decreased this blank rate without adversely affecting catalase activity. The residual blank reaction was corrected by use of a separate blank assay. Although successful, this approach does require additional steps and decreases the extent of automation.

We found that the coupled-enzyme method gave somewhat different values from those obtained with the manual method, as shown by the slope of the regression line. Although the results were correlated, the 340-nm method appears to demonstrate a somewhat greater sensitivity to changes in catalase activity in the hemolysate. This may be related to inherent differences in the response of the peroxidatic and catalatic reactions in this method, as compared with the catalatic reaction alone, as the catalase concentration in erythrocytes changes.

Unfortunately, wide application of measurements of antioxidant enzyme activity has been somewhat limited by the tedious nature of the assays. The most commonly used spectrophotometric method for catalase in erythrocytes requires a spectrophotometer capable of measuring absorbance at 240 nm and involves adjustment of initial conditions (7). Thus this technique is not suited for use as a high-volume clinical assay. The method we describe allows for a greater number of samples to be analyzed in a semi-automated fashion. This should provide a convenient means for investigating the usefulness of erythrocyte catalase measurements in assessing the potential for free-radical-induced pathology.

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Interference in an Automated Radial Partition Fluorescent Immunoassay of Thyrotropin Associated with Liver-Function Abnormalities

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In a previous evaluation of a "sensitive" radial partition fluorescent immunoassay on the Stratus™ system, thyrotropin (TSH) values exhibited a positive bias in icteric samples when compared with results of a nonsensitive radioimmunoassay. In the present study, we evaluated 366 patients' samples to assess whether any biochemical markers of liver function could identify samples for which TSH values would be falsely increased. γ-Glutamyltransferase and total bilirubin concentrations were unrelated to discrepant TSH values. In contrast, alkaline phosphatase (ALP) was significantly positively correlated with differences in Stratus and RIA TSH concentrations (P <0.001). However, this correlation explained only 34% of the observed residual variability around the estimated regression line. On average, the higher ALP values were associated with larger discrepancies between Stratus and RIA TSH values, although several samples with increased ALP did not have falsely increased Stratus TSH values. TSH measurements performed with a Stratus should be interpreted with caution in patients with abnormal biochemical markers of liver function.

Additional Keyphrases: variation, source of · radioimmunoassay compared · alkaline phosphatase

"Sensitive" assays of thyrotropin (TSH) have been introduced recently and recommended by numerous authors as a screen for thyroid disease (2–5). Several manufacturers have developed sensitive TSH assays based on a variety of methods, some of which are automated to handle large numbers of samples. Our previous evaluation of a radial

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6 Nonstandard abbreviations: TSH, thyrotropin; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; and T. Bil, total bilirubin.
partition fluorescent immunoassay (Stratus™ system demonstrated a positive interference with some icteric samples (4, 5). In contrast, we observed no interference from lutropin, follitropin, choriogonadotropin, lipemia, or moderate hemolysis. We therefore attempted to assess whether the apparent increase of TSH was related to an abnormality of hepatic function. Our objectives were to identify patients who exhibit falsely increased TSH values and to correlate the discrepancy with biochemical markers of liver function.

Materials and Methods

The sensitive Stratus TSH assay material was provided by Dade (Miami, FL). A second, "nonsensitive" radioimmunoassay (RIA) for TSH was performed with a kit purchased from Diagnostic Products (Los Angeles, CA).

Samples were selected from those submitted to the clinical chemistry laboratory at The Jewish Hospital of St. Louis and encompassed patients with normal (n = 73) and abnormal (n = 293) results for liver-function tests. The control group consisted of 48 women and 25 men, age 18 to 92 years (mean 56). The group with abnormal liver-function tests comprised 156 women and 137 men, age 17 to 94 years (mean 58). The thyroid status of the patients was not determined.

All 366 patients had TSH values measured by both assays. The hepatic biochemical measures included the determination of y-glutamyltransferase (GGT, EC 2.3.2.2) in all subjects; alkaline phosphatase (ALP; EC 3.1.3.1) and total bilirubin (T. Bil) were analyzed in only 290 patients.

Assays. TSH was measured with both the radial partition fluorescent enzyme immunoassay (6) in the Stratus and a nonsensitive RIA technic. In the former, an immobilized mouse monoclonal anti-TSH IgG is incubated with the patient's sample. A monoclonal anti-TSH (labeled with calf intestinal ALP) directed against a different site on the TSH molecule then reacts with immobilized antibody-bound TSH. All unbound sample or reagent is removed by radial elution, and the enzymatic rate of the bound fraction is measured by front-surface fluorometry. A stored calibration curve is used to convert rate to concentration.

The nonsensitive RIA, performed for comparative purposes to assess assay interference, is a conventional competitive inhibition RIA involving a polyclonal anti-TSH antibody and 125I-labeled TSH.

Manufacturers' normal TSH reference ranges are 0.4-7.0 and <4.0 milli-int. units/L for the Stratus assay and the RIA, respectively. The minimum detection limit (2 SD from the mean for the zero calibrant) of each assay is 0.15 and 0.78 milli-int. unit/L, respectively.

Statistical analysis. Unweighted linear least-squares regression analysis was used to compare results from the Stratus and the RIA. A forward, stepwise, unweighted regression was performed to determine which, if any, of the hepatic-function tests correlated with the observed discrepancies between Stratus and RIA measurements of TSH.

