A MODIFIED METHOD FOR THE QUANTITATIVE DETERMINATION OF THE THYMOL TURBIDITY REACTION OF SERUM

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It was observed by Maclagan that turbidity is produced in a barbital buffer saturated with thymol when specimens of serum from patients with diseases of the liver are added (1, 2). The degree of turbidity apparently varied with the degree of hepatic parenchymal damage. An analysis of the precipitate demonstrated that it contained globulin, phospholipid, cholesterol, and thymol. The mechanism of the reaction is unexplained, but there is some evidence to indicate that the reaction may be due to an abnormal globulin released into the blood stream in the presence of liver damage. The determination of thymol turbidity had been found useful in estimating the degree of hepatic damage in patients with diseases of the liver and in pathological states experimentally produced in animals.

In the original technique the amount of turbidity was estimated by visual comparison with the gelatin standards of Kingsbury et al. (3), which were devised for the estimation of urinary albumin. This method is subject to all of the usual criticisms of visual methods for colorimetric and nephelometric measurements. In the presence of hemolysis and of increased concentrations of bilirubin in the serum, accurate measurements of turbidity may be difficult to obtain. Moreover, the preparation and maintenance of adequate gelatin standards are associated with some difficulty. In order to obtain objective and more accurate quantitative measurement of turbidity, and to permit rigid standardization of the technique, the method has been modified in this laboratory so that turbidimetric determinations may be made in the spectrophotometer with a suspension of barium sulfate as a standard.

Method

Reagents

Thymol-barbital buffer. A barbital buffer of pH 7.8, saturated with thymol, is prepared as described by Maclagan (2). 1.03 gm. of sodium

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barbital, 1.38 gm. of barbital, and 3 gm. of powdered thymol crystals are placed in a 1000 cc. Erlenmeyer flask. 500 cc. of distilled water are added and the solution heated to the boiling point. The flask is removed from the flame and the contents mixed well by shaking. The solution is cooled to room temperature. On cooling, the solution becomes turbid. A small quantity of powdered thymol crystals is added and the solution again mixed by shaking. The flask is stoppered and is permitted to remain at room temperature overnight. Thymol crystals form at the bottom of the flask. After standing overnight, the solution is mixed once again by shaking, and is freed of crystalline deposit by filtration. The clear solution is used as the reagent and may be kept at room temperature indefinitely.

Barium chloride solution, 0.0962 N.
Sulfuric acid, 0.2 N.

Procedure

The test is carried out by adding 0.05 cc. of serum to 3.0 cc. of thymol-barbital buffer in a 10 × 75 mm. cuvette. The contents of the cuvette are mixed well and after 30 minutes turbidity is determined in the Coleman Junior spectrophotometer at a wave-length of 650 m\(\mu\). The galvanometer is adjusted to 100 per cent transmission with a blank containing 3.0 cc. of thymol-barbital buffer.

The turbidity of a given reaction is expressed in units derived from a standard curve prepared by use of barium sulfate suspensions. The turbidity standard is prepared by diluting 3.0 cc. of 0.0962 N barium chloride solution to volume in a 100 cc. volumetric flask by the addition of 0.2 N sulfuric acid at 10°. At this temperature the particle size of the precipitated barium sulfate is such that a comparatively stable suspension results. A 10 unit turbidity standard is prepared by adding 1.65 cc. of 0.2 N sulfuric acid to 1.35 cc. of the barium sulfate suspension in a 10 × 75 mm. cuvette. Similarly, a 20 unit standard is prepared by adding 0.3 cc. of 0.2 N sulfuric acid to 2.7 cc. of the barium sulfate suspension. These standards were chosen because they gave turbidimetric readings nearly equivalent to those obtained by Maclagan using visual comparison with gelatin standards. At room temperature there is some tendency for the barium sulfate suspension to settle out. For this reason cuvettes should be well shaken just before readings are made in the spectrophotometer. If a cuvette containing 3.0 cc. of distilled water is used as a blank, there is a straight line relationship between the optical density of various dilutions of the barium sulfate standard at 650 m\(\mu\) (Fig. 1).

