The rapid and nearly complete disappearance of color in the reaction with ascorbate indicates release of cobalt from cobalamin. Cooley *et al.* (*8*) offer the hypothesis that, in the case of cyanocobalamin, cyanide acts to strengthen the bond between the cobalt and benziminazole nitrogens, thus making for greater stability than is the case for hydroxycobalamin. Unusual resonance energy is imputed to the cobalt-cyanide complex, giving a positive charge to the cobalt atom and thereby strengthening the Co—N bond.

The following procedure was used to test whether cobalt is liberated in the cobalamin-ascorbate reaction. Ten mg of crystalline vitamin B_{12} was dissolved in 5 ml water. The pH was made to 3.5 with dilute HCl, and the solution was irradiated 4 hr. Conversion from the cyano form was spectrophotometrically complete. To this solution was added 100 mg of the ascorbate mixture, with immediate color change from red to brown. This reaction mixture was designated solution X. Less than 1% of the original vitamin activity remained in solution X by microbiological and rat assays.

The nitroso R salt method for cobalt determination was set up, using $CoCl_2 \cdot 6H_2O$ as a standard. Test solution X gave a characteristic color with nitroso R salt, which was read in the spectrophotometer at 5100 A. About 80% of the theoretical cobalt reacted with the reagent as measured colorimetrically against either a water blank or solution X blank. Vitamin B_{12b} in comparable concentrations of cobalt completely obscured any color resulting from addition of nitroso R salt.

In order to determine whether the cobalt of solution X was in free or combined form, extraction was carried out with CCl_4-dithizone reagent according to the standard method (*9*) for separation of cobalt. The extract, after ashing, gave a characteristic color test for cobalt. Recovery of the cobalt of the original vitamin B_{12b} was 80%. The CCl_4-dithizone solution, on the other hand, failed to extract a measurable amount of cobalt from a solution of crystalline vitamin B_{12b}. These results suggest that ascorbate actually releases cobalt from the vitamin B_{12b} molecule.

Sodium thioglycollate, cysteine hydrochloride, and thiomalic acid, in 1–4% concentration in water, all destroy vitamin B_{12b} more rapidly than they destroy vitamin B_{12}. The difference in rate or extent of destruction of the two forms of the vitamin is not, however, nearly as marked for these materials as for ascorbate. Of the reducing compounds tested, only thiosorbitol approached ascorbate in speed and completeness of destruction of vitamin B_{12b}. Thiosorbitol, like ascorbate, has much slower destructive effect for vitamin B_{12}. Sulfite also protects against destruction by these sulfhydryl compounds.

These studies indicate that the susceptibility of cobalamin to degradation by vitamin C is a function of the coordination of its key cobalt atom with various anions. Only those anions which coordinate strongly with cobalt appear capable of protection. Differentiation between strongly and weakly coordinated forms of the vitamin by reaction with ascorbate is useful and reliable, but only for highly purified concentrates free from iron and other interferences.

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The Metabolism of Betaine and Sodium Formate by Leukemic Mice[1]

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In a previous report it was shown that leukemic mice excrete increased quantities of creatine and allantoin (*1*). It was suggested that this increased creatine excretion reflected an increased rate of synthesis of creatine which was associated with the rapid rate of leucocyte production. It seemed desirable to obtain direct evidence that the increased creatine excretion of leukemic mice reflects an accelerated rate of synthesis and is not merely the result of loss of body stores of creatine. We have consistently been unable to obtain creatine synthesis *in vitro* by incubation of mouse liver preparations with the precursors of creatine. In order to measure the rate of creatine synthesis we have injected control and leukemic mice with C^{14}-labeled sodium formate and C^{14} methyl–labeled betaine and determined the excretion of labeled creatine in a subsequent 24-hr period. In addition, the excretion of C^{14}-labeled allantoin has also been determined. The results support the hypothesis that the elevated excretion of creatine by leukemic mice is the result of an increased rate of synthesis.

