A Volumetric Method for Serum Sodium

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Although the flame photometer is the method of choice for the determination of serum sodium in most large, well-equipped laboratories, there remains a place for an alternative procedure which can be used in laboratories too small to afford a flame photometer, or which can be carried out by an intern, resident, or general laboratory technician at night or at other times when the photometer operator is not available.

The need for such a method is reflected in the numerous papers which continue to appear describing new methods (or modifications of old ones) for assaying sodium in biologic materials. Most of these procedures are based on the precipitation of sodium as sodium uranyl zinc (or magnesium) acetate; the precipitate may be weighed (1), or the uranium in the precipitate or that remaining in the supernatant can be determined colorimetrically (2-5) or by titration (6). Other methods involve colorimetry of the sodium salt of violuric acid (7), titration of hydroxyl ions displaced from an ion exchange resin by a quantity of chloride equivalent to the sodium of the sample (8), and the very interesting determination of the volume of the erythrocytes after they have been equilibrated with several different concentrations of sodium chloride solution (9).

The present method is based upon the precipitation of sodium as the sodium acid salt of α-methoxyphenylacetic acid, as described by Reeve and Christoffel (10) and by Reeve (11). The precipitate has the stoichiometric composition C₆H₅CH(OCH₃)COOH·C₆H₅CH

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(OCH₃)₂COONa. It is sparingly soluble in organic solvents but dissolves fairly readily in hot water; therefore, it can be washed and re-dissolved and then titrated with alkali, each equivalent of sodium originally precipitated consuming 1 equivalent of base.

The procedure is carried out routinely on 2 ml of serum, but all quantities may be halved without increasing the error by more than 1%. Approximately 1 1/4 hours of working time are required to analyze a serum sample; as many as 6 determinations can be run simultaneously without taking much longer. Only routine apparatus (centrifuge, burette, and pipettes) is needed; it is convenient to have dry ice available.

None of the other ions normally present in serum interfere, nor would they even if present in pathologically large amounts. As Reeve has demonstrated (11), potassium may be present in quantities equivalent to 4 times the amount of sodium, and magnesium in quantities equivalent to that of the sodium, without causing an error of more than 1-2%; calcium interferes only when it is present in amounts large enough to precipitate the reagent, leaving less for the sodium (this does not occur at all below 20 mEq/L). So far as anions are concerned, the procedure was originally worked out with sodium chloride; sulfate equivalent to the sodium causes an error of only 6% (11), and phosphate can not cause any error at all. Physiologically conceivable concentrations of all the ions mentioned would, therefore, cause negligible errors.

METHOD

REAGENTS AND MATERIALS

1. Methoxyphenylacetic acid (MOPA) solution. 134 Gm. MOPA (0.8 mol.) are mixed with 300 ml. 1 N KOH. The acid may not dissolve completely, but the gradual addition of 620 ml absolute ethyl alcohol effects solution. 0.5 Gm. NaCl dissolved in 1.5 ml. of water is added to saturate the reagent and diminish losses due to solubility. The reagent is allowed to stand at room temperature (25°) until no further precipitation occurs; this requires 2-3 days. The precipitate is filtered off, and the resulting solution is stable for at least 6 months, probably indefinitely. (MOPA is available from the MOPA Chemical Co., P. O. Box 388, Rockland, Mass. The reagent solution, prepared as described above, may also be purchased.)

2. Approximately 0.05 N NaOH or KOH, standardized in any con-
venient way, e.g., against potassium acid phthalate, using phenolphthalein as indicator.

3. 0.5% phenolphthalein (in alcohol).

4. Acetone. Mallinckrodt's A.R acetone was used with no further purification; any reagent grade solvent would probably be satisfactory.

PROCEDURE

1. Two ml. of serum is treated with 2.5 ml. acetone in a centrifuge tube; the mixture is stirred and centrifuged at once to separate the protein which precipitates (about 2 minutes at 2000 rpm in a clinical centrifuge). Care must be observed not to lose enough acetone by evaporation to affect the concentration of the solution.

2. Three ml. of the supernatant solution is transferred to another centrifuge tube, treated with 4 ml. of MOPA reagent, and cooled to —20° in a cooling bath. The solution is stirred and scratched with a glass rod until a precipitate appears (about 2 minutes). The tube is held at —20° for 20 minutes more, with occasional stirring, to complete the precipitation.

