

*Comment***The nature of local chemical potentials****A comment on a letter by Professor V.P. Skulachev****R.J.P. Williams***University of Oxford, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR, England*

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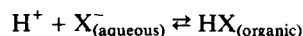
In Skulachev's FEBS Letter [1] concerning local proton energetics there are two statements relating to historical facts which I wish to correct and there is, I believe, one logical error concerning the nature of tests of local as opposed to bulk treatments of proton reactions. The comment is in regard to his distinctions between Mitchell's, Williams' and Kell's theories. Skulachev [1] begins his description of the activities of those who have 'opposed' chemi-osmotic ideas with an historical outline of Mitchell's early views and my own. In the course of the description of Mitchell's chemi-osmosis it is stated that the proton was required to move through the membrane. Reading Mitchell's early papers it is abundantly clear that no proton was required to pass through the membrane. In fact protons generated by oxidative energy or photon-energy *dragged* hydroxyl ions or *repelled* protons from the membrane to make ATP. It was this idea of field-driven chemistry which I opposed most vigorously and I still do. (Later proton movements through membranes were incorporated into chemi-osmosis but originally it was *osmotic* energies which caused chemical changes (i.e., mechanistically) and not the flow of protons – hence chemi-osmosis. I make this comment since I wish my ideas and those of Mitchell to be fairly presented. History and science both demand accuracy.)

My own views were (are) that protons generated in the vicinity of the ATP-synthetase by oxidative or photon-energy flow through the ATP-synthetase

site without equilibrating with the bulk phases. Skulachev is incorrect when he states as Williams' view that the proton did not flow through a membrane. In fact, in my first article I did not attempt to describe the observed organisation as within a membrane or otherwise but an enclosed vesicle space was stated not to be required. My condition was merely that the proton pathway was under kinetic control such that it passed through the synthetase before it discharged its energy. To remove any doubt about these facts I quote a letter from myself to Mitchell who asked me to clarify my paper before I knew of any of his views. (I now have Mitchell's permission to publish this letter.) I hope the letter convinces Skulachev and others that I had considered most of the ideas he says I opposed and that I did not reject them as ideas. Part of the letter written to Dr P. Mitchell on 5th March 1961 reads as follows:

'I hold the view that a phosphorylating system is not in equilibrium with regard to the hydrogen ion concentration. On admitting oxygen to the cytochrome chain in mitochondria the measurable pH can only refer to the bulk pH, i.e. in the vessel which contains the fluid in which the mitochondria are suspended. This pH is not related to that in the mitochondria. I wish to think about two regions of the mitochondria which are well separated in space. The region near the initial attack of oxygen – i.e. near cyt. oxidase. Here we would all agree we have the reaction $O_2 + 2 H_2O + 4 e^- \rightarrow 4 OH^-$. The electrons are trans-

ported from the end of the cytochrome chain where we have the reaction $RH \rightarrow H^+ + e^-$. In a system where electron transport is faster than hydrogen ion diffusion then there will be a rise in 'pH' near cytochrome oxidase and a fall near the terminal hydrogen carrier. The changes in 'pH' can be just as large as the redox potential difference between the couples in the two regions when the system would come into an equilibrium like that in a cell without transference. This would imply complete restriction of hydrogen and hydroxyl ion transport. One way of achieving this is to invent a membrane which is permeable to all sorts of materials such as water, carbon dioxide, and organic molecules but not to hydrogen. I can not visualise this easily but if one can, then, the 'pH' can take on its ordinary meaning in the different separate space compartments. I suggest the alternative that the two regions of space are different largely because certain groups are present in one place and not in another. The simplest system like this is a two phase liquid/liquid system. Imagine an acid partitioned across such phases. In the organic system



the acid is HX and H^+ is in low concentration but at the same thermodynamic activity as in the aqueous phase where it is in high concentration. If you ask about 'pH' I can only refer you to the traditional pH of the *equilibrium aqueous phase*. Now let this system be thrown *out of equilibrium* by addition of HX to the organic phase. It is well-known that equilibrium is then *slowly* re-obtained. However I can always say that the aqueous pH in equilibrium with the organic phase *should be*. This gives a meaning to pH anywhere.'

As this letter makes clear I have never opposed chemi-osmosis as an incorrect piece of theory in so far as proton gradients are concerned, but I have said and say again that the implied equilibration is not necessary and that the ATP-synthetase mechanism is wrong.

