a "sensitive" TSH determination or a thyroilin provocation test (5) is required.

During pregnancy or treatment with amiodarone the SimulTRAC FT/TSH assay remains a valuable tool in thyroid testing. Selection of monoclonal antibodies without cross-reaction with hCG would improve the diagnostic performance of this kit.

References

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Total Serum Protein Quantified without Interference from Dextran

To the Editor:
It has been known for many years that total protein may be grossly overestimated in dextran-containing sera when it is measured with a biuret-type reagent, because turbidity forms (1, 2). Flack and Woollen (3) studied the conditions for formation of the insoluble precipitate and concluded that a biuret reagent free from dextran interference should contain either a very high (22.5 g/L) or a very low (4.5-5.6 g/L) concentration of tartrate. The report of Barnes et al. (4), however, points to the fact that the problem of dextran interference still exists, at least with some automated analyzers. Although it is well documented that formation of the interfering insoluble complex requires the simultaneous presence of Cu²⁺, dextran, a chelating agent, and NaOH, little attention has been paid hitherto to the importance of NaOH concentration, most of the discussion centering on the appropriate concentration of chelating agent and the timing of the reaction (3, 5).

We therefore decided to examine these two constituents of the biuret reagent in conjunction. We prepared nine different biuret reagents, with three concentrations of NaOH (0.2, 0.4, and 0.6 mol/L) and three of tartrate (4.5, 9.0, and 18.0 g/L) in all possible combinations. The concentration of CuSO₄ - 3H₂O was kept constant at 3.0 g/L and that of KI at 5.0 g/L in all of these. Using a pooled specimen of human serum, a 22.0 g/L albumin solution, a 100 g/L dextran-40 solution, and a 9.0 g/L NaCl solution, we prepared a series of samples containing a constant concentration of total protein but with increasing dextran concentration ranging from 0 to 40.0 g/L. The sample reagent volume ratio in the manual assay was 1/50.

When the nine biuret formulations were tested with these samples, only the one that contained 0.2 mol of NaOH per liter and 18.0 g of tartrate per liter remained clear, and the color intensity of the reaction product was unaffected by the presence of dextran at any concentration tested. All the other biuret formulations gave visible turbidity immediately after dextran-containing serum was added. This latter observation differs from that of Flack and Woollen (3), who found that, with low tartrate concentration (4.5-5.6 g/L), turbidity takes longer than 5 min to develop.

We also tested the reagent recommended by the manufacturer for the Technicon RA-1000 discrete analyzer, which contains, per liter, 0.2 mol of NaOH, 14.2 g of tartrate, 3.0 g of cupric sulfate pentahydrate, and 5.0 g of KI. The reagent performed very well, both manually and in the instrument, at all dextran concentrations. In that respect the Technicon reagent is erroneously listed among those that give trouble with dextran (3). Evidently, the lowest concentration of tartrate that effectively prevents precipitate formation in the presence of dextran is closer to 14 g/L than to the 15 g/L previously reported (4). Nevertheless, for the rest of our experiments we maintained the concentration of 18.0 g/L for the sake of uniformity of conditions.

Finally, we examined the color yield and the time needed for completion of the reaction, because these two variables are the basis for the main arguments of Doumas (6) and Doumas and Peters (7) for choosing higher NaOH concentration. The color yield with our reagent was 89% as great as theirs for a series of samples containing from 55 to 120 g of protein per liter, so the sensitivity of the method is not appreciably compromised. The correlation between the values obtained with our reagent and those obtained with that of Doumas was also very good (r = 0.9987). The rate of color development is indeed slower, requiring about 15 min for completion as compared with about 5 min for the Doumas reagent. However, 97% of the final color developed within 7 min, so this reaction time can be used with automated instruments in which reactions of standards and unknowns are accurately timed.

In conclusion, a biuret reagent that is free from dextran interference must combine low NaOH (0.2 mol/L) with a high tartrate (between 14.0 and 18.0 g/L) concentration. A higher tartrate concentration than this is unnecessary and even undesirable, because it further decreases the color yield. The proposed reagent should be especially useful on discrete-step noncentrifugal analyzers, because in centrifugal-type instruments the precipitate that might form is automatically removed from the light path.

References

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