temperature in the presence of pyridine alone. In the presence of both pyridine and thiamine, however, destruction of the vitamin was complete after six months at room temperature and only 1 week at 45°. At 100° for four hours destruction was complete in the presence of the mixture, whereas, only 23.2% of vitamin B_{12} was lost with pyridine alone. It appears, therefore, that niacinamide or the pyridine nucleus, acts upon thiamine to form a compound that is incompatible with vitamin B_{12}.

Vitamin B_{12} has been found stable in low potency elixirs (2). Our studies on all oral products (not containing ascorbic acid) and elixir type products always have shown good vitamin B_{12} stability. No difficulty should be encountered with these products, since the thiamine and niacinamide content is relatively low.

SUMMARY

1. Studies of certain commercially available B-complex injectable products show instability of vitamin B_{12} after storage at room temperature for nine months.

2. This instability has been shown to be due to the presence of thiamine and niacinamide in combination at concentrations from 25 mg. to 100 mg./cc. of each in an aqueous solution adjusted to *p*H 4.25.

3. Destruction of vitamin B_{12} is diminished at decreasing concentrations of thiamine.

4. Both partially decomposed thiamine and the thiazole moiety of thiamine destroy vitamin B_{12} in aqueous solutions at *p*H 4.25 in the absence of niacinamide.

5. Pyridine can substitute for niacinamide in the destruction of vitamin B_{12} in the presence of thiamine.

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A Comparison of the Stability of Cyanocobalamin and Its Analogs in Ascorbate Solution*

By HASTINGS H. HUTCHINS, PATRICIA J. CRAVIOTO, and THOMAS J. MACEK

The stability of eight vitamin B_{12} products in 1% ascorbic acid solution in 1 molar acetate buffer at *p*H 4.0 has been studied during storage at constant temperatures. Cyanocobalamin (crystalline vitamin B_{12}) was shown to be markedly more stable than cyanide-free B_{12} analogs in ascorbate solutions. Vitamin B_{12} concentrates containing mixtures of cyanocobalamin and noncyano analogs were found to be less stable in ascorbate solution than concentrates containing cyanocobalamin exclusively. The stability of vitamin B_{12} concentrates thus appears to be related directly to the concentration of cyanocobalamin present in the concentrate.

CRYSTALLINE vitamin B_{12}, a coordination complex involving a cyano group and organically bound cobalt, gives rise to a series of cobalamins simply by replacement of the cyano substituent with certain other anions (1, 2). The cyanide-free analogs, originally designated by subgroupings B_{12a}, B_{12b}, etc., are now named after the anion-containing moiety, *viz.*, chlorocobalamin,[1] hydroxycobalamin, nitrocobalamin, thiocyanatocobalamin, etc. (3). All exhibit similar ultraviolet absorption spectra and are microbiologically active for *Lactobacillus lactis* Dorner and *Lactobacillus leichmannii* (2). These characteristics make it possible to compare stability of the vitamin B_{12} analogs with cyanocobalamin in aqueous solution.

A difference in stability to ascorbic acid was previously noted between cyanocobalamin and hydroxycobalamin (4–8). This reaction with ascorbic acid therefore was considered useful for measuring differences in stability between cyanocobalamin and various other analogs in the present study. For this purpose analogs in both

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[1] The term "cyanocobalamin" when used to describe the chemical composition of the vitamin B_{12} analog in concentrates is used in a chemical sense and not according to U. S. P. definition. Cyanocobalamin, as found in concentrates, is commonly amorphous; cyanocobalamin as defined in the U. S. P. XV is a pure crystalline compound.

the pure form and as various commercially-available concentrates of vitamin B_{12} were used. The presence of analogs other than cyanocobalamin in vitamin B_{12} concentrates has been suggested as a significant factor in the instability of such concentrates in pharmaceutical mixtures. Several authors have reported better stability of vitamin B_{12} in aqueous solution as a function of increasing concentration of cyanocobalamin in the vitamin B_{12} sample (4, 9).

In order to establish the cyanocobalamin content of the vitamin B_{12} concentrates used in this study the concentrates were assayed by the procedure of Bacher, *et al.* (10). Determinations made on the four samples indicated that one concentrate contained more than 99% cyanocobalamin; three others varied in cyanocobalamin content between 20 and 50%.

EXPERIMENTAL

Solutions of the vitamin B_{12} analogs were prepared at a concentration of 25 mcg./ml. in 1 molar aqueous sodium acetate-acetic acid buffer at *p*H 4.0. The solutions were assayed by the U. S. P. XIV method using *Lactobacillus leichmannii*. Aqueous solutions of cyanocobalamin were previously reported to be stable in this *p*H range (6). After removing the initial sample for assay, crystalline ascorbic acid (10 mg./ml.) was added to each of the solutions and a second sample was removed for assay for vitamin B_{12}. The samples were then stored and assayed frequently by the U. S. P. XIV method for total vitamin B_{12} activity.

The solutions of cyanocobalamin and the four vitamin B_{12} concentrates were stored at 30° in a constant temperature bath for various periods of time up to 6 days. Solutions containing nitrocobalamin, chlorocobalamin, and thiocyanatocobalamin were stored at a slightly lower temperature (25°) for three hours. During this time more than 92% of the vitamin B_{12} activity was destroyed in each of the latter samples. The results were checked in all cases both by duplication of the individual assays on separate days and by repetition of the entire experiment. The decomposition rate remained essentially the same in all experiments. During the test period no change in *p*H was observed in any of the samples.

Data indicating the stability of the various test solutions are summarized in Table I.