EXPERIMENTAL

It was necessary to determine the wave-length at which turbidity measurements could be made with least interference from those substances
in blood which absorb in the range of 400 to 700 m\(\mu\). Two substances which might be expected to interfere are hemoglobin resulting from hemolysis of red blood corpuscles and bilirubin which may occur in high concentration in the serum of patients with hepatic disease. In Fig. 2 the absorption spectrum of a turbidity standard of 15 units is compared with the absorption spectra of a 1:250 dilution of hemolyzed red blood cells and of a 1:60 dilution of serum containing 14.0 mg. per cent of bilirubin. It will be seen that turbidity determinations done at 650 m\(\mu\) are relatively free of interference from light absorption due to bilirubin and hemoglobin. This wave-length is far removed from the absorption maxima of these com-

![Graph](image1.png)

**Fig. 1.** Turbidity of barium sulfate suspensions as determined in the spectrophotometer.

![Graph](image2.png)

**Fig. 2.** Absorption spectra of a solution of hemolyzed red blood cells, a dilution of serum of high bilirubin content, and a barium sulfate turbidity standard.
The concentration of hemoglobin in the solution tested far exceeds the amount that might be present in a 1:60 dilution of serum, even in the presence of gross hemolysis. At 650 mp, light absorption by serum containing as much as 14.0 mg. per cent of bilirubin is insignificant when diluted as in the test. Therefore, it seemed that this wave-length was the one best adapted for turbidimetric determinations under the conditions of the thymol turbidity reaction.

The effectiveness of a procedure of this type is dependent upon rigorous standardization. The gelatin standards of Kingsbury et al. (3) contain a suspension of formalazin and are standardized against solutions of albumin precipitated with 3 volumes of 3 per cent sulfosalicylic acid. These standards are not easily reproducible and may change somewhat on standing. Attempts were made to obtain turbidity standards by the precipitation of solutions of albumin with 3 volumes of 3 per cent sulfosalicylic acid. Crystallized egg albumin prepared by precipitation with sodium sulfate (4), crystalline bovine albumin, and human albumin in concentrations varying from 0.05 to 0.2 gm. per cent were used. The concentration of albumin was determined by estimation of the protein nitrogen by the micro-Kjeldahl technique. There was a straight line relationship between the optical density at 650 mp and varying concentrations of albumin. However, it was found that preparations of albumin from different sources, prepared by different methods, and probably characterized by varying degrees of denaturation failed to duplicate standard curves obtained with other albumin preparations. This is demonstrated in Fig. 3, in which the turbidimetric determinations of three different albumin preparations precipitated with sulfosalicylic acid are compared. For these reasons it became apparent that an albumin standard was wholly inadequate and another type of standard was sought. The barium sulfate standard of Wadsworth (5) which is used for measuring turbidity of bacterial suspensions was found to be a sufficiently stable and reproducible standard. It was adapted for use in this procedure, as described above.

The thymol turbidity reactions of sera from forty-six normal subjects were compared with those of sera obtained from 83 patients early in the course of acute infectious hepatitis. The results are compared in Table I. The highest turbidity value in the group of normal subjects was 4.7 units and the mean for this group was 2.66 units. There was a marked increase in thymol turbidity in the presence of liver damage produced by infectious hepatitis. The mean thymol turbidity reaction in the group of patients with infectious hepatitis was 17.03 units. Only one of the 83

1 Armour and Company.
2 Plasma Fractionation Laboratory at Harvard University.
patients had reactions of less than 5.0 units, which in the experience of this laboratory represents the high limit of normal. During convalescence from the disease there was a gradual decrease from initial high thymol turbidity to results that were in the normal range. Similar increased turbidity values

![Graph showing optical density vs. Albumin concentration](image)

**Fig. 3.** Comparison of turbidity of varying concentrations of bovine albumin, human albumin, and egg albumin precipitated with sulfosalicylic acid.

**TABLE I**

Results of Thymol Turbidity Reaction in Forty-Six Normal Subjects and in 83 Patients with Infectious Hepatitis

<table>
<thead>
<tr>
<th>Subject</th>
<th>Units of thymol turbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0-2.5</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>No. of subjects</td>
<td>20</td>
</tr>
<tr>
<td>% of subjects</td>
<td>43.5</td>
</tr>
<tr>
<td>With hepatitis</td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>0</td>
</tr>
<tr>
<td>% of patients</td>
<td>0</td>
</tr>
</tbody>
</table>

have been observed with sera from patients with other types of hepatic parenchymal damage, such as that resulting from toxic hepatitis and cirrhosis of the liver. With the modified technique the normal range in units of turbidity is nearly identical with that observed by Maclagan (2) and the degree of increase in turbidity occurring in the presence of liver damage is comparable with results obtained with the original technique.
A modification of the thymol turbidity reaction has been described which permits turbidimetric measurement in the spectrophotometer. The method has been simplified, and more exact standardization of the procedure has been achieved by the use of a barium sulfate standard. Determination of the thymol turbidity reaction of sera from forty-six normal subjects gave values with a range of from 0 to 4.7 units, with a mean of 2.66 units. In 83 patients with infectious hepatitis the thymol turbidity was increased in 82 and the mean for the group of patients was 17.03 units.

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