ciliate kindly supplied by C. A. Hoare, of London—the strain described as *"Glaucoma piriformis"* (*4*)—also shows no evidence of a micronucleus. One may, perhaps, justifiably conclude that the amicronucleate condition in this species is of widespread and common occurrence. On the other hand, one investigator (*7*), also employing the Feulgen technique, has recorded the presence of a micronucleus in members of an extinct strain of this species. Also, I have observed it in preparations (belonging to E. Fauré-Fremiet) of a second bacterized strain from the Paris region, likewise no longer being maintained, in which it is typically in a depression of, or embedded in, the macronucleus. Its size is small (under 2 μ), but it can be clearly differentiated from rounded-up masses of chromatin extruded from the macronucleus during fission. At the present time, therefore, one should hesitate to assume that the American strains of *"Tetrahymena geleii"* being cultured axenically are amicronucleate until all of them have been subjected to careful examination.

In agreement with Furgason (*5*), I consider the ciliate a member of the genus *Tetrahymena* Furgason, 1940, but I have suggested (*8*) that *Tetrahymena* is the same genus as that to which Ehrenberg (*9, 10*) invalidly applied the name *"Leucophrys."* I further consider the ciliate as probably specifically identical with Ehrenberg's *"Leucophrys pyriformis."* His descriptions and figures of the organism leave something to be desired in the matter of fineness of detail, but there is nothing in them which cannot be reconciled with Maupas' (*11*) redescription of this species and with the characterization offered above. That Ehrenberg's figures show 9–11 ciliary striations on one surface, and that he never observed the occurrence of conjugation, also support—or at least do not contradict—the identity of the forms. Maupas erred in transferring the ciliate to the genus *Glaucoma,* but in his detailed description of *"G. pyriformis"* there again appear to be no characteristics given which contravene those found by Furgason (*5*) for *"T. geleii"* or by the writer for a large number of strains belonging to the same species. It is true that Maupas misinterpreted the relationships among the cytostomal organelles, very difficult to resolve without modern techniques, but he recognized their similarity to those in the closely related ciliate *"Leucophrys patula"* (*12*).

By application of the Law of Priority (Art. 25, *International Rules of Zoological Nomenclature*) all more recent names applied to the organism under consideration may be regarded as subjective synonyms of the first proposed name, keeping in mind the alleged nonavailability of the generic name *"Leucophrys"* for these particular ciliates and the suggested conclusion that *"Tetrahymena"* is chronologically the next available published name (*8*). Lwoff (*13*), without description, figures, or discussion, and spelling the trivial name with an "i," used the combination considered correct by the writer and therefore the full name would become *Tetrahymena pyriformis* (Ehrbg., 1830) Lwoff, 1947. It is the type species of the genus. Lwoff's ciliate, strain GL, cultured axenically without interruption for 30 years, may be considered as the type strain of the species.

In a longer publication a more detailed description of this species will be offered, with attention to minor variations among the various strains. Also, the relationship of *T. pyriformis* to some 5 or 6 congeneric species[5] which have been, or are now being, investigated by the writer, including in particular *T. vorax* (Kidder, Lilly, and Claff, 1940) Kidder, 1941 and *T. patula* (Müller, 1786) Corliss, 1951, will be discussed. That the 3 extant axenic strains of *T. vorax,* PP, V_1, and V_2 (only the last of which appears still capable of undergoing profound transformations in its life cycle), have all been found to be amicronucleate presents a problem of some interest regarding the phylogenetic relationship between this species and *T. pyriformis.*

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[5] Since this paper was submitted for publication, I have received from A. M. Elliott, University of Michigan, pure culture strains of the very interesting ciliate, species not yet determined, whose cytogenetics has been investigated by Elliott and Nanney (*Science,* **116**, 33 [1952]). Preliminary morphological study indicates that although the organism is similar in many respects to members of the axenic strains of *T. pyriformis,* it possesses certain characteristics which appear to differentiate it slightly from that species as the latter has been described in the present paper.

Differential Stability of Various Analogs of Cobalamin to Vitamin C

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The conversion of vitamin B_{12} (cyanocobalamin) to vitamin B_{12b} (hydroxycobalamin) through the sulfite was reported in 1949 from this laboratory (*1*). The discovery was made independently in two laboratories that vitamin B_{12b} (B_{12a}) is destroyed quickly by ascorbate, whereas vitamin B_{12} is destroyed relatively slowly (*2, 3*). Coordination of the cobalamin ion with various anions has been described (*4–6*), and these findings illuminate our early observation on the stabilizing effect of sulfite (*2*). The ascorbate reaction

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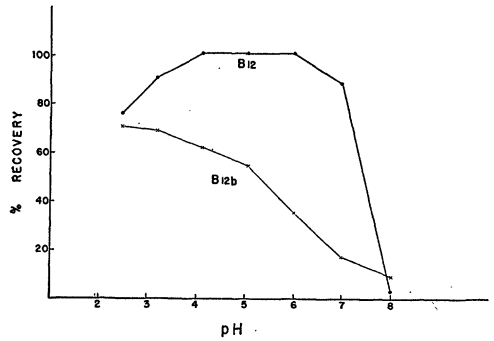

FIG. 1. Stability of vitamins B_{12} and B_{12b} at varying pH heated 3 hr at 98°–99° C.

was first used as a means of differentiating vitamin B_{12} and B_{12b} in high-potency concentrates, but was soon found to be unreliable for relatively crude preparations. The purpose of this paper is to describe experiments with known cobalamin analogs, and to point out certain of the limitations of the reaction as an analytical tool.

