolayer of bacteriorhodopsin will be formed with the hydrophobic ends of the molecules preferentially oriented towards the hydrophobic supporting membrane filter M (fig. 3) and the hydrophilic moieties drawn outwards away from it.

The data obtained on light-induced volume flow on bacteriorhodopsin (tables 1, 2 and fig. 2), which was from the illuminated compartment C to the dark compartment D, show the same trends as the data on the whole organism, i.e. increase in the magnitude of the volume flow with increase in the intensity of light (fig. 2), decrease in the magnitude of volume flow with increase in temperature (table 1) etc. As observed earlier in the case of whole organisms, in this case also the volume flow in the capillary L_1 L_2 continued as long as the light was on and ceased as soon as the light was switched off. It was also observed that the magnitude of the volume flow, comparing all the filters used, was maximum in the wavelength region containing λmax for bacteriorhodopsin (table 2). The control experiments in which no bacteriorhodopsin was used did not show any light-induced volume flow. In these experiments also, an induction time of about 10 sec was observed.

Although the present experiments were performed under controlled temperature conditions using a thermostat set at the desired temperature, it is necessary to rule out the possibility that the observed effect is not due to local heating leading to expansion of the sample. For this, experiments were performed with infra-red filters, which allowed only infra-red radiation to pass through. Since no observable flow was detected (table 2) in the experiments using infra-red filters, local heating does not appear to be a cause of the observed effect.

From the observations on whole organisms it can be inferred that there exist 2 distinct driving forces, operating simultaneously, for the observed phenomenon of light-induced volume flow through the cells of *H. halobium;* one for the volume flow from outside the cell to inside and the other for the volume flow from inside the cell to outside. Also, the rate of volume transport due to the two driving forces should be equal. What precisely the 2 driving forces are is not known, and further investigation will be required to clarify the matter. Some initial experiments were performed on bacteriorhodopsin to gain some information on the driving forces.

The purple membrane-bacteriorhodopsin from *H. halobium*, under the influence of light, is known[7] to generate an electrochemical proton gradient and an electrical potential difference across the cell membrane. The direct measurement of a light-induced membrane potential in planar black films with incorporated bacteriorhodopsin has also been claimed[8-9]. The protons are generated in the illuminated compartment. This implies that if the solution in the illuminated compartment is made more and more alkaline more and more protons will be generated in the illuminated compartment. If the proton gradient is responsible for the light-induced volume flow, making the illuminated compartment more and more alkaline should enhance the magnitude of the volume flow. To test this, the pH of the illuminated compartment was varied in the alkaline region while the pH of the dark compartment was fixed at pH = 6. For this 0.1 M tris buffer was used. It was observed that as the pH of the illuminated compartment was made more and more alkaline the magnitude of the light-induced volume flow increased (table 3). To test whether the light-induced electrical potential difference across the membrane – the supporting membrane M sandwiched between the 2 monolayers of bacteriorhodopsin generated on either side of it – is solely or partly responsible for the causation of the observed volume flow, the electrodes E_1 and E_2 (fig. 3) were short-circuited. It was observed that short-circuiting the electrodes did not stop the light-induced volume flow completely, although the magnitude was reduced (table 2), which indicates that the light-induced electrical potential difference generated across the membrane is only partly responsible for the volume flow.

Table 3. Values of light-induced volume flow Jv obtained in the case of Bacteriorhodopsin by varying the pH of the illuminated compartment and maintaining the pH of the dark compartment constant at pH = 6. (30 °C)

	pH of the illuminated compartment			
	6	8	9	10
Jv* × 10^{11} ($m^3 sec^{-1}$)	4.098	4.343	4.373	4.613

* Concentration of bacteriorhodopsin in both compartments = 0.02 ppm.

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