Dose Response Curve Linearization and Computer Potency Calculation of Turbidimetric Microbiological Vitamin Assays

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The dose response curves of various turbidimetric microbiological assays, including vitamin B₁₂, Ca pantothenate, and pyridoxine (vitamin B₆), were linear with logarithmic transformation of the responses by use of the equation derived, \( \ln[T(x) - T(\infty)] = \ln A - Bx \). A Fortran computer program which used the slope ratio potency calculation was written. The assay potencies calculated by the computer program showed excellent agreement with those obtained by the manual calculation.

An automated turbidimetric microbiological assay readout instrument (7) has been successfully used. However, the efficient use of the readout instrument was achieved only when a computer program was used to calculate assay potencies.

The method was a mathematical transformation to linearize the dose response curve. The region of linearity should cover the dose level ordinarily in use. This paper briefly describes such an equation and a computer program for the assay calculation.

MATERIALS AND METHODS

The vitamin B₁₂, Ca pantothenate, and pyridoxine (vitamin B₆) microbiological assays were studied. Assay responses were read out manually by use of the Beckman model C colorimeter (Beckman Instruments, Inc., Fullerton, Calif.) at 650 m\(\mu\), and then by the automated turbidimetric bioassay readout instrument (7) at a rate of over 240 tubes per hr.

The vitamin B₁₂ microbiological assay was performed as described in the U.S. Pharmacopeia, except that the three dose levels used for the product assay were 1, 2, and 3 ml instead of 1, 1.5, and 2 ml. Pre-punched IBM cards were used to identify products for the convenience of the analyst. The IBM cards were prepunched as STD (standard) 1, 2, and 3, followed by SMP (sample) 1, 2, 3... Each IBM card was designed to record 12 readings including three dose levels with four replicates so as to have one card per sample. The vitamin B₁₂ standard at 1-, 2-, and 3-ml levels was placed after every fifth sample, as an internal standard to check the assay system.

The Ca pantothenate assay was conducted by use of the rapid assay technique previously described (6), and the pyridoxine (vitamin B₆) assay was performed as described by Tsuji (5). Each of these assays was run in duplicate at 1-, 2-, and 3-ml dose levels. Each IBM card was made to record six readings so as to have one sample per card.

For manual calculations of the assay, standard curves were drawn by use of the data obtained from both the Beckman C colorimeter and from the automated readout instrument, and product potencies were calculated from the curves.

RESULTS AND DISCUSSION

To program a computer to determine turbidimetric bioassay potencies, it is desirable to have a linear expression of the relationship between the doses and their corresponding responses. A study was made of the standard curves for three bioassays. These were the curves for the vitamin B₁₂ assay mentioned previously (Fig. 1); the pyridoxine (vitamin B₆) assay (Fig. 2); and the Ca-pantothenate assay (Fig. 3). It was decided that the curves could be fitted satisfactorily with an exponential expression: \( T(x) = A e^{-Bx} + C \) (1) where \( T(x) = \) percentage of transmittance at dose \( x \), \( x = \) dose in concentration units, and \( A, B, C \) = parameter to be estimated; theoretically, \( T(0) = A + C \), or percentage of transmittance at dose zero, and \( T(\infty) = C \), or percentage of transmittance at dose infinite. Hence, taking the natural logarithm of \( T(x) - C \), we have \( \ln[T(x) - T(\infty)] = \ln A - Bx \) (2). The plot of \( \ln [T(x)] \) versus the dose \( x \) results in a straight line with slope \(-B\) and intercept \( \ln A \).

The region of linearity proved to be well beyond the dose level ordinarily used for assay purposes.

For a given assay, it was shown that \( A \) and \( C \) remain relatively stable as long as assay variables, medium, inoculum, incubation temperature, etc., remain unchanged. The storage of preparations...
**Fig. 1.** Vitamin B\(_12\) bioassay standard curve.

**Fig. 2.** Pyridoxine (vitamin B\(_6\)) bioassay standard curve.

**Fig. 3.** Ca pantothenate bioassay standard curve.

**Fig. 4.** Linearized vitamin B\(_12\) bioassay standard curve.
in liquid nitrogen, which would enable the use of the same batch of inoculum, assay medium, and standard for a long period of time, would help to minimize variation in the assay variables (3, 4). The \( \ln [T(x) - T(\infty)] \) transformation is based on a concept similar to the thermal penetration theory and calculation as proposed by Ball and Olson (1). The \( T(\infty) \) value of 10 for the B\(_{12}\) assay was obtained by calculating mathematically from the standard curves run daily for approximately 3 months. \( T(\infty) \) for pyridoxine was 42, and for Ca pantothenate was 25.

A typical linearized vitamin B\(_{12}\) standard curve using equation 2 may be seen in Fig. 4, and that of pyridoxine and Ca pantothenate may be seen in Fig. 5 and 6.

Since the response curves for a given assay are transformed into straight lines intersecting at a common point at zero dose, the slope ratio method (2) of calculating potency can be used. A Fortran computer program for handling up to 16 preparations was written for a 1620 computer with 20K memory. An example of a typical computer print-out is shown in Fig. 7.

Because vitamin B\(_{12}\) was considered typical of
B vitamin assays, it was taken as an example to compare the agreement of assay potencies as calculated by a computer program and by a manual method.

The vitamin B12 potencies of the Upjohn vitamin products were calculated manually from the readout data obtained from each of the two instruments. As may be seen in Table 1, the maximal difference between the potencies, as calculated from the data obtained from the two instruments, was only 3%, and there was no statistically significant difference at $P = 0.01$.

Difficulties experienced with manual readout of vitamin B12 bioassay due to the polysorbate 80, which causes a thread-like swirl upon shaking of the assay tubes and makes the dispersion of cell suspension nonuniform was not encountered with the automated readout instrument. The readings were extremely stable. This may be attributed to the design of the multiple-prong sample-mixing devices which minimizes frothing or to the intermittent flow system used in the readout instrument which allows sufficient time for the stabilization of the turbid solution (7).

As reported previously (7), the spectrophotometer used in the automated readout instrument was stable, and there were no significant differences between the internal standard values throughout the assay.

The data obtained from the automated readout instrument were processed by use of the computer program described above, and potencies obtained were compared with those of the manual calculation. As may be seen in Table 1, there was excellent agreement between the manual and computer calculated potencies.

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**LITERATURE CITED**


