New Refractometric Methods for the Determination of Total Proteins in Serum and in Urine

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New, precise, physical methods for the determination of total protein in serum or plasma and in urine are described. They are based upon the refractometric determination of total solids in these fluids before and after protein has been removed by coagulation and, in ordinary use, they require no special standardization or calibration. The urine method is applicable primarily to protein concentrations above 100 mg./100 ml. A by-product of the urine method is the direct and simple determination of the nonprotein specific gravity of proteinuric urine.

Refractometric methods have been successfully applied to the precise determination of the total solids of serum and urine, of the specific gravity of these fluids, and of related variables (1–3). Total proteins of serum may also be estimated refractometrically (4–7) but with lower accuracy (ca. ± 0.15 to ± 0.4 gm./100 ml.), depending in part upon the degree of lipemia and the variability of the other nonprotein solids.

We report here new methods for the measurement of protein in serum and in urine based upon the refractometric determination of total solids in these fluids before and after protein has been removed by coagulation. In ordinary use, no special standardization or calibration is necessary. In addition, a new and simple method is presented to obtain the nonprotein specific gravity of urine containing protein.

Briefly, the methods consist of the following. An 0.2-ml. sample of serum is drawn into an acetic acid-treated syringe. The refractive in-
dex of the acid-treated specimen is measured in a TS meter* (Fig. 1) in terms of total solids (Reading A). This measurement requires one drop of sample. The acidified specimen is then immersed in a heated water bath for 5 min. to precipitate proteins. The solution is centri-

![Temperature-compensated hand refractometer (TS meter).](image)

**Fig. 1A.** Temperature-compensated hand refractometer (TS meter). B. Scales as seen on reticle of TS meter. Reading is taken at critical boundary between light and dark fields which appears when a drop of sample is placed in the instrument.

fuged and the refractive index (total solids) of the clear supernatant fluid is then determined (Reading B). The difference between TS readings A and B (ΔTS) indicates the protein concentration, as described below. The urine method involves the same principle except that pH and salt content of a 1-ml. sample are adjusted prior to heating, a small vial being used instead of a syringe.

**Determination in Serum or Plasma**

**Materials**

Materials needed are as follows. TS Meter; water bath and appropriate racks; microhematocrit centrifuge; plain microhematocrit capillary tubes (75 × 1.3–1.5 mm.) and/or (preferably) melting-point capillary tubes (75 × 1.5–2.0 mm.) Plasticene or other sealing compound for capillary tubes; wire stylus approximately 2 in. long, of a gauge which fits into and almost occludes capillary tubes, one end having an appropriate bend or button for the finger; interval timer; disposable tissue wipes. (All the foregoing materials are needed for determinations in urine, as well.)

*Temperature-compensated hand refractometer (Cat. No. 10400), American Optical Co., Buffalo, N. Y.
Also needed are disposable plastic syringes, 1-ml. tuberculin;* hypodermic needles, No. 19, 20, 21; polyethylene tubing (0.030 in. I.D., 0.048 in. O.D. and 0.023 in. I.D., 0.038 in. O.D.†; rubber stoppers—serum, sleeve type (O.D. of plug, 7 mm. with flare cut away); reagent, 10% acetic acid (alternatively, Reagent 1 listed below under Determinations in Urine may be used).

**Procedure**

Holding a syringe vertically with the plunger all the way in, touch the open tip to the surface of the acetic acid reagent, remove gently and turn tip up. After the small drop of acid (ca. 0.005 gm.) adhering to the tip is drawn in and trapped, press the plunger in fully.

Attach a needle to the acid-containing syringe and draw up about 0.20–0.25 ml. of serum or plasma for analysis. Hold syringe with needle up and admit air by pulling plunger to about the 0.6-ml. mark. Remove needle and cap tip of syringe with a rubber stopper. Gently tap syringe while turning it tip up then tip down a few times to mix the acid and the sample thoroughly. Remove stopper and eject air from above the liquid, catching any droplets in a disposable tissue.

Introduce one drop of fluid from syringe into TS Meter and measure total solids in gm./100 gm.; designate this A. Ordinarily the mixture of acid with serum or plasma will change the TS reading not more than 0.1 gm./100 gm. or introduce no change at all. If a more accurate measure of total solids of serum is desired independently of protein content, it is advisable to take a TS reading prior to addition of acid.

Recap tip of syringe with a dry rubber stopper. Immerse syringe, stopper down, in water bath at 96°-97°, with the upper level of the sample about an inch below the surface of the water for 5 min. Do not subject the upper portion of the plunger of the syringe to this high temperature as it will become deformed; do not use higher temperatures or the barrel of the syringe will also become too distorted (a small amount of bending does no harm); at lower temperatures there is a risk of incomplete coagulation of the protein in the time allowed.

