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Effect of Inhibitors of Oxidative Phosphorylation on Biosynthesis of Cholesterol and Precursors by Liver Homogenates.* (25452)

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Essentially whole homogenates of rat liver readily convert mevalonic acid (MVA) into non-saponifiable material (NSF) and cholesterol(1). This synthetic capacity is ordinarily retained during a control preincubation period but preincubation with RNAse abolishes capacity to convert MVA to NSF(2). RNAse-treated homogenates, in contrast to controls, are devoid of ATP but homogenates pretreated with RNAse and heated liver extract from which ATP has been removed by anion exchange chromatography maintain a level of ATP and convert MVA to NSF(3,4, 5). In the liver homogenate system studied the amount of added MVA incorporated into NSF appears to be directly proportional to the level of ATP existing at the time of MVA addition. Whole homogenates of liver convert MVA to NSF under aerobic but not under anaerobic conditions(6). Biosynthesis of NSF occurs under anaerobic conditions, however, if the homogenate is first centrifuged at intermediate speed to remove "tissue fragments" relatively rich in ATPase activity. The foregoing observations emphasize the significance of systems concerned with the generation of ATP in the biosynthesis of NSF and cholesterol. ATP is known to be specifically required in the phosphorylation of MVA, the phosphorylation of MVA-5-phosphate and the concerted decarboxylation and dehydration of MVA-5-pyrophosphate(7). Oxidative phosphorylation is the primary source of ATP in aerobic homogenates of liver. Accordingly some inhibitors of oxidative phosphorylation have been studied for the extent to which they indirectly inhibit biosynthesis of NSF and cholesterol and the data

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obtained are reported in this paper.

Materials and methods. The biosynthetic experiments involved preincubation of 5 ml aliquots of 200 x g supernatant fraction of rat liver homogenate prepared as previously described with 5 ml amounts of buffer or inhibitor in buffer for a period of 30 min in stoppered 125 ml Erlenmeyer flasks(1-6). Each flask also contained 1 mg ATP and 1 mg DPN and each flask was aerated with a stream of oxygen prior to preincubation. Following preincubation each flask was opened and 1 ml MVA-2-C^{14} solution added, the contents were aerated and the flasks then reincubated for an additional 3 hrs. After the final incubation the homogenates were saponified, extracted with petroleum ether, the extracts dried with sodium sulfate, filtered, evaporated, taken up in scintillation mixture and counted. Previous studies have shown(1) that under the experimental conditions employed in these studies the counts found in the NSF fraction are essentially cholesterol or other digitonin-precipitable material. In some of the experiments sodium succinate was added to the homogenates at a level of 0.05 M. Supplementation with this substrate has been found to reduce incidence of experiments where no incorporation of MVA occurs even in control flasks.

Results. The data obtained are summarized in Table I. By far the most active inhibitor is 2,4-dinitrophenol. Biosynthesis of NSF from MVA is essentially nil at 10^{-5} to 10^{-4} M concentrations of the inhibitor. Methylene blue and acriflavin are quite active with essentially maximum inhibition at 10^{-4} to 10^{-3} M. At sub-inhibitory levels methylene blue appears to stimulate the system. Potassium cyanide is completely inhibitory at 10^{-2} M but shows no inhibition at 10^{-3} M. Sodium fluoride is inhibitory in the range of 10^{-3} to 10^{-2} M. Calcium chloride is not inhibitory at 10^{-3} M and higher concentrations were not studied. Sodium azide is only partially inhibitory at 10^{-2} M.

Discussion. The results obtained show that homogenates of rat liver preincubated with classical inhibitors of oxidative phosphorylation no longer incorporate MVA into NSF. The mechanism seems obvious that in the presence of the inhibitors ATP which is required in a number of the metabolic conversions of MVA to NSF is either not formed or is more rapidly hydrolyzed.

A number of the compounds studied were less active, presumably as inhibitors of oxidative phosphorylation, in the present system than in other systems that have been studied involving isolated liver mitochondria. Since the present system is a very crude one it is to be expected that specific components against which the inhibitors act are present in large excess and consequently require more inhibitor for inactivation.

TABLE I. Summary of Inhibition Data.

