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We have developed an accurate procedure for measurement of plasma fibrinogen. Turbidity resulting from heating (56°C, 20 min) plasma diluted with a new “zwitterionic” buffered-saline reagent (pH 5.6) is nephelometrically measured (600 nm). We evaluated the method both by confirming the accuracy of its prestandardization and calibration on a new clinical nephelometer, and by comparative standardization by use of a conventional spectrophotometer. The nephelometer is more sensitive and gives a linear response over a wide range of concentrations. Representative information supporting the broad clinical utility of the determination of plasma fibrinogen is illustrated. These assays should be a significant complement to multitest biochemical screening of both healthy and hospitalized individuals.

Additional Keyphrases diagnostic aids • spectrophotometry and nephelometry compared • stress evaluation • multitest screening • buffer as diluent

Several investigators have concluded that numerous analytical methods for plasma fibrinogen based on turbidimetry of precipitable protein with various salt-fractionation reagents (sodium sulfite, sodium sulfate, ammonium sulfate, phosphate salts, and glycine) are not reliable enough for broad clinical use (1, 2). Hence, if determination of plasma fibrinogen is to achieve its potential usefulness in clinical medicine, more suitable analytical methods must be devised. Among the better reference techniques are those involving heat precipitation and ultraviolet spectrophotometry (2), or clot formation with thrombin coupled with ultraviolet spectrophotometry (2, 4). Unfortunately, these procedures are too involved for routine analytical use, and a more accurate, rapid, turbidimetric method is desirable.

The previous turbidimetry studies (1, 2) did not include an appraisal of fibrinogen assays involving measurement of turbidity resulting from heating, at 56°C, plasma diluted with a buffer solution. Typical of such existing methods are those described by Watson (6) and Wycoff (6). Moreover, all of the workers cited measured the turbidities produced spectrophotometrically as absorbance, and none actually adhered to recommended techniques of nephelometry by using a nephelometer to measure the fibrinogen precipitates.

We describe here a new, improved buffer diluent for use in determining plasma fibrinogen via heating at 56°C. The reliability of the proposed procedure is evaluated by means of a new clinical nephelometer and with a spectrophotometer. We also present further data confirming the wide clinical applicability of this methodology.

Materials and Methods

Apparatus

A Model 2424 “Clinical Nephelometer” (Hach Diagnostics, Ames, Iowa 50010) was used for nephelometric measurements. A “Spectronic 20” spectrophotometer (Bausch & Lomb, Rochester, N.Y. 14625) was used for comparative turbidimetric studies. A Model 222 photometer (Gilford Instrument Laboratories, Oberlin, Ohio 44074) was used for ultraviolet spectrophotometry at 282 nm of fibrinogen solutions in the Grannis reference method (9).

Reagents

The nephelometric fibrinogen reagent, purchased from Hach Diagnostics, consists of a sodium chloride solution (9 g/liter) buffered at pH 5.6 with a “zwitterionic” buffer introduced by Good et al. (7). Other reagents were as described by Grannis (2) and Campbell and Hanna [as presented by Henry (8)].
Procedures

The new Hach nephelometric procedure for fibrinogen was carried out as described in the directions accompanying the reagent. In principle, the test consists of heating at 56°C for 20 min either 0.30 ml of plasma diluted with 9.7 ml reagent (if normal or supernormal concentrations are anticipated), or 0.30 ml of plasma diluted with 4.7 ml of reagent (if hypofibrinogenemia is expected). The denatured fibrinogen suspension was measured either nephelometrically against an unheated sample with the nephelometer, or turbidimetrically at 600 nm with the spectrophotometer.

The manufacturer’s standardization factor for the nephelometer was derived (by D.E.R.M.) from fibrinogen assays of 20 human plasma samples by a method involving sodium sulfite precipitation and subsequent Kjeldahl nitrogen determinations (8). The resulting “Hach” factor was checked independently (by E.W.R.) by comparing the nephelometric results of 50 samples with those obtained by the “reference” clottable-protein procedure of Grannis (8).

Results

Figure 1 illustrates the independent confirmation of the manufacturer’s procedure for plasma fibrinogen standardization: “the nephelometer is set at ‘range 10’ and the ‘150 formazin turbidity units latex turbidity standard’ is adjusted to a percent scale meter reading of 71.5.” When this is done, the plasma fibrinogen concentration, in g/liter, is obtained by simply multiplying the meter reading by 0.1.

Further information on the day-to-day precision of the nephelometric methodology was obtained by analyzing 38 samples in duplicate during a 2-week period. The standard deviation was 0.107 g/liter, which represented a relative standard deviation (coefficient of variation) of ±2.1%.

