Radioactive Vitamin B_{12} Absorption Studies: Population Distribution of Plasma B_{12} Absorption and Serum B_{12} (36752)

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In 1962, Gräsbeck *et al.* (1) reported the log-normal distribution of serum vitamin B_{12} levels in a group of 1345 persons in eastern Finland. The clinical use of serum vitamin B_{12} measurements by microbiological techniques and the simultaneous measurement of radioactive vitamin B_{12} absorption levels at the Minneapolis Veterans Hospital supplied a patient population sufficiently large for analysis of the distribution of plasma absorption levels of vitamin B_{12}, as well as a comparison with the previously reported distribution of serum B_{12} levels. All patients on whom serum vitamin B_{12} by microbiological methods and radioactive vitamin B_{12} absorption studies were done from 1965 to 1970 were included in this study. A total of 1169 patients were available for analysis. The data obtained during this period provided an opportunity to apply statistical distribution analysis to both serum B_{12} levels and radioactive B_{12} absorption in a large population. To our knowledge, this is the first reported analysis of a large series of plasma vitamin B_{12} absorption studies and the identification of a normal gaussian distribution.

Materials and Methods. The data were obtained from 1169 unselected, consecutive patients from the Veterans Administration Hospital, Minneapolis, MN. This unselected population included normal subjects, patients with alcoholic liver disease, pernicious anemia, gastric resection, and other possible causes of malabsorption. All members of the group were male with one exception, age range 25–82 years. The blood sample taken for microbiological measurement of serum vitamin B_{12} level was centrifuged after clotting and the serum separated and stored at 4° until assay using the *Euglena gracilis* method of Ross (2).

Radioactive vitamin B_{12} absorption levels were measured according to methods previously described (3, 4). An oral test dose of 0.50 μCi of ^{57}cobalt vitamin B_{12} containing 0.56 mg of vitamin B_{12} was administered to the patient after an overnight fast. Breakfast was withheld for at least 2 hr after the test dose was given. A 10-ml blood sample was drawn just prior to the patient's receiving the oral test dose and again 8 hr later. Blood samples were centrifuged and plasma removed. Plasma radioactivity was counted directly in each sample on a Nuclear-Chicago auto gamma counter which had been precalibrated for low level ^{57}cobalt counting. Counting time was for either 100 min or 100,000 counts. A comparison of activity of the base line and eight hour samples to a reference standard enabled conversion from activity in counts per minute to picogram (pg) of vitamin B_{12} per milliliter in the 8 hr plasma sample. Normal values for this laboratory range from 1.9 to 11.4 pg/ml. Values below 1.9 pg/ml were automatically repeated using added intrinsic factor. A finding of vitamin B_{12} absorption within the normal range was diagnostic of pernicious anemia. Failure of the vitamin B_{12} absorption level to correct with intrinsic factor was suggestive evidence for B_{12} malabsorption of intestinal origin.

Statistical analysis of the data was performed by the Midwest Research Support Center, Veterans Administration Hospital, Hines, IL. Plot of the cumulative distribution showed a normal gaussian and a log-gaussian distribution of the radioactive vitamin B_{12} plasma absorption levels and the serum vita-

min B_{12} levels, respectively.

Results. Because of the large sample size and simultaneous measurement of serum vitamin B_{12} and plasma absorption studies, it was possible to statistically analyze the distribution of both measurements without consideration of age, sex or genetic history. A histogram plot representing the distribution of the serum B_{12} levels on a log scale confirmed the log-normal distribution described by Gräsbeck *et al.* (1) (Fig. 1). An arithmetic plot showed the serum B_{12} distribution to be markedly skewed, but to conform quite well to a log-normal distribution, *i.e.*, the log of the serum B_{12} level had a gaussian distribution with a mean of 2.34 and a standard deviation of $\pm$ 0.40 (Fig. 1).