Vitamin B_{12b} isolated from fermentation sources (*1*) was used in early studies of the reaction with vitamin C. Advantage was later taken of the method of converting vitamin B_{12} to vitamin B_{12b} by photolysis (*5*).

Solutions of crystalline vitamins B_{12} and B_{12b}, 20 μg/ml, were made up in one-tenth standard concentration McIlvaine buffers. The pH range studied was from 2.6 to 8.0. The tightly stoppered solutions were subjected to streaming steam for 1 hr in the autoclave, cooled promptly, and assayed microbiologically. It was clear from this study (Fig. 1) and others that vitamin B_{12} is generally more stable than vitamin B_{12b}.

A standard method for the differential assay with ascorbate was worked out with known mixtures of crystalline vitamins B_{12} and B_{12b} in aqueous solution. To 2 ml of test solution, containing 20–40 μg of vitamin B_{12}, is added 80 mg of a 1:4 ascorbic acid: sodium ascorbate mixture. The pH of the solution is then 4.5–5, and no buffer is required. The solution is heated for ½ hr at 65° C, cooled promptly, and placed on microbiological assay. Under these conditions, destruction of vitamin B_{12b} is 95% or more, and of vitamin B_{12} about 5%.

Tests run on a number of commercial vitamin B_{12} concentrates revealed that many of them contained predominantly vitamin B_{12b}. Some of these concentrates were pure enough so that it was possible to check the results of the differential ascorbate method by spectrophotometric assay. The characteristic absorption spectra maxima at 3510 A for vitamin B_{12b} and at 3610 A for B_{12} was used in calculating the concentration of each form of the vitamin. There was close agreement between the two methods.

Application of the ascorbate method to liver extracts and to crude fermentation concentrates did not prove satisfactory. None of the naturally occurring amino acids, purines, pyrimidines, and metabolites tested had significant protective effect. However, as little as 10 mg of liver extract solids largely prevented the destruction of 40 μg of vitamin B_{12b} by 80 mg of ascorbic acid–sodium ascorbate mixture. Although purified liver extracts differ in their protective ability, crude liver extracts were uniformly effective. The protective property was found to reside in the ash, chiefly in the iron and copper. Iron salts are particularly effective, having demonstrable effect at .001% concentration of iron. Catalysis of the oxidative destruction of ascorbate by iron is well known, but any bearing this may have on the present phenomena is not clear.

Of the common anions tested, only sulfite, nitrite, and cyanide proved effective in stabilizing vitamin B_{12b} to ascorbate. Complete protection against ascorbate in aqueous solution is achieved in presence of approximately 100 molecular equivalents of sulfite or nitrite. At least 10 molecular equivalents of either are needed before significant protection to ascorbate occurs. Similar amounts of sulfite and nitrite are needed before the characteristic changes in spectra become apparent. This is in contrast to cyanide, where only 1 molecular equivalent is needed at pH 7 to convert vitamin B_{12b} to cyanocobalamin, as evidenced both by the shift in absorption maxima and stability to ascorbate. The effect of graded additions of materials which showed significant protection is shown in Table 1. Although thiocyanate (*7*), histidine (*8*), and ammonia (*8*) are reported to form complexes with vitamin B_{12b}, excesses of these materials did not protect vitamin B_{12b} from destruction by ascorbate.[2] An intrinsic factor concentrate of duodenum, 100:1 (Viobin), had no protective effect in a ratio of 1 mg concentrate/μg of vitamin B_{12b}.

TABLE 1

INFLUENCE OF SOME ADDITIONS ON STABILITY OF VITAMIN B_{12b} TO ASCORBATE

Addition to vitamin B_{12b}, 30 μg/ml	Activity remaining after ascorbate treatment (% of original)
None	0–4
KCN, 0.5 mol equiv*	38
" 1.0 " "	93
$NaHSO_3$, 1 mol equiv	8
" 10 " "	20
" 100 " "	94
$NaNO_2$, 1 mol equiv	2
" 10 " "	42
" 100 " "	96
$FeCl_3 \cdot 6H_2O$, .005%	7
" .02%	40
" .1%	86

* Molecular weight of cobalamin estimated as 1400.

[2] It is of interest that an excess of ferro- or ferricyanide was protective, whereas thiocyanate or cyanate appeared to have no effect.

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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