The animals were mice of the DBA strain, and leukemia was induced as previously described (*1*). Leukemic mice were taken 10 days after blood transfer; the controls were mice of the same strain which had not been inoculated with leukemic blood. Six control and 6 leukemic mice were each injected with 90 μg (4 μc/μM) of sodium formate and placed in metab-

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TABLE 1

THE EXCRETION OF LABELED CREATININE AND ALLANTOIN BY CONTROL AND LEUKEMIC MICE IN A 24-HOUR PERIOD AFTER INJECTION OF C^{14}-LABELED SODIUM FORMATE AND C^{14} METHYL-LABELED BETAINE

Substance injected	Total counts injected	Animals	Total creatinine		Allantoin	
			Counts/ min/mouse	Counts/ min/μM	Counts/ min/mouse	Counts/ min/μM
Sodium formate	1×10^6	Control	317	54	1190	80
" "	1×10^6	Leukemic	796	115	2900	142
Betaine	4.7×10^5	Control	656	94	416	20
"	4.7×10^5	Leukemic	913	92	706	21

olism cages. Pooled 24-hr urine samples were collected from each group. For the betaine experiments 10 control and 10 leukemic mice were each injected with 1 mg of betaine hydrobromide (0.53 μc/μM), and urine collections made as in the formate experiments. The urine was autoclaved with acid to convert creatine to creatinine. The creatinine was isolated by carrier isolation and purified as the zinc chloride derivative. Allantoin was isolated by carrier isolation. The total creatinine and allantoin content of the urine before carrier addition was determined in order that specific activity could be determined. The samples were placed on aluminum plates and counted with an end window tube with a window thickness of 2 mg/cm².

In addition to the carrier isolation, the urine samples were subjected to paper chromatography in a phenol water system. The specific activities of creatinine and allantoin as determined by counting the paper strips agreed quite well with the results obtained by carrier isolation procedures.

The results presented in Table 1 show that the leukemic mice excreted more labeled creatinine and allantoin after injection of labeled sodium formate or betaine than did the controls. There is adequate evidence that animals can synthesize methyl groups from one-carbon precursors (*2*), and the relative specific activities of the creatinine and allantoin excreted by these mice after labeled formate injection indicates that the mouse is able to utilize formate quite effectively as a precursor of creatine. The results also indicate that betaine may be used effectively as a precursor of allantoin. The conversion of methyl to a one-carbon fragment which can serve as a precursor of the β-carbon of serine (*3*), and which may be incorporated into purines (*4*), has been demonstrated. Betaine was more effective as a creatine precursor than as a precursor of allantoin. This is in agreement with the observations that the methyl group may be transferred *in toto* (*5*).

The fact that the leukemic mice excreted more labeled creatinine after sodium formate or betaine injection is proof that at least a part of the increased creatine excretion by leukemic mice is the result of an accelerated rate of creatine synthesis (*1*). These results in conjunction with other findings indicate that creatine (*1, 6*) and methyl groups (*7–9*) must play a significant role in white blood cell formation.

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Effect of Excess Dietary DL-Methionine on Liver and Kidney Catalase of Rats[1]

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In a preliminary investigation upon the effect of single amino acids on catalase activity, we have noted an effect of excess DL-methionine on liver and kidney catalase activity. Protein-free diets containing concentrations of 3–5% of glycine, L-cystine, arginine, leucine, tryptophan, and asparagine were without effect on the catalase activity of protein-depleted rats. DL-methionine produced a marked depression of kidney and liver catalase activity, and further data were obtained with this amino acid.

Adult female Wistar rats were placed on a protein-free diet for 2 weeks and then placed on the diets indicated in Table 1. All diets were administered *ad lib.* The protein-free diet and catalase assay method have been described previously (*1*). The toxic effect of 5% DL-methionine at 10% gelatin concentration is prevented by increased levels of dietary protein. This is in accord with previous observations wherein increasing dietary protein reversed the toxicity displayed by methionine toward growth (*2*) and nitrogen balance (*3*).

The depression and elevation of kidney catalase activity with changes in the concentration of dietary

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