Vitamin B₁₂ in Human Blood and Serum

I. Comparison of Microbiologic Assays Using Normal Subjects

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The microorganisms used for Vitamin B₁₂ (cyanocobalamin) assay are Lactobacillus leichmannii (1), Euglena gracilis (2), Ochromonas malhamensis (3–5), and a mutant strain of Escherichia coli (E. coli 113-3) (6). Improvements in protozoan assays for B₁₂ show such assays to be more sensitive and specific than bacterial assays (2, 7, 8). Ochromonas malhamensis is highly specific for true forms of B₁₂ and shows an excellent correlation with vitamin B₁₂ activity in animals (3, 9). In this article we report a comparison of results of vitamin B₁₂ assays on normal whole blood and serum using the four organisms listed above.

MATERIALS AND METHODS

Serum and citrated blood were assayed for vitamin B₁₂ by methods outlined previously (2, 4, 6, 10, 11). For E. coli 113-3, the medium of Burkholder was used (12); the prepared dry mix obtained from Difco Laboratories* was used for assay with L. leichmannii ATCC No. 7830. The blood of normal subjects was obtained from an antecubital vein, diluted 1:5 with pH 4.5 buffer;† and autoclaved 30 min. to convert bound cobalamin into its microbiologically active form; serum was treated like blood. This procedure allowed estimation of total vitamin B₁₂ (13). For inoculation of prepared specimens: (a)

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†One-half gram of trans-aconitic acid per 100 ml. of distilled water, to which triethanolamine had been added to reach pH 4.5. One milligram of sodium metabisulfite had also been added to 100 ml. of buffer for stabilization of vitamin B₁₂ analogs.
**E. coli** as a loopful from nutrient agar suspended in 25 ml. of medium, (b) **L. leichmannii**, an 18-hr. culture diluted 1:10 in basal medium, (c) **E. gracilis**, strain Z, and (d) **O. malhamensis** are inoculated directly from a 5-day culture grown in liquid maintenance medium; final growth of the protozoa is speeded by 2 days. One drop into a culture flask served as inoculum. The bacteria required 18 hr. for full growth; protozoa, 4–5 days.

**RESULTS**

Figure 1 summarizes the results obtained with the four assay organisms for serum and whole blood. As may be seen, the values for **O. malhamensis** were the lowest; serum values ranged from 135 to 643, and those for blood from 115 to 450 µg./ml. The **E. gracilis** values are the next lowest; serum values ranged from 285 to 1335, those for blood from 305 to 875 µg./ml. With **L. leichmannii**, serum values ranged from 215 to 1500, those for whole blood from 465 to 1500 µg./ml. With **E. coli** 113-3, serum ranges were 565–1400, whole blood values 700–1600 µg./ml.

In Table 1, 8 cases are listed in which complete B₁₂ analyses were carried out with the four assay organisms. With **O. malhamensis** the serum values were equal to or higher than those for whole blood; about half the cases showed the reverse on assay with **E. gracilis**.

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**Fig. 1.** Vitamin B₁₂ in whole blood and serum.
This reverse relationship, with one exception, held for all results with the *L. leichmannii* assay; with *E. coli* 113-3, the results are high in both serum and whole blood. They resembled the *O. malhamensis* results in so far as the serum values were generally higher than those for whole blood.

**DISCUSSION**

Although B$_{12}$ can be assayed biologically in mice, chicks, and rats, especially with the use of radioactive cobalt, the microbiologic method of assay is preferred because it is economical and sensitive. One serious drawback of microbiologic B$_{12}$ assay procedures is the lack of specificity and sensitivity. The extreme sensitivity (1 x 10$^{-12}$ gm.) and relative freedom of stimulation in blood, serum, and urine make protozoa the choice assay tools (4, 9, 11). The most specific is *O. malhamensis* (4, 9); *Euglena* is known to be stimulated by pseudo-B$_{12}$ (3). As seen previously and noted here, serum and blood from normal subjects have a growth-promoting effect for *Euglena* (14) above that seen with *Ochromonas*. The reason for such stimulation is obscure, since no known pseudoforms of B$_{12}$ have been found in serum or blood. Increased growth in the bacterial assay, which lacks specificity in comparison to animals and to protozoa, is probably due to nonspecific stimulants present in blood and serum such as amino acids, nucleosides or different forms of B$_{12}$.

The value of the microbiologic assay for B$_{12}$ in clinical medicine has been established (15). Before the introduction of the microbiologic assay of B$_{12}$ in human serum, deficiency could be diagnosed only by symptoms and hematologic stimulation after therapy with liver extract or crystalline vitamin B$_{12}$. A similar problem was experienced with folic acid. Because folic acid is metabolically related...
to B$_2$, microbiologic assays had to be devised to pinpoint diagnosis. This has been accomplished (16, 17). Other assays for thiamine (18), pantothenic acid (19), and nicotinic acid (20) have now been added to the diagnostic armamentarium of the clinician; they have proved their value in recent work (21).

**SUMMARY**

A comparison of microbiologic assays for vitamin B$_2$ in whole blood or serum was carried out with four B$_2$-requiring microorganisms, *Escherichia coli* 113-3, *Lactobacillus leichmannii* ATCC No. 7839, *Euglena gracilis*, strain Z, and *Ochrononas malhamensis*. B$_2$ values in whole blood and serum for normal subjects are given. The value of microbiologic assays is discussed.

**REFERENCES**