VITAMIN E-DEFICIENCY IN THE GUINEA PIG:

EFFECT OF SUCCINATE ON MITOCHONDRIAL NAD-LINKED OXIDATION

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Summary

Control of β-hydroxybutyrate oxidation by succinate occurred via two mechanisms in E^+ liver mitochondria and one of these was energy-dependent (reversal of electron transport). Addition of an energy trapping system (Pi-acceptor) to E^+ mitochondria resulted in loss of energy-dependent control by succinate. Pi-acceptor had no effect on control in E^- mitochondria, indicating that reversal of electron transport was not functional in these preparations. ATP was inefficient as an energy source for succinate control in E^- mitochondria. Menadione inhibited control of β-hydroxybutyrate oxidation in both E^- and E^+ mitochondria. The effect of menadione is consistent with its capacity to by-pass the NADH-Coenzyme Q reductase segment of the respiratory chain.

Vitamin E-deficiency has been shown to influence oxygen uptake but the effect varies with the type of tissue studied (Hummel and Melville, 1951). In addition, the response in whole homogenates (Corwin and Schwarz, 1960; Grove et al., 1965; 1966) contrasts sharply with that seen in mitochondria (Corwin and Schwarz, 1959). Respiratory decline in the latter is substrate specific whereas decline in oxygen uptake by vitamin E-deficient homogenates occurs with many different substrates.

Liver mitochondria from vitamin E-deficient rats lose respiratory activity more rapidly than control preparations only when the combination of NAD^+ and succinate are used as substrate (Corwin and Schwarz, 1959). The specificity

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of this respiratory decline suggests that a functional defect may be associated with depleting liver mitochondria of vitamin E stores. NAD-linked respiratory decline by deficient liver mitochondria begins during the second 30 minutes of incubation and is attributed to an accumulation of succinoxidase inhibitor (oxalacetate) during the first 30 minutes (Corwin, 1965).

Apparently vitamin E-deficient liver mitochondria oxidize malate more rapidly than control preparations when succinate and NAD^+ are used as substrate for the tricarboxylic acid cycle. The decline in oxygen uptake in deficient liver mitochondria is therefore an indirect measure of oxalacetate formation. The requirement for succinate in this system is twofold: (a) to provide succinoxidase activity which will be inhibited as oxalacetate accumulates, and (b) to produce reducing equivalents which will regulate the rate of malate oxidation.

Succinate can "control" NAD-linked oxidations by two mechanisms. One mechanism is energy-independent and derives from the high affinity of succinate for the respiratory chain (Krebs, 1961; Krebs and Eggleston, 1962). The second mechanism is energy-dependent and competes with other energy trapping systems for respiratory energy (Chance and Hollinger, 1960; Klingenberg and von Hafen, 1963).

Corwin has suggested that vitamin E affects the energy-dependent mechanism of succinate control, since vitamin E-deficiency decreases succinate reduction of acetoacetate in liver mitochondria (Corwin, 1965). However, these data do not differentiate between the two mechanisms of succinate control so that the observed decreased reduction of acetoacetate might be due to the energy-independent mechanism of succinate control. The primary purpose of the present study was to test the effect of vitamin E-deficiency on the two mechanisms of succinate control. β-Hydroxybutyrate was the NAD-linked substrate selected for determination of succinate control because oxidation of this substrate by liver mitochondria can be measured directly by monitoring acetoacetate formation (Lehninger, 1965).

RESULTS AND DISCUSSION

The liver mitochondria studied in the present experiments were prepared and incubated under conditions which were similar to those used by Corwin (1965). However, a substantially different dietary regimen was fed to induce vitamin E-deficiency in our animals[2]. No significant difference in succinate oxidation by liver mitochondria from vitamin E-deficient (E^-) and sufficient (E^+) guinea pigs occurred unless NAD^+ was added to the incubation medium (data not shown). In the presence of NAD^+, E^- and E^+ preparations oxidize succinate at the same rate for the first 30 minutes of incubation, but thereafter, succinoxidase activity of E^- mitochondria declines at a faster rate than the activity of mitochondria from vitamin E-sufficient guinea

