Vitamin B_{12} and protein biosynthesis. V. The site of action of vitamin B_{12} and its inhibition by a B_{12} antagonist*,**

It has been reported from this laboratory that vitamin B_{12} functions *in vivo* and *in vitro* in the incorporation of amino acids into protein[1,2,3,4], when the microsomal system of KELLER AND ZAMECNIK[5] is used. It was found[3] that much of the vitamin B_{12} is present in the pH 5 enzymes. These enzymes have now been fractionated for the separation of the vitamin B_{12}-containing enzyme(s). For this purpose 6.0 μC of ^{60}Co-labeled vitamin B_{12} were injected into a rat, and 8 h later the rat was sacrificed and pH 5 enzymes prepared from the liver by the method of KELLER AND ZAMECNIK[5]. The nucleic acids present in these enzymes were removed by protamine sulfate precipitation. Ammonium sulfate fractionation of the resultant solution showed that essentially all of the radioactivity (*i.e.*, the vitamin B_{12}) is present in the fraction precipitating between 40 and 60% saturation (Table I).

TABLE I

DISTRIBUTION OF RADIOACTIVE B_{12} IN AMMONIUM SULFATE FRACTIONS

	Counts/min	*Counts/min/mg*
Total radioactivity in 105,000 × *g* supernatant	88600	
Total radioactivity in pH 5.0 enzyme	67400	
Total radioactivity after nucleic acid separation	62000	
Total radioactivity in 0–20% ammonium sulfate fraction	Nil	
Total radioactivity in 20–40% ammonium sulfate fraction	6800	2130
Total radioactivity in 40–60% ammonium sulfate fraction	51800	17250
Total radioactivity in 60–80% ammonium sulfate fraction	Nil	
Total radioactivity in 80–100% ammonium sulfate fraction	Nil	

TABLE II

INHIBITION AND INCORPORATION OF 2-^{14}C-ALANINE INTO PROTEINS BY MICROSOME PREPARATIONS SUPPLEMENTED WITH pH 5 ENZYMES, AMMONIUM SULFATE FRACTIONS OF pH 5 ENZYMES AND ANTI B_{12}

Complete system contained 0.3 ml microsomal suspension, 0.2 ml pH 5 enzymes solution; 0.25 μmole 2-^{14}C-alanine; 10 μmoles phospho(enol)pyruvate; 0.02 mg pyruvate kinase; 0.5 μmole ATP and 0.25 μmole GTP. The incubation mixtures 5–12 contained same amount of 2-^{14}C-alanine, phospho(enol)pyruvate, pyruvate kinase, ATP and GTP. The final volume of the incubation mixture was 1.0 ml. MS = microsomes; NA = supernatant nucleic acid; ASF = ammonium sulfate fraction.

System used	*Counts/min/mg protein*
1. Complete system	93
2. Complete system minus microsomes	32
3. Complete system minus pH 5 enzymes	21
4. Complete system + anti B_{12} (250 μg)	52
5. 0.3 ml MS + 0.5 mg NA + 2 mg ASF (0–20%)	24
6. 0.3 ml MS + 0.5 mg NA + 2 mg ASF (20–40%)	67
7. 0.3 ml MS + 0.5 mg NA + 2 mg ASF (40–60%)	154
8. 0.3 ml MS + 0.5 mg NA + 2 mg ASF (60–80%)	28
9. 0.3 ml MS + 0.5 mg NA + 2 mg ASF (80–100%)	20
10. 0.3 ml MS + 0.5 mg NA + 1 mg ASF (20–40%) + 1 mg ASF (40–60%)	216
11. 0.3 ml MS + 0.5 mg NA + 1 mg ASF (20–40%) + 1 mg ASF (40–60%) + Anti B_{12} (250 μg)	122
12. 0.3 ml MS + 0.5 mg NA + 2 mg ASF (20–40%) + 2 mg ASF (40–60%) + Anti B_{12} (250 μg)	193

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** The following abbreviations are used: ATP, adenosine triphosphate; AA, amino acid; AMP, adenosine monophosphate; PP, inorganic pyrophosphate; GTP, guanosine triphosphate.

The activity of the various ammonium sulfate fractions was studied with regard to their requirement (in place of the pH 5 enzymes) for the incorporation of amino acids into protein. Table II shows that the 40–60% fraction as well as other fractions (20–40% fraction) and nucleic acids are required.

In a search for the function of this vitamin B_{12} enzyme (40–60% fraction) experiments were carried out on the initial step of the amino acid activation. In these experiments, the 40–60% fraction was incubated with ATP, amino acid and ^{32}P-labeled pyrophosphate. A large amount of radioactivity was found to be incorporated into ATP (total counts incorporated, 680/min). This was not the case when other ammonium sulfate fractions of the pH 5 enzymes were used in place of the 40–60% fraction. These results indicate that vitamin B_{12} functions in the activation reaction, AA + ATP $\rightleftharpoons^{B_{12}\ \text{enzyme}}$ AA + PP. An antivitamin B_{12} compound, the anilide of the monocarboxylic acid of vitamin B_{12}[6], was used to test further the specificity of vitamin B_{12} for this reaction. The inclusion of this antagonist in the incubation mixture, in an amount equal to 1000 times that of the vitamin, greatly decreased (55%) the incorporation of pyrophosphate into ATP (total counts incorporated, 310/min). This was partially reversed by doubling the vitamin B_{12} enzyme (40–60% fraction).

This same antagonist was then used in the usual microsomal system for amino acid incorporation into protein. Table II shows that about 44% reduction in incorporation was obtained, and this reduction was reversed by the addition of a large amount of vitamin B_{12} enzyme.

These results indicate that vitamin B_{12} is bound to an enzyme (or enzymes) which is involved in the activation of amino acid for incorporation into protein.

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Biosynthesis of ribose and desoxyribose in *Escherichia coli**

Tracer studies[1,2,3] suggest that ribose arises largely from glucose, while the origin of desoxyribose is less certain. In the present work RNA, DNA and polyglucosan[4] were isolated from *E. coli* R-2 adapted to grow on acetate as sole carbon source. RNA was converted to mononucleotides by KOH digestion, DNA was recovered by acid precipitation and degraded to mononucleotides[5]. Nucleotides were purified by ion-exchange chromatography[6,7].

Ribose. Purine ribotides were hydrolyzed with phosphatase[8] and 1 N H_2SO_4. Cytidylic acid was deaminated and added to the original uridylic acid, which was then hydrolyzed with phosphatase and uridine nucleosidase. The ribose obtained was purified on cellulose columns and then degraded[9].

Desoxyribose. The purine nucleotides were combined and hydrolyzed to desoxyribose-5-phosphate, which was then converted to acetaldehyde and lactic acid by incubation with extracts of *E. coli*[10] and rabbit muscle. Acetaldehyde was oxidized to acetic acid and the acids were degraded[9].

Glucose. The polyglucosan was isolated from a portion of the culture by the procedure used for mammalian glycogen, and hydrolyzed with 1 N H_2SO_4. Glucose was degraded by the method of BERNSTEIN *et al.*[11].

All samples were converted to $BaCO_3$ and radioactivity measurements carried out in a windowless counter, and corrected to infinite thinness. The results are shown in Table I. In experiments with 22-h cultures, the isotope distribution in ribose and desoxyribose was almost identical with that shown in the Table, and the purine- and pyrimidine-bound ribose had the same distribution.

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