Vitamin B_{12} and protein biosynthesis
VII. Activity of a cofactor form of vitamin B_{12} on amino acid incorporation into protein

It has been reported previously from this laboratory that in vitamin B_{12} deficiency there is a reduction in the incorporation *in vitro* of amino acids into protein[1]. This reduction in incorporation could be partially but never completely reversed by the addition of vitamin B_{12} to the enzyme system.

BARKER *et al.*[2] have reported that a cofactor form of pseudo-vitamin B_{12} functions in the conversion of glutamic acid to β-methylaspartic acid in the microorganism *Clostridium tetanomorphum*. More recently they have prepared similar cofactor forms of vitamin B_{12} and of the benzimidazole analogue of vitamin B_{12} by the fermentation of the same organism, in the presence of the appropriate base[3]. They state that at least 80% of the vitamin B_{12} in rabbit liver is present in this cofactor form[3]. Dr. BARKER has kindly provided some of this cofactor for our use and we are presenting herewith data on its activity in the system previously studied for the incorporation of amino acid[1].

Incorporation experiments were carried out as reported previously[4] in the system of HOAGLAND *et al.*[5]. The only change in our experimental condition is that the incubations were carried out in the dark room. The results of these investigations are enumerated in Table I.

TABLE I

THE EFFECT OF A COFACTOR FORM OF VITAMIN B_{12} ON THE INCORPORATION OF [3-^{14}C]PHENYLALANINE INTO PROTEIN BY A MICROSOMAL–pH 5 ENZYMES SYSTEM FROM NORMAL AND VITAMIN B_{12}-DEFICIENT RAT LIVERS

System used	*Nutritional status of rats*	
	$-B_{12}$ counts/min/mg protein	*$+B_{12}$ counts/min/mg protein*
Microsomes alone	18.5	18.5
pH 5 enzymes alone	19	17
Complete system (C.S.)	35.5	130.0
C.S. + 5 mμg B_{12} cofactor	45.	131.0
C.S. + 10 mμg B_{12} cofactor	85.5	137.0
C.S. + 20 mμg B_{12} cofactor	135.5	135.0
C.S. + 40 mμg B_{12} cofactor	129.0	135.0
C.S. + 50 mμg B_{12} cofactor	128.0	140.0
C.S. + 50 mμg crystalline vitamin B_{12}	90.0	

Complete system contained 0.3 ml microsomes (7.5 mg protein), 0.2 ml pH 5 enzymes (2.5 mg protein) 0.25 μmole [3-^{14}C]phenylalanine, 10 μmoles phosphoenol pyruvate, 0.02 mg pyruvate kinase, 1.0 μmole adenosine triphosphate, 0.25 μmole guanosine triphosphate in a final vol. of 1.0 ml made up by addition of 0.15 *M* KCl.

These results clearly demonstrate that the addition of the vitamin B_{12} cofactor to the deficient system completely restores the amino acid incorporating activity to the normal level. The effect produced by the addition of 10 mμg cofactor was equivalent to that produced by the addition of 50 mμg crystalline vitamin B_{12} while 20 mμg cofactor completely restored incorporating activity. These results emphasize and extend the data on the role of the vitamin B_{12} in the incorporation of amino

acids into protein and indicate that the functional form in the animal is similar to that found by BARKER *et al.* for the microorganism *Clostridium tetanomorphum.*

It was also reported earlier from this laboratory[4] that vitamin B_{12} may be involved in the activation of amino acid; however, recent studies carried out using hydroxylamine as the trapping agent show no lowering of hydroxamate formation in the deficient-liver preparations.

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Mono- and dimethylethanolamine isolated from rat-liver phospholipids

It is well known that ethanolamine and a series of "single carbon unit donors" serve as precursors of choline in the living animal. It is presumed that mono- and dimethylethanolamine are intermediates in this biosynthesis. This idea is supported by the isolation of these compounds from the phospholipids in mutant strains of *Neurospora crassa*[1], but so far there has been no report on the isolation of these compounds from animal tissue.

We have recently studied choline synthesis in the living rat, using [*Me*-^{14}C]-methionine as the precursor.

In short-time experiments we have been able to isolate small amounts of radioactive mono- and dimethylethanolamine from rat-liver phospholipids (Fig. 1). The identification of these compounds has been verified by the following procedures: (1) They are eluted off a Dowex 50 column exactly in the positions of mono- and dimethylethanolamine (Fig. 1). (2) They move identically with these compounds in a paper chromatographic system which separates ethanolamine, mono- and dimethylethanolamine and choline (50 g phenol, 50 ml *n*-butanol, 3 ml 80 % formic acid, 10 ml water). The mixture was shaken with solid KCl and the paper pretreated with 1 *N* KCl. The KCl prevents tailing on the paper. R_F values found were: ethanolamine, 0.13; methylethanolamine, 0.38; dimethylethanolamine, 0.63; choline, 0.75. (3) The compounds are converted to choline when treated with alkaline methyl iodide. The radioactive compounds were eluted from the paper, the appropriate carrier mono- or dimethylethanolamine, alkali and alcoholic methyl iodide were added and the mixtures were shaken at 45° for 2 h. The choline formed was precipitated as the reineckate and subsequently recrystallized three times from acetone with *n*-propanol. The choline reineckate from the methylethanolamine showed a specific activity of 11, 9 and 10 counts/min/mg after one, two and three recrystallizations, respectively; the choline reineckate from the dimethylethanolamine 67, 66 and 68 counts/min/mg.