the pretreated samples were placed in the ABA-100 incubator and the instrument cycle begun.

The ABA-100 dispensed 25 \( \mu l \) of Reagent B, 250 \( \mu l \) of thyroxine buffer, and 50 \( \mu l \) of working Reagent A into each sample well, an initial absorbance was read 5 and 10 min after reagent addition, and the 5-min change in absorbance was measured. For the standard curve, absorbance change for each calibrator was plotted vs. concentration on the EMIT graph paper supplied with the reagents. Unknown concentrations were determined by reading from the standard curve.

Thyroxine values by EMIT were determined (at Metpath Labs., Hackensack, N.J. 07606) with the AGA Autochemist (LKB, Bromma, Sweden) by the method of Galen and Foreman (1).

Thyroxine was also radioimmunoassayed with reagent kits obtained from Corning Medical, Medfield, Mass. 02052. In this assay system a solid-phase separation technique is used, thyroxine antibody being covalently attached to glass beads and packaged in premeasured assay tubes.

Patients’ sera were assayed in duplicate by both EMIT technique with the ABA-100 and by radioimmunoassay. The period between analyses was less than 24 h, during which the samples were stored at 4°C. Linear regression analysis (EMIT-ABA method on the y axis) of the paired data from 198 sera gave a correlation coefficient of 0.96, a slope of 0.96, and a y-intercept of -0.25. The normal ranges for these two methods are similar (45–120 \( \mu g/liter \) for EMIT and 45–125 \( \mu g/liter \) for the radioimmunoassay). 95% of our comparative values were diagnostically consistent with these normal ranges. Fifty patients’ sera were also assayed by the EMIT technique with the ABA-100 and with the AGA Autochemist. Linear regression analysis of these paired data (EMIT-ABA method on the y axis) gave a correlation coefficient of 0.95, a slope of 0.70 and a y-intercept of 1.98.

Determination of the within-day precision of the EMIT thyroxine assay for three sample concentrations gave coefficients of variation of 11.0% (mean, 25.0 \( \mu g/liter \); range, 73.9 \( \mu g/liter \); and 3.1% (mean, 125.7 \( \mu g/liter \)). Determination of day-to-day precision by assaying two control sera on each of 20 days gave coefficients of variation of 3.5% (mean 53.4 \( \mu g/liter \)) and 3.3% (mean 103.9 \( \mu g/liter \)). Analytical recoveries of thyroxine added to thyroxine-free sera averaged 95% over the range of 25–160 \( \mu g/liter \).

To determine the stability of the EMIT thyroxine reagent, we compiled the absorbance changes for each calibrator from 26 standard curves over a 20-day period. The variations in the calibrator raw data were determined and coefficients of variation in the range 2.4–2.9% were obtained.

The EMIT ABA-100 thyroxine assay yields results that agree well with those obtained by a radioimmunoassay. Agreement was also found with the original application of the EMIT thyroxine method on the AGA Autochemist, although there was more bias. Precision of the assay was excellent and the analytical recoveries were satisfactory. The major advantage in using this enzyme immunoassay for thyroxine is the stability of the standard curves within a given lot of reagents. The reagent stability allows longer use of a reagent kit than is possible with radioimmunoassay.

We conclude that the EMIT ABA-100 thyroxine assay is a viable alternative to radioimmunoassay.

Reference


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Antisera as a Source of Inaccuracy in Protein Immunoassays

To the Editor:

The Letter from Pratt (1) on the antisemum requirements for accurate assays of peptide hormones prompts me to point out that similar problems arise in the measurement of protein concentrations by automated immunoprecipitation and related techniques. Many human serum proteins, such as \( \alpha_1 \)-antitrypsin, display genetic variations in structure. It is relatively easy to produce antisera that are specific and do not give unwanted reactions with other proteins. However, for concentration measurements to be accurate, all the genetic variants of the prot-in should react to the same extent with the particular antisemur under the reaction conditions chosen—that is, an equal number of antibody molecules must be bound. It follows that a suitable antisemur must not contain antibodies to antigenic determinants present in some, but not all, of the protein variants.

In a single serum a large number of IgG proteins of widely different structure are present. Again, an antisemur for quantitative applications should contain only antibodies specific for the antigenic determinants present in all human IgG proteins. Grubb (2), using monoclonal IgG proteins, showed that selected antisemur gave reasonably accurate immunoassay results for several preparations containing single IgG proteins, as judged by an independent chemical assay, and would therefore be likely to give accurate results for sera containing oligoclonal and polyclonal IgGs. We have suggested one technique by which such antisemurs might be prepared (3). However, the cost of producing antisemur guaranteed to give accurate results is likely to be too high for some protein antigens as to restrict the use of immunoassays severely.

References


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Source of Error in Determination of Erythrocyte Folate by Competitive Binding Radioassay—Comments

To the Editor:

Netteland and Bakke (1) have identified problems in preparing whole-blood samples for radioassay of erythrocyte folate. In the protocol for our folate radioassay kit, the methodology for release of folate reactive in our competitive protein binding system involves a 21-fold dilution of ethylenediaminetetraacetate-treated blood in ascorbic acid, 2 g/liter solution, (pH ~4.2) and subsequent incubation of the diluted sample for 90 min at room temperature. According to Netteland and Bakke (1), this kind of procedure yields suboptimal values for erythrocyte folate. However, cells are lysed and oligoglutamates hydrolyzed to monoglutamate under these conditions, and these effects, which were established in our laboratory, are based on the following findings (2):

(a) In binding reactions with milk protein at pH 9.3, pteroylmeglutamate (PteGlu) and 5-CH3FH4 are equally reactive (3). However, PteGlu3 and PteGlu7 (gift of Dr. S. Waxman, Mt. Sinai School of Medicine, New York; 1975) are, respectively, 2.9- and 5.6-fold more reactive than the monoglutamates (2). In view of the reactivities of these oligoglutamates and the increased re-
activity with increasing number of glutamyl moieties, conversion of erythrocyte folates to a homogeneous form (monoglutamate) for recognition by binding protein was required. Endogenous conjugase, active at an acidic pH, provided for the enzymatic conversion of oligoglutamates to monoglutamate form(s).

