References and Notes

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An in vitro Effect of Vitamin D on Citrate Oxidation by Kidney Mitochondria

The possibility that an *in vitro* effect of vitamin D may be demonstrable appears evident from the effects on citrate metabolism which have been reviewed in recent publications (*1, 2*). In these it was reported that additions of vitamin D to rachitogenic and nonrachitogenic diets reduced the oxidation of citrate by kidney homogenates and mitochondria, thereby accounting for an increase in the citrate content of certain tissues and an increase in the citrate excretion in urine. Lately, we have succeeded in demonstrating a reduction in citrate oxidation by kidney mitochondria when vitamin D was added *in vitro*.

Young, male rats of the Sprague-Dawley strain were made vitamin-D deficient by the feeding of either a rachitogenic or a nonrachitogenic diet as described earlier (*1*). They were killed by decapitation, and kidney mitochondria were prepared, essentially by the method of Schneider (*3*).

The oxidations were carried out in a Warburg apparatus at 30°C with air as the gas phase. The incubation mixture, 3 ml in volume, contained 40 μmole of phosphate buffer (*p*H 7.3), 20 μmole of $MgCl_2$, 6 μmole of adenosine triphosphate, 0.08 μmole of cytochrome *c*, isotonic sucrose, and the indicated additions. Forty micromoles of glucose and excess hexokinase (Sigma) were added from the side arm of the flasks to prevent limitation of oxidation by a lack of phosphate acceptor (*4*). All substrates were added in amounts of 15 μmole, except citrate and succinate, which were added at 45 μmole, and oxalacetate, which was added at 10 μmole with an addition of 15 μmole of pyruvate.

When desired, the flask contents, prior to and following incubation, were deproteinized with 10-percent trichloroacetic acid. The filtrates, for the calculation of P/O ratios, were analyzed for P by the method of Lowry and Lopez (*5*). Citrate was determined by the method of Speck, Moulder, and Evans (*6*), and keto acids by the method of Friedmann and Haugen (*7*). Vitamin D and other compounds tested for their effect were added in propylene glycol or occasionally in the ethanol-serum albumin-phosphate buffer suspension of Nason and Lehman (*8*). In all cases, only 0.05 ml of each of these preparations was added to the contents of a flask. Control flasks received an equivalent amount of appropriate carrier.

Table 1. The *in vitro* effect of vitamin D on the oxidation of various substrates by kidney mitochondria.

Substrate	Without vitamin D (μl O_2)	With vitamin D* (μl O_2)	Decrease (%)
Citrate	85	32	62
Isocitrate	81	28	65
α-Ketoglutarate	75	80	0
Glutamate	69	55	20
Succinate	115	117	0
β-Hydroxybutyrate	35	31	11
Pyruvate plus oxalacetate	88	90	0

* One hundred and twenty-five micrograms of vitamin D_2 in 0.05 ml of propylene glycol per flask; other flasks received 0.05 ml of propylene glycol alone; 0.7 mg of mitochondrial nitrogen was added to each flask.

The comparative effect of vitamin D on the oxidation of various substrates (Table 1) clearly shows that vitamin D had a pronounced effect on citrate and isocitrate oxidation. Its effect on glutamate oxidation was small, but significant, while on the oxidation of α-ketoglutarate, succinate, β-hydroxybutyrate, and on pyruvate in the presence of oxalacetate, it had little or no effect. It is interesting to note that only the triphosphopyridine nucleotide systems are affected if one considers glutamate oxidized by both tri- and diphosphopyridine nucleotide pathways.

Table 2 illustrates the action of vitamin D in reducing citrate oxidation and α-ketoglutarate production while not affecting coupled phosphorylation efficiency to any degree.

In experiments not shown here, vitamins D_2 and D_3, when added *in vitro* in either of the carrier systems used, were equally active in reducing citrate oxidation, while equal quantities of 7-dehydrocholesterol, ergosterol, Δ^7-cholestenol, and cholesterol were inactive. However, it should be noted that in view of the different solubility characteristics of the sterols as compared with vitamin D, the possibility that they did not enter the mitochondria cannot be ruled out.

Table 2. The *in vitro* effect of vitamin D on the accumulation of α-ketoglutarate during citrate oxidation. The values represent an average of at least six determinations.

	Citrate oxidized (μg)	Oxygen consumed (μl O_2)	α-Ketoglutarate accumulated (μg)	P/O
Without vitamin D	630	83	14.4	3.0
With vitamin D_2*	180	46	8.5	2.7

* One hundred and twenty-five micrograms of vitamin D_2 in 0.05 ml of propylene glycol per flask; other flasks received only 0.05 ml of glycol; 0.7 mg of mitochondrial nitrogen was added per flask, and the oxidation was continued for 10 minutes.

The possibility that the resulting accumulation of citrate in kidney, and possibly in other tissues, may be an important factor in calcium transport and deposition appears increasingly alluring. Studies on these phases are in progress, especially with relation to specific enzyme systems.

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Insect Nutrition and Metabolism of Sterols

The importance of cholesterol and related steroids in insect nutrition was first demonstrated in 1935 (*1*), and it is now well established that insects in general require a dietary sterol (*2*). The insects' nutritional requirement for sterols is indicative of an inability to synthesize the steroid nucleus, at least in physiologically adequate amounts. This characteristic is in direct contrast to that of the higher animals, in which steroids are apparently synthesized from simple com-