Remove syringe containing the heavy coagulum, drying it around the stopper with tissue. Remove rubber cap and replace it with a glass capillary-plastic tube-needle unit prepared as follows:

The gauge of the needle is determined by the size of glass capillary

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*Disposable syringes with plastic plunger and glass barrel (0.5 ml.) may also be adapted to this method.
†No. 60 and 50, respectively, Clay-Adams, Inc., New York, N. Y.
to be used; melting point tubes generally require No. 60 polyethylene tubing on a Gauge 19, 20, or 21 needle, while microhematocrit tubes take No. 50 polyethylene on a Gauge 21 or 22 needle. Cut a length of polyethylene tubing at an angle to produce a beveled end. Insert the selected needle into this end just enough to encompass fully the bevel of the needle. Cut the tubing within 1/8 in. and parallel to the bevel of the needle, thus leaving it with a tubular plastic tip. The distal bevel of this tip may be readily inserted into the proper-size glass capillary. The fit, as the plastic-covered needle enters the glass lumen, should be snug, slight resistance being overcome by gently rotating during insertion; do not force. A loose fit calls for a different gauge needle, plastic tube, and/or capillary. Plastic-tipped needles can be prepared in quantity beforehand and, just prior to use, connected to capillary tubes and set aside. In general, the larger melting-point tubes are preferred to microhematocrit tubes.

Holding the needle firmly on the syringe, fill the glass capillary by pressing the plunger. The open tip of the capillary should be held near a disposable tissue to catch overflow. Remove glass capillary from plastic-tipped needle and close one end with about 1/8 in. of Plasticene or other sealing material. Centrifuge for approximately 2 min. (Some samples require less than 1 min., others 5 min. or more; melting point tubes require less time than microhematocrit tubes.) Remove capillary from centrifuge, insert wire stylus into the sealed end, and, holding the capillary between the thumb and third finger, press stylus with forefinger, ejecting clear supernatant fluid into the TS Meter. Take reading on serum solids scale; designate this B. The difference between TS readings before and after coagulation (A - B) equals ΔTS, which measures total protein (Fig. 2) according to the equation

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\text{Total protein of serum (gm./100 ml.)} = 0.95 \Delta TS
\]

The syringe, which may be slightly deformed during heating, is discarded. Needles can be cleaned and dried by suction and those with plastic tips may be used several times before it is necessary to renew the polyethylene tip.

Occasionally a sample rich in protein produces so heavy a coagulum that it cannot easily be forced through a needle. In such cases, instead of a needle, one may employ a rubber stopper such as is used to close the syringe during heating. Prepare it with a fine hole so that the contents of the syringe may be squeezed through it while one end of a glass capillary is pressed against the hole in the stopper.
Determination in Urine

In addition to those materials shown (under Determination in Serum or Plasma) as required for both serum and urine, the following are required: screw cap vials, 15 × 45 mm.; vial or tube rack; indicator solution (0.1 gm. of brom cresol green and 0.1 gm. of brom cresol purple mixed in a mortar with 33 ml. of 0.01 N NaOH, the mixture diluted to 250 ml. with water); Reagent 1 (20% aqueous solution of acetic acid saturated with sodium chloride); alternate Reagent 1a (30 to 40% aqueous solution of acetic acid saturated with salt); Reagent 2 (saturated aqueous solution of sodium chloride); dropping pipets for indicator solution and reagents, each chosen to deliver close to 0.025 gm. or milliliter per drop uniformly; other dropping pipets.

Procedure

Pipet 1 ml. of urine into a vial and add 1 drop of indicator solution. If the mixture is not greenish yellow (pH slightly less than 5) add 1–3 drops of Reagent 1 as necessary to develop and maintain this color, tapping or shaking the vial to mix the contents well. If less than 3 drops of Reagent 1 is required, add Reagent 2 to bring the total of the
two reagents to three drops (e.g., 1 drop of Reagent 1, two drops of 2; three drops of 1, none of 2; etc.). If an occasional urine proves so alkaline that it cannot be properly acidified without more than 3 drops of Reagent 1, it may be necessary to repeat the analysis using Reagent 1a. Thus a total of four drops or about 0.1 gm. or milliliter of fluid is added to 1.0 gm. or milliliter of urine, diluting the urinary protein, if any, by about 10 per cent.

Place a drop of this mixture in the TS Meter, determine the total solids reading on the serum scale and designate this $A$.