(b) Conditions for optimal conjugase activity and maximal conversion were established by radioassay of hydrolysates, with PteGlu as the standard. As the hydrolysis proceeded, the "PteGlu" concentration decreased and reached a minimal concentration of measurable PteGlu when only monoglutamate was present (2). Minimal values were obtained at 90 min, with no significant further decrease by 120 min. Higher concentrations of ascorbic acid, 5 and 10 g/liter, were less effective in reducing "PteGlu" concentrations and this was probably related to the lower pH values, pH 3.8 and 3.5, respectively, for these 21-fold dilutions of whole blood.

(c) Blood samples diluted in phosphate buffer, pH 7.4, and lysed by freezing and thawing showed "PteGlu" concentrations two- to four-fold the concentrations of dilutions with 10 g/liter ascorbic acid solution incubated for only 20 min (2). The results indicated a need for an acidic pH for conjugase activity and confirmed the higher reactivity of intact oligoglutamates in the binding reaction.

The data in Table 1 of Netteland and Bakke (1) may be accounted for by the effect of conjugase activity during additional freeze-thaw cycles and at the various incubation temperatures. Considered in terms of the above observations, the lower values cited may more correctly represent whole-blood folate.

References
2. Unpublished data, this laboratory.

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Dr. Bakke responds:

To the Editor:
The new information provided by Dr. Gutcho on the rationale of incubating whole blood with ascorbic acid before binding to milk protein is greatly appreciated. I agree that decreased hydrolysis of oligoglutamates in erythrocytes to monoglutamate when the incubation step is omitted could give rise to erroneously high estimation of erythrocyte folate if the standard PteGlu is less reactive in binding assays. However, Schreibner and Waxman, using commercially available bovine beta-lactoglobulin, found PteGlu to be somewhat more reactive than PteGlu2 and PteGlu3 at pH 7.4. They preferred to use PteGlu as a standard in the assay of erythrocyte folate, and hemolysis was produced by freezing and thawing (1). The manufacturers of the kit used in the experiments reported in our Table 1 (Diagnostic Products Corp.) have not provided data on the relative reactivity of different folates for their kit in which beta-lactoglobulin is used at alkaline pH.

As pointed out in our Letter, we repeatedly found 10 to 17% higher values as compared to the standard procedure when whole blood was frozen and thawed and then incubated with ascorbic acid solution for 90 min. Unless freezing and thawing should affect the conjugase that hydrolyzes the oligoglutamates during the subsequent incubation, Dr. Gutcho's data cannot explain this finding. The information provided by the manufacturers of folate kits contains no warning that freezing and thawing might interfere in this manner. It is more likely that freezing and thawing is a more efficient method of producing lysis of the cells and making the folates available for the reaction with beta-lactoglobulin. The increased values found when the dilution was changed from 21-fold to 32-fold remains to be explained.

We have collected the results of our analyses of serum and erythrocyte folate during a period of time for the incubation procedure for sample preparation and during a subsequent period for freezing and thawing and binding assay after dilution of the samples. 32-fold with ascorbic acid solution (10 g/liter). The correlation between results for serum and erythrocyte folate was poor during the first period (r = 0.42, n = 33). A better correlation (r = 0.59, n = 59) and a much steeper regression line (erythrocyte folate on the y-axis) was found when the latter method was used. Other investigators using freezing and thawing and subsequent analysis by competitive binding assay or by the Lactobacillus casei assay have also found such a correlation. The improved correlation observed after we changed the sample preparation procedure adds to the suspicion that the method recommended by the manufacturer is not optimal.

The means are not available in our laboratory to test whether freezing and thawing yields the "correct" value for erythrocyte folate. However, our findings, together with those of Gutcho and of Schreibner and Waxman (1), have identified a number of possible sources of error in the determination of erythrocyte folate. It is now up to the manufacturers to show that the sample preparation methods they describe for their assays and the choice of standards are adequate. While we wait for these problems to be sorted out in detail we prefer to continue using the modified procedure, which gives higher values for erythrocyte folate and better correlation between the concentration of folate in serum and in erythrocytes. We have determined these values in 49 healthy volunteers of either sex with normal hemoglobin concentration. The mean values were 12.3 nmol/liter in serum and 1556 nmol/liter in erythrocytes. Elimination of extreme observations, leaving 95% of the values, led to a suggested normal range of 5 to 22 nmol/liter in serum and 800 to 2300 nmol/liter in erythrocytes.

References

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An Assessment of Hospital Routine Urinalysis

To the Editor:
In a recent evaluation of urinalysis methods in 35 Israeli laboratories (1) it was found that some of the methods in use were inaccurate and that others were obsolete. Despite this, there were no falsely positive results and all but three falsely negative results were attributable to the nature of the (lyophilized) control urine used as a sample. These findings contrast markedly with those of Kirkland and Morgan (2), who surveyed the routine ward and clinic urine testing for protein and glucose in a U.K. teaching hospital in the early 1960's and found that two-fifths of all proteinurias and half of the glycosurias were not detected, and that in addition there were several falsely positive results.

To assess whether the differences between the findings of these two reports reflected differences in the experience and training of the staff performing the tests or a genuine improvement in routine urinalysis during