Oxidative Phosphorylation in Acute Hyperthermia

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The possibility that uncoupling of oxidative phosphorylation is a mechanism for development of acute hyperthermia during anesthesia is discussed in relation to thermodynamic balance. Uncoupling agents, such as 2,4-dinitrophenol (DNP), increase the rate of oxygen uptake and inhibit formation of adenosinetriphosphate (ATP). However, such agents would have to increase oxygen uptake many times by their secondary effects on energy metabolism to produce sufficient heat for acute hyperthermia. Intrinsic factors could also increase oxygen uptake with liberation of sufficient heat. Initial sustained muscular hyperactivity with marked heat production appears to be a possible factor in clinically observed hyperthermia. Biochemical studies of energy metabolism of muscle may lead to better understanding of this syndrome.

ACUTE HYPERTHERMIA during anesthesia is being reported with increasing frequency as knowledge of and concern for this complication become widespread. Uncoupling of oxidative phosphorylation is one of the mechanisms that have been proposed as a possible cause of this syndrome.[1] The objective of the present paper is to discuss this possibility in relation to thermodynamic balance.

Mitochondria form adenosinetriphosphate (ATP) from adenosinediphosphate (ADP) and inorganic phosphate (P_i), the required energy being supplied by respiration through the electron-transport mechanism. A simplified diagram of the process is shown in figure 1. Substrates other than succinate in the citric acid cycle have a P:O ratio (P_i to ½ O_2) of 3. This means that 3 moles of ADP and P_i are converted to 3 moles of ATP in the overall oxidation of two metabolic H atoms in the chain of reactions between nicotinamide adenine dinucleotide (NAD) and oxygen. The P:O ratio of succinate is 2. Speculation regarding P:O ratios greater than 3 had been presented recently by Smith and Hansen.[2] Numerous observations suggest that the en-

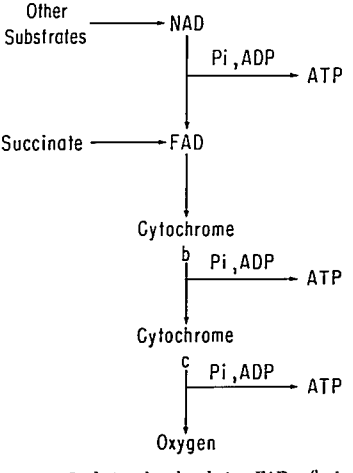

FIG. 1. Oxidative phosphorylation; FAD = flavin-adenine dinucleotide; NAD = nicotinamide adenine dinucleotide. (Modified from Schneider, W. C.: Mitochondrial Metabolism. Advances Enzym. 21: 1–73, 1959.)[3]

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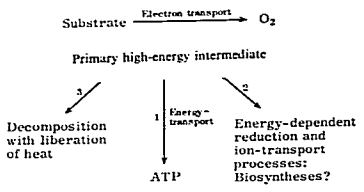

FIG. 2. Schematic diagram of possible reactions of the primary high-energy intermediate of oxidative phosphorylation. (From Schatz, G.: Mitochondrial Oxidative Phosphorylation. Angew. Chem. [Eng.] 6: 1035–1046 [Dec.] 1967. By permission of Academic Press, Inc.)

ergy generated during respiration in the electron-transport chain is not trapped directly as ATP but first appears in a high-energy intermediate compound or in a high-energy "state" of the mitochondrial membrane. The energy of this still-uncharacterized primary intermediate can be utilized either for ATP formation (pathway 1 in figure 2) or for energizing directly (without passing through ATP) other endergonic reactions in mitochondria (pathway 2 in figure 2). Finally, the intermediate may decompose spontaneously without providing useful chemical energy, the equivalent free energy probably being lost as heat (pathway 3 in figure 2).[4]

Normally, such a dissipative reaction is negligible. However, it can be greatly stimulated either by damage to the mitochondrial structure or by "uncouplers" such as 2,4-dinitrophenol (DNP).[4] Figure 3 demonstrates the possible sites of action of uncoupling agents.[5] The postulated high-energy intermediate (A ~ X) will cause an inhibition of electron transfer unless it is removed continuously. This is achieved by adding sufficient P_i and ADP to drive the reactions toward ATP formation. These conditions explain the phenomenon of cellular respiratory control—that is, the stimulation of respiration by addition of ADP and the inhibition of respiration by removal of ADP (phosphate acceptor). It is apparent from figure 3 that stimulation of respiration and inhibition of P_i esterification will occur when any one of the three high-energy compounds (A ~ X, X ~ P, and ATP) shown at points a, b, and c in the figure is degraded by hydrolysis. The system is then said to be "uncoupled" and is usually characterized by an increased rate of oxygen uptake and inhibition of phosphate esterification. The mode of action of uncoupling agents of oxidative phosphorylation is not known. However, the net result of uncoupling of oxidative phosphorylation is a speed-up in the transfer of the hydrogen from AH_2 (for example, NADH) to form BH_2 and the hydrogen from BH_2 down the cytochrome chain (as H^+ and e^-) to form H_2O from $\frac{1}{2}$ O_2. Without the disposal of the hydrogen of BH_2 and regeneration of B, the oxidation of AH_2 and the formation of A ~ X could not occur. In the absence of sufficient oxygen for regeneration of B, especially as a consequence of damage to the mitochondrial structure, other metabolic routes for regeneration of B, such as the conversion of pyruvate to lactate or of glucose and glycogen to triglycerides, may be stimulated.

