THE SITE OF VANADYL INHIBITION OF CHOLESTEROL BIOSYNTHESIS IN LIVER HOMOGENATES

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Cholesterol (CHL) biosynthesis both in vitro (1,2) and in vivo (3-6) is reported to be inhibited by vanadium salts. This inhibition appears to involve reactions between mevalonic acid (MVA) and CHL but the exact locus has not been more specifically defined. Data reported in this communication demonstrate that with rat liver homogenates vanadium is an inhibitor of oxidative phosphorylation and that vanadyl inhibition of CHL biosynthesis is, in all probability, a reflection of the inability of a system to maintain a functional level of ATP.

The experiments to be described involved: (a) a preliminary period where essentially whole homogenates of liver were preincubated with and without the addition of a vanadium salt, followed by, (b) determinations by anion exchange chromatography of the level of ATP maintained in the supplemented and unsupplemented homogenates at the end of the preincubation period, and, lastly, (c) a final biosynthetic period in which paired flasks that had preincubated with and without added vanadyl salt were supplemented with MVA-2-C^{14} and reincubated for determinations of the capacity to synthesize CHL from the added precursor.

Rat liver homogenate was prepared by fragmenting in a loose (1 mm clearance) Potter-Elvehjem homogenizer 1 part of liver with 2 parts of pH 7.0, phosphate (0.1 M)-magnesium (0.006 M)-nicotinamide (0.03 M) buffer.

The homogenate was centrifuged (200 xg, 3 min) before use. Each incubation flask contained in a total volume of 11 ml the following addenda: 1 mg ATP, 10 mg DPN, 100 mg sodium succinate, 5 ml of homogenate and the indicated level of vanadyl sulfate ($VOSO_4 \cdot 2H_2O$, Fisher). Each flask was aerated (15 sec) with a stream of oxygen prior to preincubation and those flasks supplemented with MVA were again aerated prior to the final incubation period. Following the biosynthetic period involving added MVA the contents of these flasks were saponified with alcoholic KOH, extracted with petroleum ether, the ether extracts dried with sodium sulfate, filtered, evaporated to dryness, taken up in scintillation mixture, and counted. Previous studies have shown that under the experimental conditions employed the counts found in the non-saponifiable fraction (NSF) are essentially CHL or other digitonin-precipitable material. Determinations of ATP content were carried out on perchloric acid extracts of supplemented and unsupplemented flasks prepared at the conclusion of the preincubation period by treating the homogenates with an equal volume of 4% perchloric acid. Excess perchloric acid was removed as the insoluble potassium salt. ATP was determined spectrophotometrically following extended gradient elution of the extracts on Dowex-1 formate as described by Allfrey and Mirsky (7).

The results summarized in Table I demonstrate that complete inhibition of CHL biosynthesis is obtained by preincubating homogenates with about 0.005 - 0.010 M vanadyl sulfate. As shown in Figure 1, homogenate preincubated with 0.0075 M vanadyl sulfate was devoid of ATP. The activity of the NSF found in a paired flask supplemented with MVA after the preincubation period and reincubated for a determination of the capacity to utilize MVA was 23 cpm. Homogenate preincubated without vanadyl sulfate contained a level of about 2 mg ATP at the end of the preincubation period and the activity of the NSF in a paired flask reincubated with MVA was 12,731 cpm.

The results presented indicate that the site of vanadyl inhibition of CHL biosynthesis, at least as far as liver homogenate is concerned, is in reactions involving the maintenance of ATP levels in the system studied.

Table I. Biosynthesis of NSF by Liver Homogenate as Influenced by Preincubation with Various Levels of Vanadyl Sulfate.

Exp #	Vanadyl sulfate, M	NSF, cpm
1	0	5745
	0.001	3637
	0.003	721
	0.005	57
	0.007	37
	0.010	23
2	0	6207
	0.001	4086
	0.003	1312
	0.005	229
	0.007	35
	0.010	33

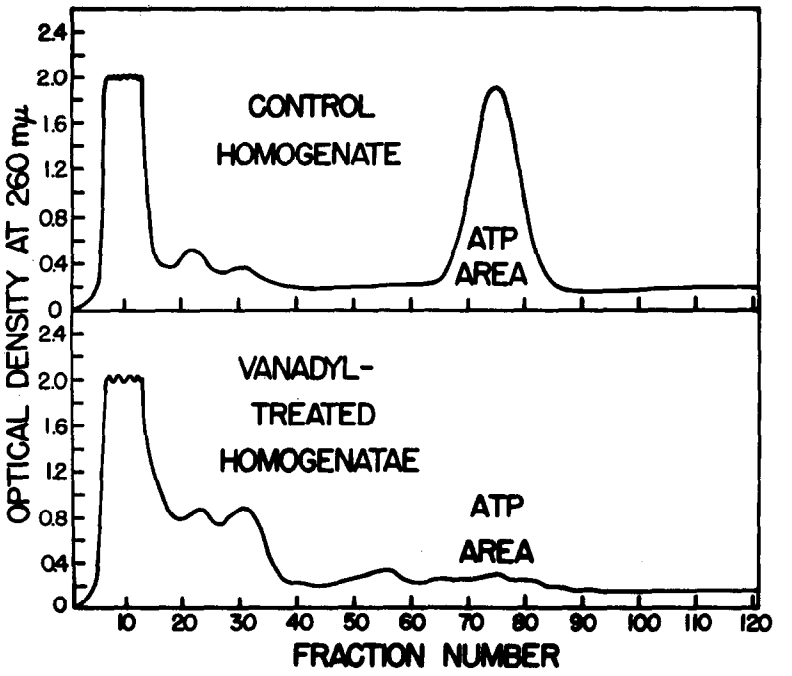

Figure 1. ATP content of rat liver homogenate preincubated with and without added vanadyl sulfate. The level of vanadyl sulfate was 0.0075 M.

ATP is, of course, essential in the phosphorylation of MVA, the phosphorylation of MVA-5-P and the concerted decarboxylation and dehydration of MVA-5-PP. If the preliminary results demonstrating a reduction of CHL biosynthesis *in vivo* both in man and lower animals following administration of

vanadium salts can be confirmed and extended and if the mechanism described here is operative in the intact animal, it follows as a corollary that ATP levels of tissues in vivo may be altered by dietary means.

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