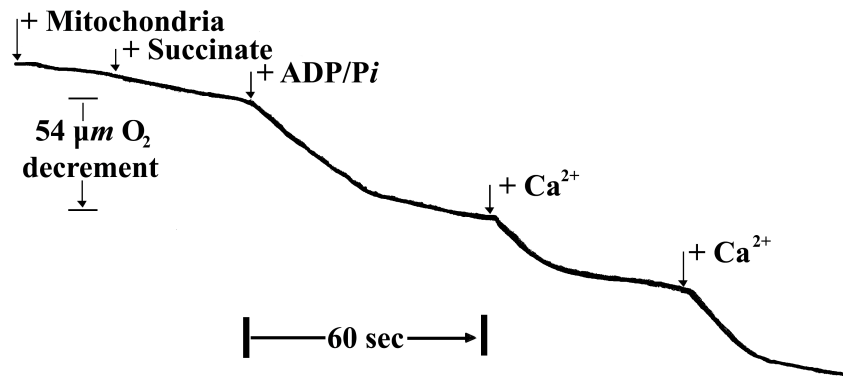


2. (30 pts) During the classical period of bioenergetics research (1960's), Britton Chance and his students characterized what was then considered a peculiar response of isolated, actively respiring mitochondria to the addition of calcium ions. Consider the data from one of these experiments and answer all the following questions.

Mitochondria, freshly isolated from guinea pig kidney, were resuspended in a well-buffered isosmotic medium. An aliquot of this suspension was then assayed polarographically, to determine the effect of various additives on mitochondrial respiration (measured as oxygen reduction). The strip chart recording below indicates changes in the level of oxygen dissolved in the suspension as a function of time.



A. (8 pts) Briefly describe the experimental results, and explain why the addition of succinate and ADP and inorganic phosphate produce the effects indicated.

B. (6 pts) The addition of calcium seems to mimic the effect of ADP and inorganic phosphate. Propose a hypothesis to explain this effect.

C. (5 pts) What special membrane mechanism must mitochondria possess to account for the calcium effect?

D. (5 pts) Suggest a concrete test of your hypotheses for either B. or C. and describe clearly what the results would indicate.

E. (6 pts) What would be the effect of adding dinitrophenol (DNP) or a proton ionophore just before the addition of ADP and inorganic phosphate? Of adding it later, just before the addition of calcium?