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Short Communication

Isolation of Tightly Coupled Mitochondria From Acidic Plant Tissues

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This communication emphasizes the need for continuous control of pH when macerating acidic plant tissues for the purpose of isolating tightly coupled mitochondria. A useful device for such control is described.

Mitochondria capable of oxidative but not phosphorylative activity have been isolated from apple fruits when either alkaline homogenates (5) or polyvinylpyrrolidone (PVP) (1) was used to suppress phenolic compounds. However, it was not until a pH slightly below 7, plus 0.5 % PVP, were used that phosphorylating, "coupled" mitochondria were obtained from the parenchymatous tissues of both pear and apple fruits (7, 8). Loomis and Battaile (6) have since shown that PVP is less effective at an alkaline pH. Wiskich (9) utilized an isolation medium at pH 7.4, but containing 1 % PVP, to obtain apple mitochondria with respiratory control ratios (RCR) of somewhat less than 2. Hulme *et al.* (2) reported results similar to those obtained by Wiskich.

Tightly coupled mitochondria (RCR's approaching infinity) were obtained from pear tissues only when additional precautions were taken to control pH during maceration. This was accomplished with the device illustrated in figures 1 and 2. The surface of the stainless steel screen (fig 2) has been ground slightly to provide shearing edges. Downward pressure applied as the tissue is pushed manually across the screen results in an effective rupture of cells and a good yield of mitochondria. Isolation medium is maintained at a level slightly above the screen so that the mitochondria and other intracellular materials are bathed in the solution immediately upon cell rupture. The pH is continuously monitored and held within 0.1 pH unit of the desired value with additions of 1 M KOH. We have found a pH range of 6.7 to 6.9 to yield best results. Based on the earlier experiments of Wiskich *et al.* (10) Lance *et al.* (4), and Hulme *et al.* (1), we have successfully used an isolation medium comprised of the following: 0.25 M sucrose, 50 mM tris or potassium phosphate (pH 6.8), 6 mM EDTA, cysteine (2 μmole/g tissue) or 10 mM β-mercaptoethanol, 0.5 % PVP (40,000 MW) and 1 mg/ml fatty-acid-poor bovine serum albumin. A 3:1 (v/w) proportion of medium to tissue has been used. The extent of coupling in mitochondria that may be obtained with this method is shown by the oxygen electrode trace in figure 3.

Ku *et al.* (3) have also isolated tightly coupled mitochondria from tissues of intermediate acidity,

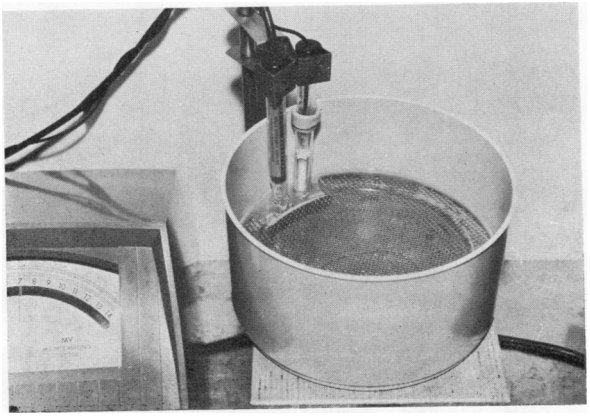

FIG. 1. Device used to macerate tissues under carefully controlled pH.

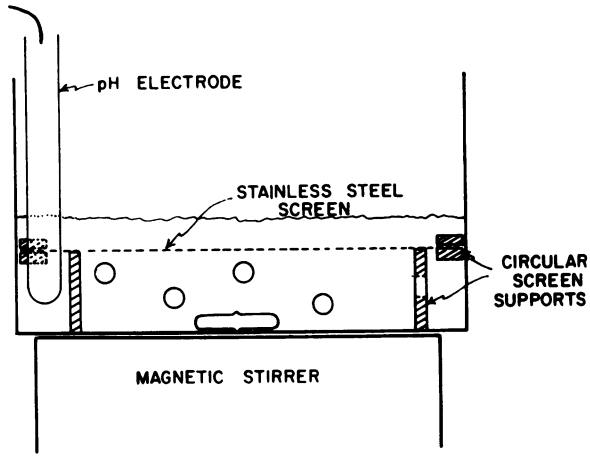

FIG. 2. Schematic cross section of the device pictured in figure 1. The 12 mesh screen is positioned to suit volume of material. A 25 mm screen height in a 160 mm diameter vessel has been used to macerate 200 g tissue in 600 ml of medium.

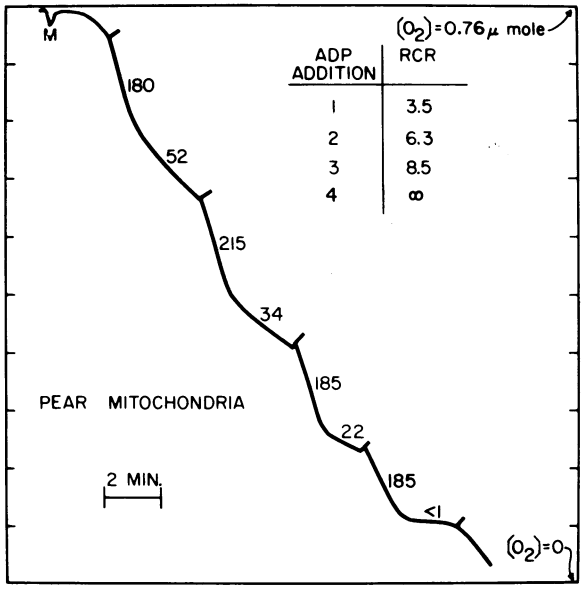

FIG. 3. Oxygen electrode trace obtained with mitochondria from parenchymal tissue of pear fruits. Repeated additions of 0.3 μmole ADP are indicated by the slant lines. Numbers along trace refer to rate of O_2 uptake in μl/hr. Reaction mixture: 1.5 μmole sucrose, 60 μmole P_i (pH 6.8), 30 μmole α-ketoglutarate, 0.03 μmole DPN, 0.1 μmole thiamine pyrophosphate, 3 μmole $MgCl_2$, 0.013 μmole CoA, 3 mg bovine serum albumin. Final volume—3 ml + 0.03 ml with each addition of ADP. Temperature 25°.

such as tomato or melon fruits, by gently slicing the tissues into the isolation medium with reference to pH paper or a pH meter to monitor acidity. Though effective as a gentle technique, slicing seriously limits the number of cells broken and the consequent yield of mitochondria. The shearing effect obtained with a screen is also obviated as fruits soften. However, reasonable cell breakage is achieved by merely pressing the tissues through the mesh.

It would be fallacious to imply that the procedure described herein unfailingly results in mitochondria with high or infinite RCR's. Too many unknowns are yet operative; and, indeed, one must question whether respiratory control is the ultimate index of mitochondrial integrity. The capacity of isolated mitochondria to maintain respiratory control at elevated temperatures, or to recover from damage, (unpublished results) may yet be a more sensitive index to mitochondrial integrity than is respiratory control *per se*.

Acknowledgment

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