THE 'ENERGISED STATE' IN BACTERIORHODOPSIN SHEETS
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It has recently been proposed /1/ that the 'energised state' of
energy-transducing biomembranes may perhaps best be viewed as a
charging of the interfacial membrane/solution capacitances, and that
the protic current flow coupling such processes as electron transport
and ATP synthesis may be largely confined to the interfacial regions
/1,2/ in vivo. Such coupling protons would not be osmotically active.
Whilst the uptake of lipophilic substances has been widely used as a
probe of energisation in biological membrane systems /3/, the inter­
pretation of such data is complicated by the fact that, for thermodynamic
reasons, most bioenergetic systems form vesicles upon isolation, and it
is unclear to what extent the probe molecules are binding to membranes
or are osmotically active. Recent experiments using resonance Raman
spectroscopy indicate that a great deal of binding may in fact occur /4/. However, purple membrane sheets /5/, isolated from Halobacterium halobium
do not form vesicles and the effect of illumination of this system on
its ability (or otherwise) to interact with 'probe' molecules should
provide much information on the requirement or otherwise of an intact
vesicular structure for membrane energisation. The purpose of the present
communication is to describe a novel polarographic assay for commonly-used
'probes' of energisation, and to report that the energised state is
readily observed in bacteriorhodopsin sheets by this method.

In a typical experiment differential pulse polarograms or linear sweep
voltammograms were performed on a solution of the probe of choice, in the
dark and under illumination, and then an appropriate concentration of
bacteriorhodopsin sheets were added, polarography being again performed
in the dark and in the light. Of those probes currently used to monitor
membrane energisation we have tested safranine, 9-amino acridine (9AA),
1-anilinonaphthalene-8-sulphonate (ANS) and Tl, since all are electro­
active at the mercury electrode. The electrochemical apparatus has been
described elsewhere /6/.

Figure 1 shows linear sweep voltammograms at the hanging mercury­
drop electrode of a safranine solution (dark or light) in the absence
(trace a) or presence (traces b, c) of bacteriorhodopsin sheets. Of
the 4 peaks given by the native solution, two are not observed in the
presence of bacteriorhodopsin, and the major peaks (II and III) are
reduced, implying that some of the safranine is bound by the sheets.
This binding is increased upon illumination. In the case of the
negatively-charged ANS, illumination causes a desorption of probe
molecules bound in the dark (data not shown).
In conclusion, we feel that care should be exercised in the interpretation of experiments using lipophilic probe molecules, and that the bacteriorhodopsin sheet system provides a useful control for the energy-dependent binding of such molecules to biomembranes.