Exp. #	Inhibitor	Conc., M	cpm	% inhibition
1	None		3483	
	Potassium cyanide	1×10^{-3}	3592	0
	2,4-Dinitrophenol	"	12	100
	Sodium azide	"	3698	0
	Calcium chloride	"	3228	7
	Methylene blue	"	74	98
2	None		3546	
	2,4-Dinitrophenol	1×10^{-3}	3	100
	"	1×10^{-4}	10	100
	Methylene blue	"	20	100
	Sodium fluoride	1×10^{-1}	30	99
	Idem	1×10^{-2}	1496	58
3*	None		4095	
	Methylene blue	1×10^{-3}	17	100
	Idem	1×10^{-4}	4539	0
	"	1×10^{-5}	5239	0
4*	None		3731	
	Methylene blue	1×10^{-3}	0	100
	Idem	1×10^{-4}	6538	0
	Acriflavin	1×10^{-3}	0	100
	"	1×10^{-4}	4035	0
	"	1×10^{-5}	3751	0
5	None		4308	
	Sodium fluoride	1×10^{-1}	339	92
	Idem	5×10^{-2}	544	87
	"	1×10^{-2}	1735	60
	"	5×10^{-3}	1547	64
	"	1×10^{-3}	3559	17
	"	1×10^{-4}	3769	13
6	None		5329	
	Sodium fluoride	1×10^{-1}	53	99
	Idem	1×10^{-3}	4459	16
7	None		5549	
	2,4-Dinitrophenol	1×10^{-4}	251	96
	"	1×10^{-5}	5111	8
8	None		1513	
	2,4-Dinitrophenol	1×10^{-4}	6	100
	"	1×10^{-5}	19	99
	Potassium cyanide	1×10^{-2}	16	99
	Sodium azide	"	793	48

* Signifies experiments carried out in the presence of 0.05 M sodium succinate.

Although some of the compounds studied are quite active as indirect inhibitors of NSF and cholesterol biosynthesis there is little possibility that an inhibitor of oxidative phosphorylation would have utility in practical control of cholesterol biosynthesis. Oxidative phosphorylation is of such fundamental significance in the metabolism of most cells in an aerobic environment that no basis of selective action against cholesterol biosynthesis in particular would appear to exist.

Summary. Classical inhibitors of oxidative phosphorylation particularly 2,4-dinitrophenol inhibit the biosynthesis of non-saponifiable material and cholesterol from mevalonic acid by rat liver homogenates. Presumably in the presence of inhibitors a level of ATP is not maintained for the phosphorylations and the concerted decarboxylation and dehydration essential in utilization of mevalonic acid.

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Utilization of Pyridoxine N-Oxide by Rats.* (25453)

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When pyridoxine was treated with a mixture of glacial acetic acid and hydrogen peroxide, addition of one atom of oxygen resulted (1). Based on analogous reactions observed with a variety of pyridine derivatives(2), the product was believed to be pyridoxine N-oxide(1). The currently available data indicate that some biological systems are capable of reducing a heterocyclic compound containing N-oxide functions(3,4). In addition, the biological formation of N-oxides as a normal constituent has been reported(4,5). By means of bioautography and microbiological assay, pyridoxine N-oxide retained some Vit. B_6 activity for growth of *Saccharomyces carlsbergensis*, suggesting that yeast could reduce a portion of the N-oxide(1). In the present study, the metabolism of pyridoxine N-oxide by rats has been investigated.

Methods. Male rats 3 weeks of age were placed on basal diet† for 10 days and then evenly divided into 5 groups of 6 rats each with respect to body weights. During assay period, the basal ration and water were fed *ad lib.* In addition, rats were supplemented once each week for 4 weeks with distilled water, or distilled water which contained 20 μg of pyridoxine hydrochloride, 100 μg of pyridoxine hydrochloride, or equivalent amounts of pyridoxine N-oxide hydrochloride. *Paper chromatography and bio-autography.* Paper chromatography was conducted on Whatman No. 1 filter paper at room temperature in a descending system. The solvent was the upper layer of a mixture of isoamyl alcohol, pyridine and water (2:1:2, *v/v*). The spots of Vit. B_6 components as well as pyridoxine N-oxide were detected by spraying the papergram with

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† Each 10 lbs. of basal ration free from both fats and vit. B_6 contained 7.8 lb glucose, 1.8 lb vitamin-free casein, 0.4 lb Wesson salts(6), 9 g choline chloride, 4.5 g inositol, 10 mg thiamine chloride hydrochloride, 15 mg riboflavin, 100 mg nicotinic acid, 90 mg D-calcium pantothenate, 45 mg menadione, 0.5 mg biotin, 15,000 I. U. vit. A, 0.15 mg calciferol and 30 mg alpha-tocopheryl acetate.