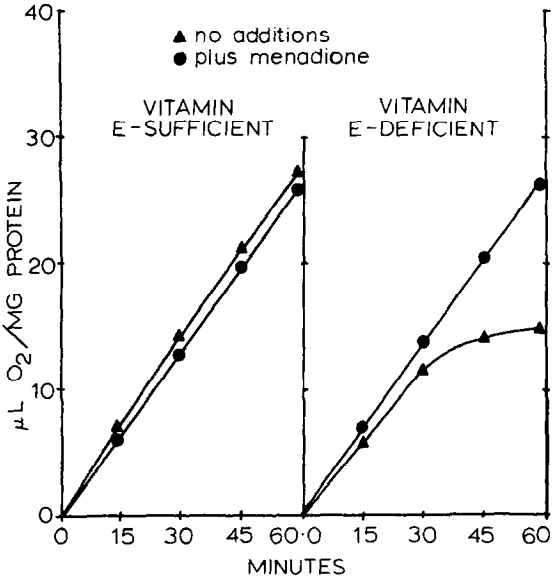

Fig. 1. Effect of menadione on NAD-linked succinate oxidation. The medium contained 1.5 mM NAD^+, 0.22 M NaCl, 2.9 mM KCl, 9.7 mM potassium phosphate (pH 7.5), 1.5 mM $MgCl_2$, 66.7 mM sucrose and 2 to 4 mg mitochondrial protein in a final volume of 3.0 ml. Succinate was present at a concentration of 14.8 mM. Menadione was added at 0.02 mM as indicated and incubation was at 37°.

[2]The dietary regimen used by Corwin had 30% torula yeast as the sole source of protein, whereas a 20% casein diet was used in the present studies (Bird and Carabello, 1966).

pigs (figure 1). This NAD-linked decline in succinate oxidation is in accord with data reported by others (Corwin and Schwarz, 1959) and is prevented by menadione. Menadione causes E^- and E^+ preparations to consume oxygen at equivalent rates.

The effect of succinate on β-hydroxybutyrate oxidation by E^- and E^+ mitochondria can be determined by measuring acetoacetate formed in the presence of varying concentrations of succinate (figure 2). It is evident that succinate inhibits (or controls) β-hydroxybutyrate oxidation in both E^- and E^+ mitochondria, and the % control by succinate increases with increasing concentrations of succinate. However, vitamin E-deficiency decreases succinate control of β-hydroxybutyrate oxidation.

Control of β-hydroxybutyrate oxidation is partially due to competitive inhibition at bifurcation points within the respiratory chain (Krebs, 1961; Krebs and Eggleston, 1962) and partially to energy-dependent reversal of electron transport (Chance and Hollinger, 1960; Klingenberg and von Hafen,

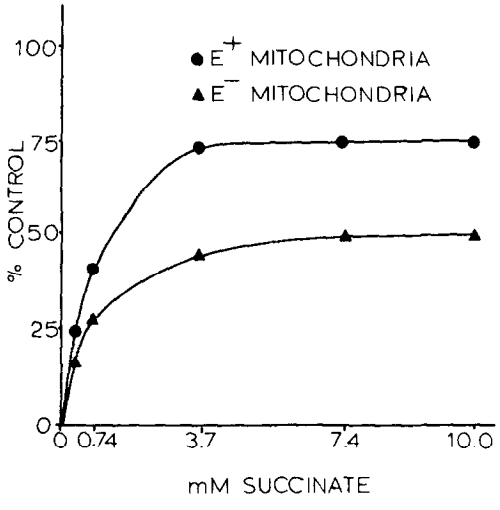

Fig. 2. Effect of vitamin E-deficiency on succinate control of β-hydroxybuty rate oxidation. The medium was as described in figure 1. β-Hydroxybutyrate was present at a concentration of 7.4 mM. Incubation was at 37° for 60 minutes. The concentration of succinate was varied as indicated.

$$\% \text{ control} = 100 \times \left[1 - \frac{\text{acetoacetate formed with succinate}}{\text{acetoacetate formed without succinate}}\right]$$

1963). In order to determine which of these two mechanisms is influenced by vitamin E-deficiency, the effect of Pi-acceptor on succinate controlled acetoacetate formation was studied. Addition of Pi-acceptor has been shown to prevent energy-dependent reduction of acetoacetate by succinate (Azzone et al., 1963). Thus the only mechanism of succinate control operating when Pi-acceptor is included in the incubation medium is control via energy-independent inhibition at a point of bifurcation.

