THE USE OF POTASSIUM CYANIDE IN THE LACTOBACILLUS LEICHMANNII ASSAY FOR VITAMIN B₁₂

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Studies by several investigators (Stokstad, Dornbush, Franklin, Hoffmann, Hutchings, and Jukes, 1949; Skeggs, Nepple, Valentik, Huff, and Wright, 1950) have indicated that an increased growth response was obtained to liver concentrates with thioglycolic or thiomalic acids when vitamin B₁₂ activity was determined with *Lactobacillus leichmannii*. This was attributed to the prevention, by such reducing agents, of the destruction of vitamin B₁₂ in the *L. leichmannii* assay medium. Recently Hendlin and Soars (1951) have shown that it is vitamin B_{12a} which is readily destroyed when autoclaved in the assay medium and that such destruction is prevented either by aseptic addition of the sample to sterile medium or by addition of reducing substances. Vitamin B₁₂ was unaffected under these conditions. Brink, Kuehl, and Folkers (1950) have reported that vitamin B₁₂ is a cyano-cobalamin complex; and Kaczka, Wolf, Kuehl, and Folkers (1950) have demonstrated that vitamin B_{12a} differs from vitamin B₁₂ by the absence of the cyano group. Furthermore, vitamin B_{12a} could be converted to vitamin B₁₂ by reaction with potassium cyanide. It appeared likely, on the basis of the foregoing data, that the assay of liver concentrates and other substances in a potassium cyanide supplemented medium would result in the conversion of vitamin B_{12a} like components to the more stable vitamin B₁₂, thus eliminating the use of reducing agents. This would be a definite advantage since the reducing agents themselves elicited a response from *L. leichmannii*. The experimental data presented here substantiate this hypothesis.

METHODS

Lactobacillus leichmannii ATCC 4797 was used in these studies. The culture was carried in an agar stab medium, and the inoculum was prepared by the procedure previously described (Hendlin and Soars, 1951). The medium used was a modification of the one employed by Skeggs, Huff, Wright, and Bosshardt (1948).¹ A total volume of 10 ml in 20 by 180 mm pyrex test tubes was employed. The medium was sterilized by autoclaving for 13 minutes at 120 C. Assay tubes were incubated at 37 C for 40 hours after which time growth was determined by titrating the acid produced with 0.1 N sodium hydroxide.

RESULTS

The concentration of potassium cyanide required to completely protect vitamin B_{12a} from destruction by autoclaving was determined by comparing the

¹ The medium was modified by eliminating pyridoxal and adding *p*-aminobenzoic acid (20 µg per 100 ml double strength medium).

dosage response curves obtained with vitamin B_{12a} in the presence of varying concentrations of potassium cyanide with the response curve obtained by the aseptic addition of the vitamin. The data indicated that 5 to 20 μ g of potassium cyanide per 10 ml medium completely protected vitamin B_{12a} from destruction. For this reason, 10 μ g of potassium cyanide per 10 ml medium was chosen for

TABLE 1
Comparative microbiological activity of several liver concentrates

	VITAMIN B ₁₂ EQUIVALENT		
	A*	B*	C*
Vitamin B _{12a} μ g per μ g	0.1	1.0	0.9
Liver Concentrate #1† μ g per ml	1.7	10.3	9.5
• #2 μ g per ml	2.9	3.0	3.4
#3 μ g per ml	2.3	7.7	7.0

* Treatment A) autoclaved with medium

B) added aseptically to medium

C) autoclaved with medium supplemented with KCN (1 μ g per ml)

† Each number designates different injectable liver (15 U.S.P. units).

Figure 1. Effect of KCN on vitamins B₁₂ and B_{12a}.

assay purposes. Vitamin B₁₂, which is not destroyed under similar conditions, was not affected by potassium cyanide supplementation (figure 1). To further check the validity of cyanide protection, several liver concentrates were assayed for vitamin B₁₂ content under the following three conditions: (a) addition to basal medium prior to autoclaving, (b) aseptic addition to previously autoclaved medium after sterilization by filtration through ultrafine sintered glass

filters, and (c) addition to medium supplemented with potassium cyanide (10 μ g per 10 ml). The results obtained are presented in table 1. It will be noted that in the case of several liver concentrates and vitamin B_{12a}, microbiological response was markedly reduced when the samples were autoclaved in the basal medium as previously demonstrated (Stokstad, Dornbush, Franklin, Hoffmann, Hutchings, and Jukes, 1949; Skeggs, Nepple, Valentik, Huff, and Wright, 1950; Hendlin and Soars, 1951). The values obtained in medium supplemented with potassium cyanide showed close agreement with those obtained by the aseptic addition of the samples to sterile medium.

The above data indicate that potassium cyanide effects the same protection of liver concentrates as has previously been obtained with thioglycolic and ascorbic acids. This is no doubt, due to the conversion of vitamin B_{12a} like substances, which are destroyed when autoclaved in the *L. leichmannii* medium, to the more stable vitamin B₁₂. In addition, the use of cyanide eliminated the consistently high titers (5.0 to 6.0 ml 0.1 N NaOH) obtained with protective levels of reducing agents (0.01 and 0.02 per cent of ascorbic and thioglycolic acids, respectively) in the absence of vitamin B₁₂. It should be pointed out, however, that in several instances titers as high as 3 ml were observed in potassium cyanide supplemented tubes (figure 1).

SUMMARY

The destruction of vitamin B_{12a} during autoclaving in the *Lactobacillus leichmannii* medium which has previously been prevented by the addition of reducing substances can also be prevented by the addition of potassium cyanide.

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