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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TABLE I

ISOTOPE DISTRIBUTION IN SUGARS SYNTHESIZED BY *E. coli* FROM $CH_3{}^{14}COOH$

The growth medium contained per l: Na_2HPO_4, 11.35 g; KH_2PO_4, 2.7 g; $(NH_4)_2SO_4$, 2.0 g; $MgSO_4$, 0.2 g; $CaCl_2$, 2 mg; $FeSO_4 \cdot 7H_2O$, 1 mg; sodium acetate, 5 g. A total of 300 μC 1-^{14}C-sodium acetate was also present. The medium was inoculated with 10% of its volume of a 22-h culture grown aerobically in non-isotopic acetate. After 8 h aerobic growth at 37°, when the culture had reached half the maximum growth possible in this medium, the cells were harvested.

Glucose		*Ribose*		*Desoxyribose*	
C-atom	*RSA**	*C-atom*	*RSA*	*C-atom*	*RSA*
1	1.6				
2	3.1	1	23.7	1	21.9
3	100	2	49	2	47
4	97.5	3	100	3	100
5	(0.08)	4	8.7	4	1.67
6	0	5	3.4	5	3.2

* Relative specific activity. For each sugar the carbon atom with the highest specific activity is arbitrarily assigned a value of 100.

BERNSTEIN[2] found that *E. coli* converts 3,4-$^{14}C_2$-glucose to ribose labelled chiefly in carbons 2 and 3 with relative specific activities of 85 and 100 in these positions. He has also found[12] that 1-^{14}C-acetate added to cells growing in glucose labels positions 1, 2 and 3 of the ribose in a ratio of 22:75:100. When glucose is the sole carbon source, ribose seems to be formed by the oxidative decarboxylation of hexose monophosphate. Our present findings do not appear to be explained solely on this basis. The labelling of ribose suggests either oxidation of hexose monophosphate coupled with the operation of transketolase and transaldolase[13], or condensation of 2-carbon and 3-carbon compounds, or a combination of these two pathways. The different labelling of glucose and ribose suggests that the polyglucosan and pentose synthesized may reflect the labelling of different hexose phosphate pools either in terms of time or intracellular location. The identical labelling of ribose and desoxyribose suggests that one may be a precursor of the other, or that both are derived from identically labelled precursors. Similar studies with other labelled substrates with these cells and with bacteriophage-infected *E. coli* are now in progress.

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