Forty-eight of the 50 calibration samples were measured with the spectrophotometer (two tubes were inadvertently discarded). A multiplication factor of 12.3 was calculated by the method of least-squares for converting the absorbance into plasma fibrinogen (g/liter). The results of the Hach method via this “spectrophotometric” quantitation are presented in Figure 2. Although the overall mean for both methods was 5.05 g/liter, results obtained with the spectrophotometer for samples with less than about 4.8 g/liter averaged 0.15 g/liter less, and results for samples more concentrated than this averaged 0.10 g/liter more than the corresponding reference values. Clearly, results obtained with the nephelometer are the more nearly accurate.

Added data on the precision of the spectrophotometric readings were collected on the same 38 duplicate samples used in the nephelometric day-to-day precision results cited above. The standard deviation was 0.064 g/liter, or a relative standard deviation of ±1.3%. This is better than the corresponding precision, ±2.1%, obtained with the nephelometer and reflects the fact that absorbances, if calculated from care fully established “%T” readings on the spectrophotometer scale, can be more nearly accurate than “percent scale” values on the nephelometer. The latter scale may be read to 0.5%, which corresponds to 0.05 g of fibrinogen per liter of plasma (when using a 0.3:10.0 dilution).

Discussion

These experiments prove that the Hach fibrinogen methodology rapidly yields results that are entirely comparable to those obtained by more laborious “reference” procedures.1 Nephelometric data are more reliable than spectrophotometric data, largely because of the excellent linear response with the former instrument. The proposed nephelometric method is being used with hospitalized patients and has proved to be most valuable in helping to assess the status of numerous pathological states. Unfortunately, despite an ample body of relevant literature (e.g., 2, 9–18), many clinicians are unaware of conditions associated with hyperfibrinogenemia and how this parameter can be a valuable biochemical test in the clinical evaluation not only of diseased and injured patients, but of chronically stressed individuals as well. Serial determinations, in particular, are valuable in following the course of many disease states characterized by tissue destruction, inflammation, or stress: rheumatic fever, septicemias, respiratory diseases, diabetes, thrombophlebitis, cardiomyopathy, pulmonary and myocardial infarctions, rheumatoid arthritis and arteriosclerosis, acute pancreatitis, cancer, alcoholism, and schizophrenia, and postoperative recovery. Recovery and survival frequently seem

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1 Plasma fibrinogen methods involving precipitation at 56°C include turbidity owing to fibrin degradation products and (or) fibrin monomers and complexes found in certain patients with fibrinolytic or defibrinating processes. The quantities of such proteins are typically small in comparison to fibrinogen.
Fig. 2. Correlation between results of fibrinogen assays made with the Hach fibrinogen reagent and a spectrophotometer, and the reference 2 method

Each of the 48 points represents the means of duplicate analyses of each method
to be intimately related to the patient’s plasma fibrinogen response.
Table 1 illustrates representative plasma fibrinogen concentrations in unselected patients at the time of admission to a general hospital; 44% were hyperfibrinogemic. This compares favorably with a previous study (9) in which 61% of randomly collected samples from 280 patients showed elevated fibrinogen during hospitalization. These surveys support the view that fibrinogen determinations should be used more widely in patient management, and that this procedure might be a useful addition to the battery of biochemical hospital admission tests.

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Screening of 563 Students for Cholinesterase Variants

Charles E. Becker

Of 563 dental students screened for cholinesterase variants, 97.2% were homozygotes for the usual cholinesterase (E1E1,E1), 2.1% were heterozygotes for the usual and the dibucaine-resistant cholinesterases (E1E2,E1), and 0.7% were heterozygotes for the usual and the fluoride-resistant cholinesterases (E1F1,E1).

Additional Keyphrases “Ro 2-0683” and “Sernylan” used in distinguishing variants • incidence of variants in a subpopulation

Plasma cholinesterase (acyleholine acyl-hydrolase, EC 3.1.1.8) destroys the muscle relaxant, succinylcholine, and the local anesthetic, procaine. Individuals possessing a variant cholinesterase are endangered by exposure to these compounds. This paper describes how 563 dental students were screened for plasma cholinesterase variants, and the results.

References


Table 1. Plasma Fibrinogen Concentrations of 250 Unselected Patients upon Scheduled Admission to a 537-Bed General Hospital

<table>
<thead>
<tr>
<th>No. samples</th>
<th>% of total</th>
<th>Plasma fibrinogen, g/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group mean</td>
<td>Group range</td>
</tr>
<tr>
<td>1</td>
<td>&lt;1%</td>
<td>1.82</td>
</tr>
<tr>
<td>140</td>
<td>56%</td>
<td>2.00–4.09 (“normal”)</td>
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<td>70</td>
<td>28%</td>
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<td>28</td>
<td>11%</td>
<td>5.38</td>
</tr>
<tr>
<td>11</td>
<td>4%</td>
<td>6.84</td>
</tr>
</tbody>
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|                      |           |                          |
|                      | 2-0683    | “Sernylan” used           |
|                      | in        | distinguishing variants   |
|                      | variants  | incidence of variants     |
|                      | in a      | subpopulation             |

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