The effect of Pi-acceptor on E^+ mitochondria to decrease succinate control of β-hydroxybutyrate oxidation to the level present in E^- mitochondria (Table I). These data suggest that vitamin E-deficiency has no effect on succinate control that operates via inhibition at bifurcation points. In contrast, these data indicate that vitamin E-deficiency acts to prevent succinate control mediated by reversal of electron transport. Presumably, E-deficiency affects energy transfer reactions that are necessary to drive the reversal process. There is no evidence to suggest that E-deficiency acts on electron transfer reactions.

ATP can serve as an energy source for the reversal of electron transport and if the energy transfer reactions involved in reversal are intact in E^- mitochondria, ATP would be expected to restore energy-dependent control by succinate. However, addition of ATP had very little effect on succinate control of β-hydroxybutyrate oxidation (Table I).

The effect of menadione on succinate control was also studied (Table I). Menadione eliminated the effect of vitamin E-deficiency on acetoacetate formation and this is consistent with our observations on oxygen uptake (figure 1). Menadione stimulated acetoacetate formation in both E^- and E^+ mitochondria, and apparently reversed both energy-dependent and independent succinate control in these preparations (Table I). The effect of menadione to reverse both mechanisms of succinate control may result from the ability of this agent to by-pass the NADH-coenzyme Q reductase segment of the respirator chain. Menadione has been shown to link NADH oxidation directly to cytochrome

TABLE I

EFFECT OF Pi-ACCEPTOR, ATP AND MENADIONE ON

SUCCINATE CONTROL OF β-HYDROXYBUTYRATE OXIDATION

The medium was as described in figure 2. When added, succinate was at 7.4 mM, ATP at 0.33 mM and menadione at 0.02 mM. Pi-acceptor consisted of: 1.4 mM AMP, 22 mM glucose and 0.5 mg hexokinase. Incubation was at 37° for 30 minutes. Figures in parentheses indicate the number of animals used in each experiment.

Additions	Acetoacetate (μg/mg protein ± s.e.m.)		% control	
	E^-	E^+	E^-	E^+
none	33.7±1.2(7)	34.8±1.8(8)	-	-
succinate	*13.2±0.8(7)	8.4±1.2(8)	60.8	75.8
succinate + Pi-acceptor	13.7±0.9(6)	12.8±1.3(7)	58.9	61.1
succinate + ATP	12.5±1.2(6)	9.8±1.2(7)	62.8	71.8
menadione	39.8±2.4(5)	38.7±2.5(8)	-	-
succinate + menadione	29.3±1.6(5)	26.9±2.0(8)	26.4	30.5

* Significantly greater than E^+ at $p<0.01$

b, thereby circumventing the first site of oxidative phosphorylation (Conover and Ernster, 1960; 1962). Menadione-mediated respiration exhibits respiratory control so that menadione does not uncouple phosphorylation from cytochrome b to oxygen.

In light of the present studies, the theory that vitamin E functions solely as a biological antioxidant should be re-evaluated. If the effect of vitamin E-deficiency in liver mitochondria were mediated by lipid peroxidation, a non-specific denaturation effect of vitamin E-deficiency on electron and energy transfer reactions would be anticipated. Instead our data indicate

that the site of action of vitamin E-deficiency is rather specific and that E-deficiency acts on the energy transfer reactions of liver mitochondria.

EXPERIMENTAL

Mitochondria were prepared by a modification of the method used by Corwin and Schwarz (1959). In all experiments, 0.8 ml of mitochondrial suspension were used and this represented 2 to 4 mg protein. Protein was determined by the method of Lowry et al. (1951) with Lab-trol (Dade Reagents Inc.) as a standard. Oxygen uptake was measured in a Gilson respirometer which was modified so that each active flask was paired with its own thermobarometer and reference flasks. Acetoacetate was determined by the method of Walker (1954).

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