It is a fact that today the idea of the flow of protons through the ATP-synthetase has become incorporated into chemi-osmosis and Mitchell's view has been dropped. Mitchell and Williams did not differ on this point by 1970.

So much for the early history. I turn next to the logical problems. Skulachev believes that the development by Kell of a theory of ATP-formation using local proton distribution is still open to test but he supposes that Williams' views need no longer be examined. The basis of this view is stated

to be that no system which does not have vesicle form is known to make ATP. I dispute the logic of the argument in so far as it separates Kell from Williams. None of the tests mentioned by Skulachev can distinguish the views of Kell and Williams for the following reasons.

Kell's and other's views on local proton energies are really variants of Williams' views. (Kell stresses this in all his articles.) There is then one simple *positive* test of the distinction between all variants of local circuits and bulk phase osmotic circuits. If a local circuit is the correct description then there will be found to be a kinetic route to the ATP-synthetase which does not require the bulk aqueous phase of mitochondria or thylakoids. As soon as this path is demonstrated in vesicles then it must be a matter of time only before it will be shown that ATP-synthesis does not require a vesicle. That no such clear-cut evidence is now available is not relevant. Einstein, when faced with experiments which showed apparently that relativity theory was incorrect, stated simply that since the theory was correct, incorrect experimental tests must have been tried. Negative results do not exclude a theory. May I state that the difference between local circuits and chemi-osmosis requires studies of the rates of ATP synthesis, of proton flow, and of the rates of rise of bulk ΔpH and $\Delta \Psi$. Today, so far as these experiments have been done, the evidence is in favour of local circuits [2]. If these conclusions are accepted somebody some time *must* be able to show that vesicles are not necessary and it then will be known that chemi-osmosis is incorrect in all essentials and not just in the ATP-synthesis step. Both Williams and Kell stand or fall by this test. Skulachev's proposed tests are in fact variants of the tests which have already been done. Curiously perhaps, scientific progress in this and other fields depends on a consensus view. It takes time and repeated demonstration or falsification for ideas to gain acceptance or to be rejected. The greatest difficulty in distinguishing Mitchell's, Kell's and Williams' ideas is the design of the correct experiments to test the kinetic as opposed to the thermodynamic relationships in energy transduction.

In order that those who can do experiments on the problem may be encouraged may I draw attention to two striking features of the organisation of the thylakoid membrane. In a paper on chloroplast membranes (next but one to Skulachev's letter)

Andersson and Haehnel [3] show that in the thylakoid membranes photosystem I accumulates next to the ATP-synthetase but photosystem II fills the long-stacked regions (fig. 3 in [3]). Now photosystem I can drive ATP formation via cyclic phosphorylation and QH₂ oxidation. Photosystem II does not drive ATP-formation but acts as an alternative reductant for quinone; i.e., it supplies Q' H₂ to photosystem I. It can be suggested that QH₂ diffuses to the end sections of the membranes to react with photosystem I directly adjacent to the ATP-synthetase. This will provide a very local system. Such organisation is not a proof of Williams' and Kell's scheme but it has one simple explanation. ATP is made best by protons generated locally. If experimenters make vesicles by sonication they will not see these effects. I note that mitochondria are invaginated in a similar way to thylakoids, but mitochondrial and thylakoid particles are small spheres. My second piece of experimental evidence concerns even these spheres.

In a recent paper Tiemann and Witt, who use chemiosmotic formalism, have shown that 99% of the protons generated are membrane-bound [4]. It is not possible to describe these protons as belonging to any *osmotic* energy component. They clearly belong to the energised membrane. Even at equilibrium which will be attained rapidly in small vesicles 99% of the energy is associated directly

with the membrane and 1% is chemiosmotic. Observations using displacement techniques, e.g., movements of organic bases or of lipophilic cations, will not uncover this distinction yet it introduces the possibility of membrane structure control during energisation which is totally absent from chemiosmosis. This feature must be put together with local kinetics and could occur before equilibration with the bulk.

A final point: if decisive experiments are to be done then we must describe the two models with their appropriate equalities. No local field, local binding or local pH is part of a *chemi-osmotic* system. Skulachev must not try to protect the 'Oracle of Edinburgh' (the first statements were not from Bodmin) from a cathedral close. Oracles belong to an earlier historical period, and a different kind of religion from today's.

REFERENCES

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