1. Utilizing macerated flight muscle isolated from the fly, Calliphora sp, Wanzemann and Buzz report the production of approximately 20 moles of ATP for every mole of pyruvate completely degraded to CO_2 and H_20. In the same study the authors also conclusively demonstrate the presence of the Krebs Cycle and an electron transport system similar to the one found in other cells (1969 Acta Artifacta 239:316-332).

Consider these data, the following equations and redox potentials, and answer all the questions.

$C_6H_{12}0_6 + 6O_2$ ------> $6C0_2 + 6H_20$

$\Delta G'^o$ = - 686 Cal/mole

$CH_3C0C00H + 5/2 0_2$ -----> $3C0_2 + 2H_20$

$\Delta G'^o$ = - 263 Cal/mole

ADP + Pi ----------> ATP + H_20

$\Delta G'^o$ = 7.3 Cal/mole

Redox Potentials, mVolts

-0. 32	NAD/NADH, H^+
0. 03	FAD/$FADH_2$
0. 03	Fumarate/Succinate
0. 08	Fe^{2+}, Fe^{3+} , Cytochrome b_K
0. 22	" " , Cyt. c_1
0. 25	" " , Cyt. C
0. 29	" " , Cyt. a
0. 53	" " , Cyt. a_3
0. 82	$0_2/H_20$

A. (6 pts) Suppose a pulse-labeling experiment were performed with the macerated tissue using ^{14}C- succinate (every carbon labeled). What compounds would you expect to contain the label initially? after 30 minutes? Why?

B. (8 pts) Draw a section of an inner mitochondrial membrane, illustrating the components of the electron transport system and the flow of electrons during the NAD and FAD oxidation.

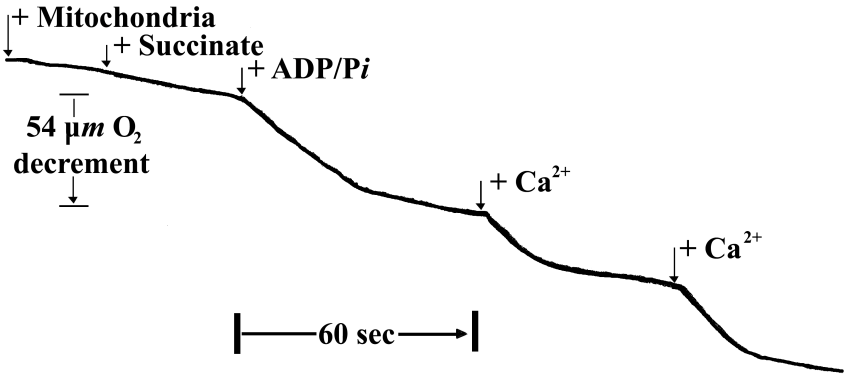
C. (6 pts) If under standard conditions, the energy stored in pyruvate is converted to ATP with 45% efficiency in rat liver mitochondria, is energy conversion in fly flight muscle mitochondria more or less efficient? Why? How many moles of ATP are produced from the complete oxidation of one more of pyruvate in rat live mitochondria? Show your calculations.

D. (6 pts) Postulate a mechanism to account for the difference in ATP production exhibited by flight muscle mitochondria.

E. (6 pts) Describe one test of your hypothesis and the result you expect to obtain.

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?