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This Week's Citation Classic

Wiskich J T & Bonner W D, Jr. Preparation and properties of sweet potato mitochondria. *Plant Physiol.* 38:594-604, 1963.
[E.R. Johnson Foundation, University of Pennsylvania, Philadelphia, PA]

The conditions necessary for isolating plant mitochondria exhibiting respiratory control were examined in detail. The activation of succinate oxidation by ATP was shown to be unrelated to oxidative phosphorylation. A study of respiratory inhibitors suggested the presence of an oxidase, other than cytochrome oxidase, in sweet potato mitochondria. [The *SCI*® indicates that this paper has been cited in over 130 publications since 1963.]

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"At the time, studies on plant mitochondria were diverse, and apart from one report,[1] with little detail, no evidence of respiratory control had been provided. It was important to establish that respiratory control was an intrinsic property of plant mitochondria—a reflection of their efficiency of energy conservation and membrane integrity. I had felt that the equations used to explain oxidative phosphorylation did not really explain much, but worse, they appeared to ignore the membrane. My belief in the role of the membrane and charge separation had been greatly influenced by R.N. Robertson[2] and Peter Mitchell[3]—although I did not fully comprehend the latter paper.

"We set about examining the procedures and precautions necessary to prepare tightly coupled mitochondria, and what better place could have been chosen than the Johnson Foundation with its superb technical equipment and personalities like Britton Chance, Ronald Estabrook, and Walter Bonner—even encouragement to play cricket for a local club.

"The choice of material was difficult because of a lack of plant growth facilities. Potato was available all year and is still useful, but difficult, tissue. Today, one would use better grinding techniques and some sort of density gradient to 'purify' the mitochondria. Nevertheless, our paper showed others what could be done and how one could assess the biochemical integrity of plant preparations. It was important to establish criteria by which plant mitochondriacs could compare, and assess, the quality of their preparations. Furthermore, the paper provided some results on cyanide-insensitive respiration which could not be explained by the 'excess-cytochrome-oxidase' hypothesis. This further strengthened the suggestion of a cyanide-insensitive alternate oxidase.

"I don't really know why the paper has been cited so often—we misinterpreted the ATP-activation of succinate dehydrogenase and the coupled NADH oxidation. Now we recognise the NADH oxidation as being due to a separate enzyme on the external face of the inner membrane. However, the P/O ratios still tend to be rather low.

"The current state of affairs of plant mitochondria has been recently reviewed.[4] Our knowledge has increased greatly, but with that knowledge has come a greater appreciation of our ignorance—there is probably more confusion and more disagreement than there was in 1963. We now worry about malate oxidation and the role of NAD-malic enzyme; about the rotenone-insensitive pathway, the cyanide-insensitive pathway, and the interaction between the two. We don't worry enough about the proper operation or activation (?) of Site I phosphorylation. All of these are features which appear to be different from animal mitochondria. It is pleasing to reflect that we have progressed from a stage of following the 'animal' workers to independence, and in some instances, to leading them."

1. **Bonner W D & Voss D O.** Some characteristics of mitochondria extracted from higher plants. *Nature* 191:682-4, 1961.
2. **Robertson R N.** Ion transport and respiration. *Biol. Rev.* 35:231-64, 1960.
3. **Mitchell P.** Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* 191:144-8, 1961.
4. **Day D A & Hanson J B.** Plant mitochondria. (Tolbert N E, ed.) *The biochemistry of plants.* New York: Academic Press, 1980. Vol. 1. p. 315-58.

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