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Oscillations of the trans-membrane potential difference in the alga *Hydrodictyon reticulatum*

The effect of alternating light and dark on the resting membrane potential was investigated when studying ion transport in *Hydrodictyon reticulatum*. The present communication discusses the resulting oscillations which were observed.

The potential difference was measured with Pyrex glass microelectrodes of the Ling–Gerard type. The outer saline contained 0.5 mM NaCl, 0.368 mM KH_2PO_4, 0.287 mM K_3PO_4, 0.2 mM $CaCl_2$, 0.05 mM $MgSO_4$, 0.55 mM KNO_3 and ferric citrate.

Fig. 1a shows a typical change in the intracellular potential when the light was switched on and off at about 3-min intervals. The "intracellular potential" represents essentially the sum of any potential differences across the plasmalemma and the tonoplast, the tip of the microelectrode being introduced approximately into the centre of the Hydrodictyon cell, *i.e.* presumably into the central vacuole. The changes

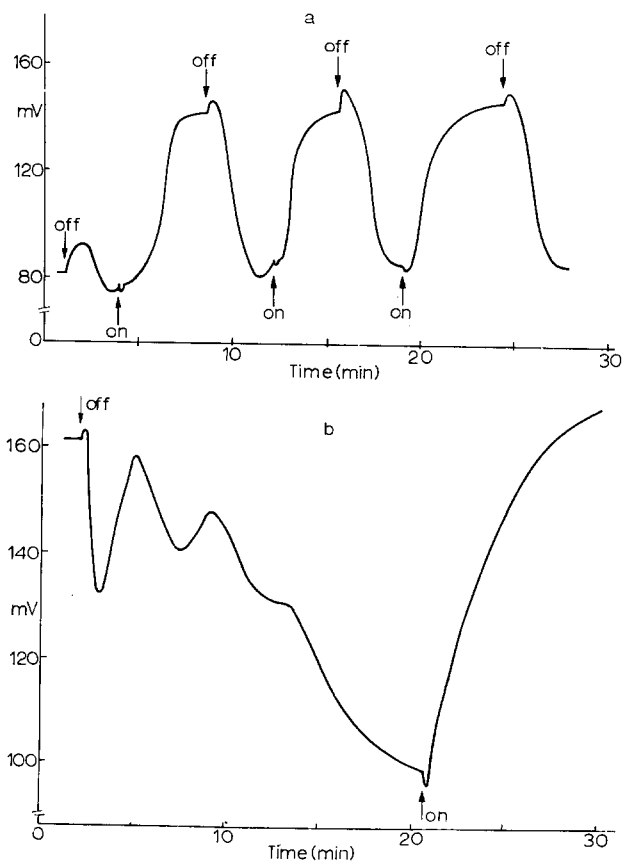

Fig. 1. a. The effect of light and dark on the membrane potential difference in *H. reticulatum*. b. Oscillation of the membrane potential in *H. reticulatum*.

of potential, an increase in light and a decrease in dark, occur within 1 min of altering the light conditions and are of about 50 mV. Although these changes are comparable with those observed by earlier authors[1], a different behaviour, not yet discussed, was observed when the intracellular potential of Hydrodictyon was allowed to proceed to a steady state. Oscillations could be observed, appearing either in light or in darkness, more rarely in both phases in any one experiment (Fig. 1b). Not more than four oscillations were obtained in an experiment under the conditions used. The period varied from 1.7 to 7.2 min, the average period being 3.7 min.

BANNISTER[2] described oscillations in the evolution of photosynthetic oxygen by *Chlorella pyrenoidosa*. This finding may be relevant to the results described here as the light-dependent phenomena shown in Fig. 1 may be associated with photosynthesis. The following mechanisms may be responsible for changes of the potential difference and for oscillations: (i) a change of membrane permeability due, for example, to varying internal ionic composition during active photosynthesis[3]; or (ii) a change in the local concentration of some ionic species (*e.g.* K^+) in a layer close to the membrane. In this connection, it has already been shown by DILLEY AND VERNON[4] that the light-dependent photosynthetic transfer of electrons is accompanied by movements of ions across the chloroplast membrane.

An attempt was made to find conditions which would be favourable for increasing the number of oscillations. In isolated mitochondria, valinomycin-induced oscillations of H^+ and K^+ fluxes, of the movement of water and oxygen consumption

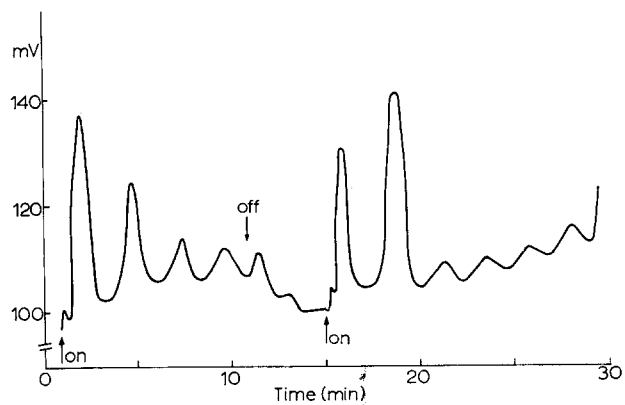

Fig. 2. Oscillations of the membrane potential difference in *H. reticulatum* in the presence of valinomycin.

have been demonstrated (*e.g.* ref. 5). Since chloroplasts and mitochondria have much in common as regards electron transport and energy transfer, the effect of valinomycin on the number and shape of oscillations was studied. With this substance added to the outer saline (2 μg/ml), as many as 14 oscillations were obtained in one such experiment. A typical response of the membrane potential difference to valinomycin is shown in Fig. 2. The initial one or two peaks, with an average period of 2.1 min (max. period 3.2 min, min. period 1.1 min) and average amplitude of 45 mV, were followed by oscillations (mostly damped) with the same period but con-

siderably lower amplitude. The oscillations occurred more often in the light phase and began within 60 sec after addition of the valinomycin.

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The phosphorus content of cell walls of *Micrococcus lysodeikticus*

The cell wall of *Micrococcus lysodeikticus*, reported by SALTON[1] to contain 0.09% phosphorus (P), has more recently been stated to contain no P (refs. 2, 3). In this laboratory, preparations of the cell wall of *M. lysodeikticus* NCTC 2665 have always contained 0.11–0.13% P and in this note we present evidence for believing that the P is an integral component of the wall and not a contaminant.

ARCHIBALD *et al.* could not isolate teichoic acid from *M. lysodeikticus* cell walls[4]. Thus, the low amount of P found in the wall could be due to a contaminant which might originate from membrane or nucleic acid. Due to the presence of carotenes, the membranes (and thus cells) of *M. lysodeikticus* are highly coloured and the preparation of "clean" walls is facilitated. The morphological purity can be checked by staining and by electron microscopy. Walls used in our experiments were prepared from cells in the stationary phase by shaking with glass beads and were thoroughly washed with Tris buffer and water.

Extraction of dry walls with refluxing ether or with chloroform–methanol (2:1) did not remove any P (Table I). Chloroform–methanol is used widely to remove phospholipid from bacterial membranes, in particular that from membranes of *M. lysodeikticus*[6]. Thus, any contamination of the wall did not appear to be due to membrane. This possibility was also checked by establishing how much membrane would be expected to be present in the wall. Membranes were prepared by lysing protoplasts[6,7] and were then shaken with glass beads under the same conditions employed for wall preparation. 20–25% of the membrane (or membrane fragments) was sedimented under the conditions used to sediment walls. Moreover, with respect to colour and P content (0.5–0.6%), the material sedimented had properties identical