The total free energy produced by oxidation of 2 H atoms through the chain between NAD and oxygen is approximately 53 kcal.[6] The formation of 3 moles of ATP represents 21 kcal of total energy produced. Under steady state conditions, no net ATP accumulation occurs. Hence, if not utilized for direct biosynthesis, the total free energy appears as heat (fig. 2). In the presence of "uncoupling agents," pathways 2 and 3 predominate. But the free energy available still remains 53 kcal. In other words, with or without the presence of uncouplers, at most 53 kcal are added to the body as heat per $\frac{1}{2}$ O_2 utilized in the process of oxidation of 1 NADH. This amount of heat is utilized normally to maintain body temperature, replacing the heat lost by the body to the environment.

Under standard conditions, $\frac{1}{2}$ O_2 represents 11.2 l. Normally, the average oxygen uptake of an adult under anesthesia is approximately 250 ml/min. Accordingly, 45 minutes are necessary to provide 11.2 l of oxygen (11.2/0.25). On the average, the specific heat of the tissue is 0.83 kcal/kg/degree C. For a 70-kg man, approximately 56 kcal of energy are needed to increase the body temperature 1 C, if the tissues are evenly heated. Since 45 minutes are required to provide at most 53 kcal to the body per $\frac{1}{2}$ O_2 utilized, almost

50 minutes are necessary to increase the body temperature 1 C ($45 \times 56/53$). In acute fulminating hyperthermia during anesthesia, the body temperature almost invariably increases more than 2 C within the first hour of anesthesia.[7] Therefore, the heat produced by normal oxidative phosphorylation alone is unlikely to be the only factor causing acute hyperthermia, even in the presence of an impairment of heat-eliminating mechanisms. For the "uncouplers" to produce sufficient heat to cause this complication, they would have to increase oxygen uptake multifold by virtue of their secondary effects on energy metabolism, unless some agent other than oxygen served as a hydrogen acceptor. At present there is no evidence supporting the participation of an exogenous uncoupling agent in acute hyperthermia. Lesions of the hypothalamic area, endocrinopathies of various types, and some unknown pathologic metabolites may increase oxygen uptake, and hence liberation of heat, considerably.

On the other hand, in almost every instance of reported malignant hyperthermia some degree of generalized muscular rigidity preceded the observed increase in body temperature. It seems possible that these patients had some type of myopathy. When all the muscles of the body are contracted at the maximal rate of activity, the metabolic rate of the whole body can increase to 1,500 to 2,000 per cent above the basal level. The thermodynamics of muscular contraction can be summarized in the following reactions[6]:

(1) $ATP + HOH \rightleftharpoons ADP + P_i + H^+ + 7.4$ kcal

(2) Creatine $\sim$ P + ADP + $H^+ \rightleftharpoons$ ATP + creatine + 2.8 kcal

(1) + (2) creatine $\sim$ P + HOH $\rightleftharpoons$ creatine + P_i + 10.2 kcal

Thus, the hydrolysis of 1 mole of creatine phosphate liberates about 10 kcal. However, neither stored creatine phosphate nor the respiratory metabolism of muscle is adequate to meet the energy demand of muscle for intense activity. Some of the demand is satisfied by glycolysis of glucose to lactate (glucose + 2 ADP + 2 $P_i \rightleftharpoons$ 2 ATP + 2 lactate). Yet the supply of ATP decreases and some ADP is accumulated. Most of the ADP is further metabolized by the enzyme adenylic acid kinase, which catalyzes the following reaction and supplies additional ATP.

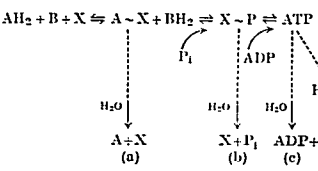

FIG. 3. Possible sites of action of uncoupling agents. HMP = hexosemonophosphate; AH_2 = substrate; B = hydrogen receptor; X = unknown substance; A $\sim$ X and X $\sim$ P = high-energy intermediates. (From Griffiths, D. E.: Oxidative phosphorylation. *In* Campbell, P. N., and Greville, G. D.: Essays in Biochemistry. New York, Academic Press, Inc., 1965, vol. 1, pp. 91–120. By permission.)

$$2\ ADP \rightleftharpoons ATP + AMP$$

Most of the AMP is further converted to IMP and ammonia, driving the adenylic acid reaction to completion. These mechanisms demonstrate the ability of muscle to maintain the supply of ATP necessary for activity. However, the quantitative capacity of this mechanism is difficult to assess. Formation of pyruvate from lactate or glucose can supply the AH_2 substrate (fig. 3) but may result also in the accumulation of pyruvate. A net formation of H^+ would accompany the formation of lactate or pyruvate from glucose, provided ATP and NAD did not accumulate. In fact, severe metabolic acidosis has been frequently observed in the survivors.

The metabolic rate and its associated heat production increase about 9.5 per cent for each 1 C increase. Thus, an increased temperature tends to produce a still higher temperature. The initial heat production, regardless of cause, leads to the development of a vicious circle. Among the possible changes in energy metabolism, the initial sustained muscular hyperactivity associated with heat production and anaerobic glycolysis seems to be a likely factor. The cause of the muscular rigidity is obscure. However, an inborn error of drug metabolism or an excessive response due to primary myopathies must be consid-

ered. Thorough biochemical studies may provide better understanding of this syndrome. These studies may include the determinations of rates of oxygen utilization, accumulation of lactate or other organic acids, and triglyceride synthesis and perhaps instantaneous measurements of glycogen, ATP, NADH, and creatine phosphate in muscle specimens.

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