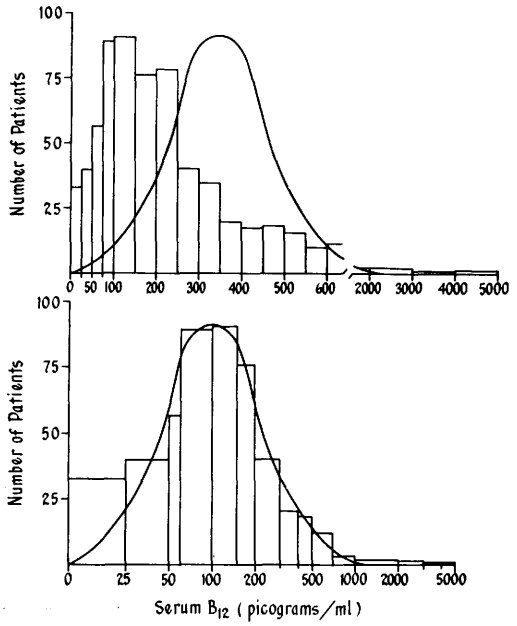

FIG. 1. Comparison of the arithmetic distribution of the serum B_{12} levels (upper) to the logarithmic distribution of serum B_{12} levels (lower). A normal gaussian curve is superimposed on the histogram plots to show that the results most closely approximate a log-normal distribution.

The radioactive vitamin B_{12} plasma absorption studies seen in the histogram in Fig. 2 followed a gaussian distribution except for slight skewing. This skewing was probably explained by the impossibility of negative values which one would expect in a true gaussian distribution. The mean absorption was 3.36 pg/ml with a standard deviation of 2.08 pg/ml.

TABLE I. Percentiles of Interest (Estimated and Observed) for the Plasma B_{12} Absorption and the Serum Vitamin B_{12} Levels.

Percentile	Estimated	Obsd.
Plasma B_{12} absorption (pg/ml)		
2.5	—0.80	0
5.0	—0.06	0.20
95.0	7.78	7.15
97.5	7.52	8.10
Log serum B_{12} (pg/ml)		
2.5	35	24
5.0	48	36
95.0	1000	1125
97.5	1380	1587

The correlation coefficient between the plasma vitamin B_{12} absorption levels and the serum vitamin B_{12} was 0.1328, while the correlation coefficient between vitamin B_{12} absorption and the logarithm of the serum vitamin B_{12} was 0.3722. This again supported the findings of a gaussian distribution for vitamin B_{12} absorption levels and a log-gaussian distribution for the serum vitamin B_{12} levels.

The estimated and actually observed percentiles of interest for the plasma B_{12} absorption and serum B_{12} levels are shown in Table I. Eighteen patients had vitamin B_{12} absorption levels and serum vitamin B_{12} levels be-

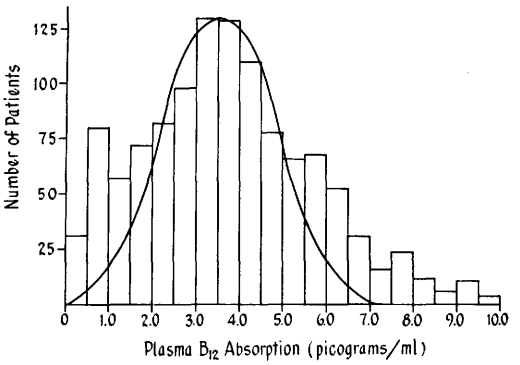

FIG. 2. Distribution of vitamin B_{12} absorption studies most closely approximates a normal gaussian distribution.

low the 5 percentile (0.236 pg/ml), eight patients had plasma vitamin B_{12} absorption levels and serum vitamin B_{12} levels below the 2.5 percentile (0.024 pg/ml). Eight had plasma vitamin B_{12} absorption levels below the 5 percentile and above the 95 percentile for serum vitamin B_{12} levels. Seven patients had plasma vitamin B_{12} absorption levels and serum vitamin B_{12} levels above the 95 percentile (72 and 1125 pg/ml). There were no patients found who had a high plasma vitamin B_{12} absorption level and a low serum vitamin B_{12} level.