Cap vials tightly (hermetically) and place in rack in water bath above 95° for 3 min. Lower half of vial should be immersed. Depending upon the amount of protein in the original sample, a coagulum of varying density will form. Remove rack, allow the vials to cool. Just before use, shake vial with cap on to bring into solution any water condensed on the wall. Draw a sample of fluid into a microhematocrit tube, seal one end, and centrifuge for about 1 min.*

As with serum, obtain TS meter reading, $B$, on the supernatant fluid. The difference ($A - B$) gives $\Delta TS$ which, for practical purposes, may be considered numerically equal to the urinary protein content in gm./100 ml. (Fig. 3).

*Actually, most samples can be centrifuged directly in the vial at 2000 rpm for about 1 min., to obtain a supernatant fluid, obviating the need of transfer to microhematocrit tubes.
If urine is too rich in protein, its coagulum may have to be taken into a syringe and expressed into the microhematocrit tube, or the original urine can be diluted with water, e.g., 1:1, and the mixture rerun and corrected for the dilution.

Nonprotein Urinary Specific Gravity

The value of the TS Meter in this method is further enhanced because the nonprotein specific gravity of the urine can now be obtained quite simply.

Calling the TS meter reading of the original urine sample (on the serum scale) \(I\), it is easy to ascertain the value \(I - \Delta TS\), which corresponds to the reading of that urine, minus protein and added reagents. Locate the value of \(I - \Delta TS\) in the "Serum Total Solids" column of the conversion tables provided with the instrument. In the "Urine Specific Gravity" column of the tables find the corresponding value, which now represents nonprotein specific gravity (see examples, Table 1).

The classic correction, no longer needed, of 0.003 specific gravity units per gram per 100 ml. of dissolved protein is not applicable here unless the specific gravity of the proteinuric urine is obtained by hydrometer, since the specific gravity scale of the TS meter will not yield the true specific gravity of urines rich in protein. In the refractometric method proposed here, it is actually unnecessary to know the protein concentration of the urine or its true specific gravity when proteinuric, in order to obtain the nonprotein specific gravity.

*TS meter.

Table 1. Sample Calculations of Nonprotein Urinary Specific Gravity

<table>
<thead>
<tr>
<th>Apparent s.g. of proteinuric urine*</th>
<th>% protein in urine</th>
<th>(I)</th>
<th>(A)</th>
<th>(B)</th>
<th>(\Delta TS)</th>
<th>(I - \Delta TS) Nonprotein s.g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.007</td>
<td>0.0</td>
<td>1.7</td>
<td>3.0</td>
<td>3.0</td>
<td>0.0</td>
<td>1.7</td>
</tr>
<tr>
<td>1.009</td>
<td>0.3</td>
<td>2.0</td>
<td>3.0</td>
<td>3.0</td>
<td>0.3</td>
<td>1.7</td>
</tr>
<tr>
<td>1.014</td>
<td>1.3</td>
<td>3.0</td>
<td>4.2</td>
<td>2.9</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>1.023</td>
<td>1.3</td>
<td>4.8</td>
<td>5.8</td>
<td>4.5</td>
<td>1.3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Discussion

These new methods, physical rather than chemical, have a characteristic simplicity made possible by the new technology in refractometry. Although this serum method has few advantages over conventional ones, it is fairly rapid and, for practical purposes, requires no standardization or calibration since this is built into the TS meter. Actually,
it is far simpler and only slightly less accurate ordinarily to use only
the TS reading of untreated serum, and to determine protein from the
conversion table supplied with the instrument; or, to use the TS meter
model (Cat. No. 10401) whose reticle provides the scale for direct
reading of protein concentration. However, it is reasonable to suppose
that the difference method (ΔTS) will retain its basic accuracy despite
extreme variations of nonprotein solids, in which case the regression
between total solids and total protein loses value accordingly.

A small amount (about 0.1 gm./100 ml.) of serum glycoprotein fails
to be precipitated in the acid-heat treatment used here.

The urine method is applicable primarily to the range above 100
mg./100 ml. in which it lends itself to precise and accurate determina-
tion of protein concentration, as illustrated in Fig. 3.

Although the TS meter is a precision optical instrument readable to
± 0.1 gm./100 gm., a difference between two readings, can, of course,
be in error by ± 0.2 gm./100 gm., and such factors as time and tem-
perature of heating, pH, etc., if not properly attended, can introduce
further error.

It is planned to discuss theoretic relationships between ΔTS and
actual protein content of plasma elsewhere (8). Suffice it to mention
here that, although our results are presented so as to relate to protein
determinations made by conventional methods which employ the nitro-
gen-protein conversion factor of 6.25, an analysis of the refracto-
metric correlations with total solids of serum before and after depro-
teinization appears at this time to be more consistent with the con-
version factor 6.54 given by Sunderman et al. (9).

References
5. Sunderman, F. W., and Boerner, F., *Normal Values in Clinical Medicine*. Saunders, Phila-