Results

Ordinary least-squares regression analysis of Stratus vs RIA on 366 paired observations gave the following equation: Stratus = 0.73 + 1.24 RIA with a correlation coefficient (r) of 0.977 and standard deviation of the residuals about the regression line (Sy|x) of 2.20. Evaluation of the data by omitting the five TSH values >20 milli-int. units/L reduced the correlation (as would be expected), but had little effect on the regression line or standard deviation. Figure 1 shows a scatterplot of all the data; the inset, which emphasizes results for TSH concentrations from 0 to 20 milli-int. units/L, points out several data points that are significantly above the best fit of the line.

We performed a forward, stepwise, unweighted linear least-squares regression analysis for Stratus TSH concentration as a function of RIA TSH, plus the hepatic-function indicators ALP, GGT, and T. Bil, to determine whether these additional variables could account for the large discrepancies in some of the Stratus–RIA TSH pairs. Alkaline aminotransferase (EC 2.6.1.2) was also considered, but deleted from statistical analysis because we measured it in only 34 of the 366 samples. After accounting for the correlation between Stratus and RIA TSH, the stepwise statistical analysis indicated that for the 290 subjects for whom all hepatic measurements were available, ALP was significantly positively correlated with the differences in Stratus and RIA TSH concentration (P <0.001). None of the other hepatic markers provided any additional significant discriminating power.

Figure 2 shows the scatterplot of the difference between the observed Stratus TSH concentration and the least-squares predicted concentration (regression line in Figure 1) as a function of ALP activity. As illustrated, some increased ALP values are associated with large differences between observed and predicted TSH (shown by plus signs). The estimated slope of the relationship is 0.0029 (SD 0.00024). Thus, on average, every 100 U/L change in ALP activity is associated with a 0.29 milli-int. unit/L difference between the observed and expected Stratus TSH concentration. ALP as an independent factor accounted for 33.6% of the residual variability about the regression line of Stratus vs RIA TSH concentration (Figure 1). However, several samples with increased ALP activity exhibited no significant difference between observed and predicted TSH.

A three-dimensional plot of Stratus TSH, RIA TSH, and ALP (Figure 3) shows that an increased ALP activity is frequently, but not invariably, associated with samples for which TSH values are higher by the Stratus assay than by the RIA.

We tested a few patients' samples with apparent falsely increased TSH values in the Stratus TSH assay system by using an anti-β-choriogonadotropin antibody-coated tab instead of anti-TSH-coated tabs. When measured against an antibody that does not recognize TSH, these samples also gave falsely high values, expressed as TSH.
Fig. 2. Effect of ALP on TSH measured by the Stratus assay.
The difference between the observed Stratus TSH concentration and the
least-squares predicted Stratus concentration based on the observed RIA
concentration is indicated on the y-axis. +, differences having absolute
standardized residuals > 2.

Fig. 3. Effect of ALP on Stratus vs RIA TSH correlation, exhibited in
dimensionality. The circles represent the points in three dimensions of Stratus TSH, RIA TSH,
and ALP. The two-dimensional scatterplot of Stratus TSH vs RIA TSH (Fig. 1,
inset) has been placed below the three-dimensional plot. Each circle in
the three-dimensional plot has a corresponding dot on the Stratus/RIA plot. Seven
of the circles were selected to demonstrate the projection of each onto its
corresponding dot. The solid line is the projection to the zero ALP plane and
the dashed line continues the projection to the corresponding point on the
Stratus/RIA scatterplot. The lengths of the solid lines reflect the magnitude
of ALP activity. ALP values (U/L) for the seven selected points are shown in
parentheses.

Discussion

During our initial evaluation of an automated assay
capable of analyzing large numbers of samples, we
observed a positive interference in the icteric samples that
did not correlate with bilirubin concentration (4, 5). This
positive interference could result in a failure to detect
hyperthyroid patients, as well as lead to falsely diagnosing
some euthyroid patients as hypothyroid.

We undertook a new study to determine the cause of the

positive assay bias, which we postulated was related to an
abnormality of hepatic function. This study confirms our
previous observation that falsely increased TSH values are
independent of T. Bil concentration. Another liver-function
test, GGT, also does not correlate with the falsely increased
TSH values. However, some of the observed TSH discrepancy
appears to be related to an increased activity of ALP
in the patients. The concentration of ALP does not predict
absolutely whether the interference will be present (see
Figure 2), because some samples with markedly increased
ALP values fail to demonstrate falsely increased TSH. A
similar interference has been observed in the Stratus
method for creatine kinase MB isoenzyme (7) and may be
associated with a high-molecular-mass form of ALP that is
membrane bound. In both our study (4,5) and that of Butch
et al. (7), increases of ALP could not account for all the
falsely increased TSH values. Dade has modified the sub-
strate wash solution to correct the problem of ALP inter-
ference for the MB assay by including a better inhibitor of
human ALP. However, this product is not available for the
TSH assay.

The evaluation of biochemical markers of liver function
demonstrates that increases in ALP partly explain the
positive bias for Stratus TSH values when compared with a
nonsensitive RIA method. However, we are unable to
explain the false increases for all patients' samples in
which TSH discrepancies are noted. Until this problem is
corrected, TSH values measured by the Stratus assay
should be interpreted with caution when determined for
patients with hepatic dysfunction.

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