Discussion. The findings of a log-normal distribution of serum vitamin B_{12} levels in a large population confirmed the work previously reported by Gräsbeck *et al.* (1). An explanation for this distribution is not readily apparent. As reported by these workers, when the normal mean level is small compared to the standard deviation, as is found in our results, asymmetric distribution must necessarily occur. As we indicated earlier, the skewing of the results may be explained by the absence of negative values, since a symmetrical distribution would imply the presence of negative numbers. The finding of logarithmic distribution in biologic materials is not unusual. The effects of cumulative concentrations of drugs, radiation and other physical factors frequently have a logarithmic association. It may be that most biological measurements are really log-normally distributed, but because of wide biological variation the logarithmic and arithmetic distributions are difficult to identify.

In contrast to the log-normal distribution of the serum vitamin B_{12} levels, the plasma vitamin B_{12} absorption levels were found to have a normal gaussian distribution. Again the data only approximated a gaussian distribution showing a slight skewing, which also may partially be due to the lack of negative numbers. Explanation for this population distribution is not known.

Demonstration of a positive correlation between vitamin B_{12} absorption and serum vitamin B_{12} levels of 0.1328 is in agreement with previous reports (4). Of significance is the increase in correlation to 0.3722 when vitamin B_{12} absorption levels are compared to the logarithm of the serum vitamin B_{12} levels. This again confirms the findings of a log-normal distribution of serum vitamin B_{12} levels and a normal or gaussian distribution for vitamin B_{12} absorption levels.

It is not the intent of this report to compare the relative significance of serum vitamin B_{12} measurements by microbiological techniques to the use of vitamin B_{12} plasma absorption levels in the diagnosis of pernicious anemia or other forms of vitamin B_{12} malabsorption. Suffice it to say, in our hands the plasma absorption measurements have been useful in detecting malabsorption of vitamin B_{12}. Instrumentation now makes it feasible to accurately detect the low levels of radioactivity that one finds in these plasma absorption measurements. We have not been troubled by the serious errors in the 8-hr plasma absorption test which have been described by others (5). In our particular hospital, the use of the radioactive vitamin B_{12} plasma absorption level has supplanted the measurement of urinary or fecal radioactivity for diagnosis of vitamin B_{12} malabsorption.

Summary. Simultaneous vitamin B_{12} plasma absorption levels and serum vitamin B_{12} levels were measured in 1169 consecutive unselected patients. Analysis of the population distribution confirmed the findings of Gräsbeck *et al.* (1) of a log-normal distribution of the serum vitamin B_{12} levels. To the contrary, a plot of the cumulative frequencies for distribution of the vitamin B_{12} plasma absorption levels showed a normal gaussian distribution, except for slight skewing at both extremes. This skewing would be anticipated because of the impossibility of negative values. The mean vitamin B_{12} plasma absorption level was 3 ± 3.6 pg/ml with a standard deviation of 2.08 pg/ml. Correlation between the plasma vitamin B_{12} absorption level and the serum vitamin B_{12} level was 0.1328. When plasma vitamin B_{12} absorption levels were compared with the logarithm of the serum vitamin B_{12} levels, the correlation was 0.3722. Eighteen patients were found who had plasma absorption and serum vitamin B_{12} levels below the 5 percentile. Eight had plasma absorption and serum vitamin B_{12} values below the 2.5 percentile. An additional

eight had plasma absorption measurements below the 5 percentile, while having serum vitamin B_{12} levels above the 95 percentile. Seven had plasma B_{12} absorption and serum vitamin B_{12} levels above the 95 percentile. In no patient was a high plasma vitamin B_{12} absorption and a low serum vitamin B_{12} level found.

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