DISCUSSION

The data summarized in Table I indicate stability of eight vitamin B_{12} products in 1% ascorbic acid solution buffered with 1 molar sodium acetate-acetic acid at *p*H 4.0. This *p*H was used since it generally provides maximum stability for solutions of vitamin B_{12} alone and in the presence of other B vitamins. In a separate test, incidentally, it was observed that the rate of decomposition of thiocyanatocobalamin and nitrocobalamin was the same in unbuffered solution at *p*H 2.75 as is reported in buffered solution at *p*H 4.0.

Temperature was observed to have a noticeable effect on vitamin B_{12} analog stability. At 30° de-

TABLE I.—STABILITY OF VITAMIN B_{12} AND VITAMIN B_{12} ANALOGS IN ASCORBATE SOLUTION

	Micrograms per ml. (L. L.)							
	Stored at 30°					Stored at 25°		
Test Period	Cyanocobalamin, U. S. P.	Vitamin B_{12} Concentrate, Sample A	Vitamin B_{12} Concentrate, Sample B	Vitamin B_{12} Concentrate, Sample C	Vitamin B_{12} Concentrate, Sample D	Chlorocobalamin	Nitrocobalamin	Thiocyanatocobalamin
Cyanocobalamin content	97.7% (dry basis)	100%	20%	50%	50%	4.5%	Less than 2%	4.5% or less
Initial assay	29.6	29.8	24.5	26.2	28.8	16.8	24.0	24.0
Ascorbic acid added								
Initial assay	28.1	27.4	12.2	24.4	26.8	3.7	21.2	5.3
15 min.	..	..	..	..	..	1.19	6.3	2.0
30 min.	..	..	7.9	..	..	0.87	3.0	1.8
1 hr.	..	..	7.5	..	..	0.72	0.31	2.0
2 hr.	23.6	27.5	6.4	16.5	13.8	..	..	..
3 hr.	..	..	..	..	22.4	0.68	0.22	1.7
4 hr.	..	26.4	4.8	19.5	17.5	..	..	..
6 hr.	22.3	26.4	4.9	14.5	14.8	..	..	..
8 hr.	22.5	27.1	3.8	18.7	16.7	..	..	..
12 hr.	18.1	27.7	3.7	14.8	16.1	..	..	..
16 hr.	15.6	24.5	3.3	12.3	13.0	..	..	..
20 hr.	..	25.1	2.3	11.1	12.5	..	..	..
24 hr.	23.3	21.8	2.4	10.2	11.1	..	..	..
2 days	17.9	24.5	0.49	5.7	9.5	..	..	..
3 days	15.8	..	0.44	..	..	..	..	..
4 days	19.0	23.5	..	6.9	10.6	..	..	..
5 days	13.2	26.2	0.44	..	..	..	..	..
6 days	16.7	17.6	0.37	4.0	7.6	..	..	..

composition of the nitro-, chloro-, and thiocyanato-analogs was sufficiently rapid to cause difficulty in sampling. For this reason it was found better to study the stability of the analogs at a slightly lower temperature, i.e., 25° instead of 30°. Vitamin B_{12} decomposition was observed to be completely arrested during storage of samples at −10°. This observation enabled the authors to store samples and reassay them for confirmation of results on subsequent days.

Data given in Table I indicate that vitamin B_{12} concentrate stability in ascorbic acid solution is greater with increasing concentration of cyanocobalamin present. The presence of noncyano analogs in vitamin B_{12} concentrates resulted in a correspondingly greater vitamin B_{12} instability. Thus vitamin B_{12} concentrate (Sample B) containing only 20% cyanocobalamin proved to be less stable than B_{12} concentrates containing 50% cyanocobalamin (Samples C and D). The latter samples also were less stable than vitamin B_{12} concentrate containing all cyanocobalamin (Sample A).

The effect of ascorbic acid on stability of the different forms of vitamin B_{12} was quite pronounced. Cyanocobalamin and cyanocobalamin-containing B_{12} concentrates were found to be most stable to ascorbic acid. Noncyanocobalamins in pure form on the other hand were quite unstable. Marked decomposition even occurred in these solutions during the brief interval between addition of ascorbic acid and immediate sampling for initial assay.

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The Effect of Prednisolone and Prednisone on the Oxygen Uptake of Inflamed Tissue*

By JOHN G. McDONALD and HAROLD C. HEIM†

Inflammation was induced in rats by injecting air followed by a solution of croton oil in corn oil under the skin of the backs of the animals. Forty-eight hours after the injection of the croton oil some of the animals received 5.0 mg. of prednisone or prednisolone by injection into the inflamed area. Forty-eight hours after injection of the steroids the animals were sacrificed, the inflamed tissue was removed, homogenized, and the Q_{O_2} determined by conventional manometric techniques. Some of the animals received no steroids and served as the controls. It was found that tissue from the animals treated with the steroids showed a higher Q_{O_2} than did tissue from animals which received no steroids. The results were found to be statistically significant.

ALTHOUGH cortisone and hydrocortisone are widely used in the clinical management of certain inflammatory conditions, the mechanisms by which these drugs exert their antiphlogistic effects are not well understood. Recently the Δ^1 analogs of cortisone and hydrocortisone have been introduced as therapeutic agents under the names of prednisone and prednisolone, respectively, and clinical reports indicate that they are powerful antiphlogistic agents (1).

In a previous study (2) it was observed that the oxygen uptake of homogenates prepared from inflamed tissue was increased if the inflamed tissue had been previously treated with hydrocortisone. This effect was not observed with certain steroids which do not exhibit antiphlogistic activity. Because prednisone, prednisolone, and hydrocortisone all elicit similar therapeutic effects, it appeared of interest to determine the effects of prednisone and prednisolone on the oxygen uptake of homogenates prepared from inflamed tissue which had previously been treated with these

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