Vitamin K_1 and oxidative phosphorylation*

Recently MARTIUS AND NITZ-LITZOW[1,2] have shown that the phosphorylation accompanying the oxidation of β-hydroxybutyrate is dependent on vitamin K_1. This conclusion was based on the observation that the P:O ratio of vitamin K-deficient chick liver mitochondria was 30% lower than normal mitochondria and that addition of vitamin K_1 *in vitro* could almost completely restore the lowered P:O ratio. MARTIUS[3] has interpreted these results as indicating that vitamin K_1 is concerned with only one of the three "high energy" phosphates formed in the course of the reaction. This reasoning would appear correct if it is assumed that the vitamin K-deficient mitochondria were 100% devoid of the vitamin, but this is questionable. We have reexamined the problem and have found that if rat liver mitochondria are exposed to ultraviolet (U.V.) light the P:O ratio resulting from the oxidation of β-hydroxybutyrate is lowered by about two-thirds without affecting the oxygen uptake and that the addition of vitamin K_1 to the treated system almost completely restores its P:O ratio. Because of this reversal and since vitamin K_1 can be altered by the exposure to U.V. light, we have tentatively concluded that the U.V. light is in some way destroying the vitamin in the mitochondria. The specificity of the U.V. light for vitamin K in the mitochondria must, however, await better assay methods and more data.

If we interpret our data in the same way as MARTIUS AND NITZ-LITZOW, *i.e.* on the basis of the proportional change in the P:O ratio, it can be concluded that the vitamin is concerned with two of the three phosphorylations accompanying the oxidation of β-hydroxybutyrate. However, on the basis of the absolute changes in the P:O ratios, it could be concluded that the vitamin-deficient experiments only accounted for *one-half* of a "high energy" phosphate while the present experiments accounted for *one* "high energy" phosphate.

Rat liver mitochondria were isolated by differential centrifugation in a mixture of 0.44 *M* sucrose and 10^{-4} *M* ethylenediaminetetraacetate. The isolated mitochondria were suspended in 0.1 *M* tris(hydroxymethyl)aminomethane buffer (pH 7.2) by homogenization. Oxygen consumption was measured manometrically at 30° C and inorganic phosphorus was determined by the method of LOWRY AND LOPEZ[4]. The homogenate was divided into two portions; one served as the control and the other was exposed to U.V. light, 2537 A (Mineralight, Ultra Violet Products, Inc., Calif.) while being stirred slowly for 30 min. The control sample was treated in the same manner without the U.V. exposure. The U.V.-treated mitochondria were also divided into two portions; one served as the control and the other was incubated with vitamin K_1 while being stirred slowly for 30 min. The control sample was treated in the same manner without the addition of the vitamin. All preparations and treatments were carried out at 3° C.

Our results support the original finding of MARTIUS AND NITZ-LITZOW, that vitamin K_1 participates in respiratory chain phosphorylation. We feel, however, that the lowered P:O ratio of the U.V.-treated rat liver mitochondria is more indicative of the stoichiometry of the phosphorylative reactions accompanying the oxidation of β-hydroxybutyrate than the lowered P:O ratio of the mitochondria from the vitamin K-deficient chicks.

TABLE I

EFFECT OF U.V. RADIATION AND VITAMIN K_1 ON RAT LIVER MITOCHONDRIAL P:O RATIOS*

System	*No. of Expts.*	*Average P:O*	*Range*
Control mitochondria	9	1.53	1.95–1.22
U.V.-treated mitochondria	5	0.51	0.86–0.17
U.V.-treated mitochondria + vitamin K_1	3	1.27	1.53–1.13
Control mitochondria + vitamin K_1	3	1.47	1.62–1.25

* Test system: 0.01 *M* β-hydroxybutyrate, 0.001 *M* DPN^+, $1.5 \cdot 10^{-5}$ *M* cytochrome *c*, 0.005 *M* ADP, 0.0125 *M* inorganic P, 0.01 *M* KF, and 0.01 *M* $MgCl_2$. Vitamin K_1 where added, $1.8 \cdot 10^{-6}$ *M*.

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