as we described in Clin. Chem. 23, 754 (1977). The latter procedure enables one to determine the true protein content. In all cases, the absorbance was read after 30 min and protein content was calculated from the absorbance of standard sera treated similarly. We obtained the following results: 94.0 (Yatzidis), 92.2 (our a), and 68.3 (our b), all expressed as the mean values in g/liter. Evidently both reagents, when directly used, gave practically the same but falsely high results. However, when the absorbance was read within 1–5 min after mixing the respective reagent with lipemic serum, a result nearly corresponding to the true protein content was found.

The absorbance of the reaction mixture containing lipemic serum is the resultant of two components: the colored biuret product and the turbidity. The reaction velocity of the biuret reaction is apparently greater then the increase in turbidity. The clearing effect attributed to the modified biuret reagent is mostly caused by the time factor only. Our detailed results will be published elsewhere.

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Enzyme Immunoassay

Theophylline with a Centrifugal Analyzer, and Comparison with an Ultraviolet Method

To the Editor:

Measurement of theophylline in plasma is clinically useful in evaluating patient dose–response and toxicity (1).

We report here a rapid, micro procedure for measuring theophylline in plasma (or serum with the EMIT reagent system (Syva, Palo Alto, Calif. 94304) and a centrifugal analyzer (Centrifichem, Models 330 and 440; Union Carbide, Rye, N. Y. 10580). For comparison, theophylline was determined by a modification of an ultraviolet method (2).

Theophylline may be determined with the EMIT reagents after a single dilution of samples and standards. All reagents are reconstituted according to kit instructions except for the modifications noted below.

The reagents are EMIT theophylline antibody/substrate (Reagent A), and EMIT theophylline coupled to glucose-6-phosphate dehydrogenase, EC 1.1.1.49 (Reagent B).

The standards are EMIT theophylline calibrators, 0–40 mg/liter, and the controls are plasma with weighed-in amounts of theophylline: 10, 20, 30 mg/liter.

The procedure is as follows:

Pipet 100 µl of sample, calibrator, or control into a 12 × 75 mm test tube, and add 200 µl of EMIT buffer [tris(hydroxy-methyl)methylamine, 55 mmol/liter, pH 7.9]. Prepare the substrate by pipetting 0.6 ml of Reagent B into 10 ml of EMIT buffer. For the pipettor, dial in 10 µl for sample, 50 µl for sample and diluent, and 350 µl for reagent. Start the pipettor. The last sample plug is used. (Note: When using Centrifichem 400, leave position 1 empty.) Manually pipet 20 µl of Reagent A carefully into the sample section of the transfer disc.

Set the analyzer as follows:

Filter = 340 nm
Terminal/Rate = Terminal
Auto/Store = Auto
T0 = 30 s
Print = 9, all
Temp. = 30 °C
Absorbance/Conc = ABS
Calib/operate = Operate (300 series only)
ΔT = 15 s

Select the print-out that yields a ΔA of 65 to 135 units between the 2.5 and 40.0 mg/liter calibrator, and a ΔA of at least 20 units between the 0 and 2.5 mg/liter calibrator. Before plotting the data, subtract the zero-standard absorbance value (A0) from the absorbance value of standard, sample, and calibrator. The final value (A – A0) is used to construct a curve on the log-log graph paper provided with the kit.

The accuracy and precision of the method was determined by assaying plasma with weighed-in amounts of theophylline corresponding to 10 and 20 mg/liter. The coefficient of correlation (r) between the 10 and 20 mg/liter sample and results obtained respectively for A – A0 for 15 specimens was 0.988 and 0.977, respectively. The CV for 15 specimens (within-day run) was 4.47% (10 mg/liter) and 4.67% (20 mg/liter).

The ultraviolet method (2) was modified for pediatric samples by reducing the sample volume from 3 to 1 ml and scaling down other reagents accordingly. Satisfactory results were obtained with 0.5 ml of sample, but sensitivity was better with the 1-ml sample. For 15 patients’ samples (3.0–15.0 mg/liter) the r was 0.976.

We conclude that the EMIT system adapted to the Centrifichem is a satisfactory, rapid, micro-scale procedure for measuring theophylline in plasma. It requires only 100 µl of sample and about 20 min of technician time. The correlation with an established ultraviolet method is excellent, and no extraction procedure is necessary. In contrast, the ultraviolet method requires a larger sample volume, more technician time (1–2 h), and two extraction steps.

References


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Effect of Antibody Binding on the Mobility of Serum Lysozyme

To the Editor:

The electrophoretic mobility of urinary lysozyme has been thoroughly characterized, particularly in patients with monocytic and myelomonocytic leukemia (1), but references to the mobility of lysozyme (EC 3.2.1.17) in serum are scarce. In 1973, Finkle et al. (2) found complexes of a monoclonal IgG with lysozyme in the serum of a patient with acute myelomonocytic leukemia, and they presented evidence that the enzyme and the monoclonal protein had the same mobility. In 1975 we described a second patient with monocytic leukemia whose serum lysozyme had the same mobility as the gamma globulin fraction, which in this case was polyclonal (3). At that time it appeared that serum lysozyme had a strong tendency to form complexes with the gamma globulins. In three subsequent cases, however, one of monocytic leukemia, one of myelomonocytic leukemia, and one of myeloma, we found no evidence of IgG–lysozyme complexes, and in these cases the mobility of serum lysozyme was identical to that of free urinary lysozyme.

In an attempt to determine whether soluble complexes of lysozyme and anti-lysozyme antibody could account for the shift of serum lysozyme to the gamma globulin region, as seen by Finkle et al. (2) and in our earlier case (3), we added 500 µg of purified human lysozyme to 1 ml of serum obtained from a sheep hyperimmunized with human lysozyme. Details of the preparation of this antisera were reported previously in this journal (4). An identical mixture was prepared with the serum from a sheep immunized with human IgG. Both mixtures were incubated for 1 h at 37 °C and then separated by cellulose acetate