PARTICIPATION OF CLOSELY JUXTAPOSED SULFHYDRYL GROUPS IN FATTY ACID BIOSYNTHESIS

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The observation that arsenite inhibited the enzymatic synthesis of long chain fatty acids was reported several years ago (Brady, Mamoon, and Stadtman, 1956). Jakoby (1958) has recently presented evidence for the requirement for closely juxtaposed enzyme sulfhydryl groups in the oxidation of aldehydes. We have reinvestigated the effect of arsenite in a fatty acid synthesizing enzyme preparation obtained from the high speed supernatant solution of extracts of rat liver tissue. The enzyme was partially purified by ammonium sulfate fractionation, followed by adsorption and elution from calcium phosphate gel. The requirements for fatty acid synthesis in this enzyme preparation are malonyl CoA (Brady, 1958; Wakil and Ganguly, 1959a) and TPNH* (DPNH is approximately two-thirds as effective as TPNH). It was observed that the addition of acetyl CoA increased the incorporation of malonyl CoA into fatty acids confirming the observation of Wakil and Ganguly (1959b); however, butyryl CoA caused an even greater increase in the incorporation of malonyl CoA and accordingly was added to the incubation mixtures in Experiment 1 (cf. Shaw, Dituri, and Gurin,

*The following abbreviations are used: DPNH, TPNH, reduced di- and triphosphopyridine nucleotide; acyl CoA, the respective thiol ester derivatives of coenzyme A; BAL, 2, 3-dimercaptopropanol.

1957; Long and Porter, 1959; Wakil and Ganguly, 1959b).

As shown in Table 1, the addition of a mercaptan such as 2-mercaptoethanol or BAL significantly increased the incorporation of malonyl CoA and acetyl CoA into fatty acids. The

Table 1

The Effect of Mercaptans and Arsenite on the Conversion of Malonyl-1,3-C^{14} CoA and Acetyl-1-C^{14} CoA to Long Chain Fatty Acids

Exp.	Additions	Incorporation of labeled substrate
		mμatoms C^{14}
1.	None	28
	2 μmoles of $NaAsO_2$	14
	6 μmoles of 2-mercaptoethanol	67
	6 μmoles of 2-mercaptoethanol + 2 μmoles of $NaAsO_2$	0.1
	4.7 μmoles of BAL	116
	9.4 μmoles of BAL	105
	4.7 μmoles of BAL + 4 μmoles of $NaAsO_2$	1.3
	9.4 μmoles of BAL + 4 μmoles of $NaAsO_2$	48
2.	None	3.5
	2 μmoles of $NaAsO_2$	2.9
	6 μmoles of 2-mercaptoethanol	5.3
	6 μmoles of 2-mercaptoethanol + 2 μmoles of $NaAsO_2$	0.4

The incubation mixtures contained 100 μmoles of potassium phosphate buffer (pH 7.0), 1.8 μmoles of TPNH, and enzyme (0.5 mg. of protein). To the flasks in Experiment 1 were added 0.30 μmole of unlabeled butyryl CoA and 0.52 μmole of malonyl-1,3-C^{14} CoA (0.1 μC per μmole). To the flasks in Experiment 2 were added 0.3 μmole of acetyl-1-C^{14} CoA (1.0 μC per μmole) and 0.50 μmole of unlabeled malonyl CoA. The final volume was 1 ml. The mixtures were incubated for 1 hour at 37°. The reaction was terminated by the addition of 0.2 ml. of 1 M KOH, allowed to stand 15 minutes to hydrolyze the CoA esters, followed by acidification, extraction and counting the long chain fatty acids.

addition of arsenite in the absence of exogenous mercaptan caused some inhibition in the conversion, whereas the same concentration of arsenite in the presence of mercaptoethanol completely blocked the process. Inhibition by arsenite was reversed by BAL.

Further experiments indicate that the condensation of acetyl or butyryl CoA with malonyl CoA is unaffected by the addition of arsenite. These results suggest that the closely-juxtaposed sulfhydryl groups participate in the reductive process and may resemble to a degree the reverse of the sequence of reactions described by Nirenberg and Jakoby (1960) for the oxidation of aldehydes.

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