Preliminary Notes

Function of vitamin B_{12} in methylmalonate metabolism I. Effect of a cofactor form of B_{12} on the activity of methylmalonyl-CoA isomerase

The study of BARKER *et al.*[1] showing that a derivative of pseudo-vitamin B_{12} functions in the reversible conversion of glutamate to β-methylasparate in *Clostridium tetanomorphum* suggested to us the possibility of a similar involvement of vitamin B_{12} in the isomerization of methylmalonyl-CoA to succinyl-CoA in the propionate metabolism pathway[2] in the animal.

Studies carried out using enzyme preparations and incubations essentially according to the methods of FLAVIN AND OCHOA[2] for the incorporation of $^{14}CO_2$ into succinate with propionyl-CoA used as substrate showed a several-fold reduction in vitamin B_{12}-deficient rats[3]. The difference in incorporation between the B_{12}-normal and the deficient rats was primarily due to a reduction in the isomerase step, since methylmalonate formation was essentially the same for the deficient and normal groups. This is in agreement with similar results obtained by SMITH AND MONTY[4].

Attempts to restore the activity of the isomerase from B_{12}-deficient animals by

TABLE I

INCORPORATION OF $^{14}CO_2$ INTO METHYLMALONIC, SUCCINIC, AND OTHER ACIDS FORMED FROM PROPIONYL-CoA BY MITOCHONDRIAL PREPARATION FROM THE LIVERS OF VITAMIN B_{12}-NORMAL AND DEFICIENT RATS

System	*Total incorporation**		*Incorporation in succinate**	
	Nutritional status of rats		*Nutritional status of rats*	
	$-B_{12}$	$+B_{12}$	$-B_{12}$	$+B_{12}$
Expt. I				
Complete system (CS.)	1312	1446	81	1236
CS — propionyl CoA	9	7	1	0
CS + B_{12} (100 mμg)	1292	1272	82	763
CS + 2 μmoles CN	1377	1451	92	1106
CS + DMBC (10 mμg)	1195	1374	208	1167
CS + DMBC (50 mμg)	1209	1289	308	1101
CS + DMBC (100 mμg)	1253	1336	599	1121
Expt. II				
Complete system	1160	1043	96	757
CS + B_{12} (100 mμg)	1025	1154	64	773
CS + 2 μmoles CN	1042	1207	64	858
CS + DMBC (100 mμg)	1108	1191	747	1087
CS + DMBC (100 mμg)	1103	1151	807	881
CS + DMBC (400 mμg)	1156	1116	907	871

* In counts/min/mg protein added. Total incorporation includes activity present in methylmalonate, succinate, malate and fumarate.

Abbreviations: B_{12}, vitamin B_{12}; DMBC, 5,6-dimethylbenzimidazole-B_{12} coenzyme; Tris, tris(hydroxymethyl)aminomethane; GSH, glutathione; CoA, coenzyme A; ATP, adenosine triphosphate.

addition of vitamin B_{12} *in vitro* were unsuccessful, as were attempts to inhibit with cyanide; however, when 5,6-dimethybenzimidazole-B_{12} coenzyme[5] was used the isomerase activity was completely restored to normal (Table I).

Two experiments were carried out, each in duplicate, involving two normal and two deficient rats. The reaction mixture complete system contained Tris buffer, pH 7.4, 400 μmoles; $MgCl_2$, 10 μmoles; GSH, 10 μmoles; ATP, 10 μmoles; propionyl-CoA[6], 1 μmole; $NaH^{14}CO_3$, 10 μmoles (10^6 counts/min); and buffer extract of acetone-dried rat-liver mitochondria[7,8], containing 18–25 mg protein. The final volume was 1.7 ml. The mixtures were incubated at 28° in an atmosphere of $O_2 + CO_2$ for 1 h and the reaction was stopped by the addition of 0.3 ml 2 *N* $HClO_4$. Direct scintillation counting of acid-deproteinized aliquots gave the total radioactivity incorporated while counting after heating aliquots for 10 min at 100° with a few drops of 3% $KMnO_4$, and removal of excess permanganate by reduction with methanol gave the radioactivity due to succinate, since succinate is stable to this permanganate oxidation, while methylmalonate, as well as malate and fumarate, is oxidized to volatile products. The precipitated MnO_2 was removed by centrifugation before counting of the filtrates. All results are expressed in counts/min/mg protein (enzyme preparation) added.

The data given in Table I clearly indicate that vitamin B_{12} coenzyme is a cofactor for the isomerization of methylmalonyl-CoA to succinyl-CoA.

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Department of Animal Science, University of Illinois, Urbana, Ill. (U.S.A.)

SHANTOO GURNANI
S. P. MISTRY
B. CONNOR JOHNSON

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Nitrogen fixation in cell-free extracts of *Clostridium pasteurianum*

Biological nitrogen fixation is of fundamental importance to the existence of all living things, yet there has been little progress in understanding the chemistry of the process. Investigations at the enzyme level to define the mechanism have hitherto been blocked by failure to find adequate ways for extracting the enzymes in active