3. The precipitate of sodium acid salt, which is quite voluminous, is centrifuged (2 minutes at 2000 rpm) and washed 3 times with 3-ml. portions of acetone at —20°. The last centrifugation may take a little longer.

4. The precipitate is dissolved in 5-10 ml. of hot, distilled water and washed into a small Erlenmeyer or beaker with 2 small portions of water.

5. A drop or two of phenolphthalein is added, and the solution is titrated to a pink color with 0.05 N alkali, using a 5- or 10-ml. burette. Each mEq of alkali consumed is equivalent to 1 mEq of sodium in the precipitate. Because of adsorption of sodium to the plasma proteins precipitated in step 1, an empirical correction of 7% is added to the sodium found to obtain the true quantity of sodium in the serum.

NOTES ON THE TECHNIC

Step 1. The serum may be obtained by any standard hospital procedure. It must not contain large amounts of added acid or alkali, however (such as would be present in unneutralized trichloroacetic acid filtrates, for example), because the acid salt of MOPA is soluble in acids and in alkali. Precipitation should occur between pH 3 and pH 7. Solutions which do not fall within this range may be adjusted with HCl or KOH. Because the reagent is itself a buffer (MOPA and
its potassium salt), some deviations from neutrality can be tolerated—in particular, any natural serum can be used.

Step 2. It is convenient to use a Dry Ice cooling bath containing ethanol-water (40:60). This sets to a slush at approximately −20° and maintains the proper temperature for a long time. Cooling in ice water is not satisfactory because precipitation is not complete in 20 minutes. If there is no hurry, the mixture may be kept overnight at 0-2°, and precipitation will then be complete.

Step 3. The precipitate is centrifuged at room temperature; once formed, it does not redissolve to too great an extent during centrifugation. It is of the utmost importance, of course, to wash the precipitate thoroughly to remove excess reagent, which would consume alkali.

Step 4. It is well to analyze a standard sample or a known NaCl solution simultaneously with the unknown until one acquires confidence in the technic. The 7% correction does not apply to pure NaCl solutions, because no sodium is lost by adsorption on protein.

RESULTS AND DISCUSSION

The method was first tested by repeated analyses of 2 serum samples whose sodium content had been determined with a flame photometer. The results of repeated determinations are given in Table 1 (the values obtained by the MOPA method always include the 7% correction, both here and hereafter).

Twenty-six unselected samples of sera from hospital patients previously analyzed by flame photometry were analyzed by the MOPA procedure. The results are shown in Table 2 (the samples are arranged in order of their sodium content, to make it easier to check for a systematic trend in the results obtained; the sample number gives the chronological order in which the analyses were performed).

These data cover a range of 103-175 mEq/L; except for 2 extremely high results (which may actually be errors in the flame photometer determinations; see footnote to Table 2) the errors are never greater than 6% and usually not more than 3%. The errors are dis-

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Table 2.

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* Determined on 2 different flame photometers in different laboratories.

** This value was first determined as 159; since this would have meant that the MOPA value was 16% high, it was rechecked and the photometer now gave 175.

tributed randomly, and no systematic trend is seen when the amount of sodium in the sample increases or decreases.

Finally, a pooled serum sample which had been analyzed by a large number of clinical laboratories in and around Philadelphia was analyzed by the present procedure. Seventeen flame photometers gave an average value of 142.4 (with a standard deviation of 2.3); duplicate determinations by the MOPA procedure were 142 and 144 mEq/L.

We have stated that an empirical correction of 7% must be added to the results to correct for sodium lost by adsorption on the protein precipitated from the serum. To demonstrate that adsorption really was the fate of the lost sodium, the protein precipitates from 2 determinations were washed thoroughly, and ashed with nitric and perchloric acids. The clear solutions thus obtained were assayed for sodium by the MOPA procedure; the results were equivalent to 14 mEq/L for both samples, with a correction of 3 mEq/L for the acid reagents, leaving 11 mEq/L of sodium adsorbed to the protein. Since both sera assayed approximately 130 mEq/L, the 11 mEq of adsorbed sodium corresponded to 8%. Because of the uncertainties involved in the assay of the precipitate and because a correction of 7% gave better general agreement with the flame photometer determinations, we prefer to use 7% as an empirical correction.
SUMMARY

A simple, rapid method for determining sodium in serum is described; it requires no special equipment and no special training. Ions normally present in serum do not interfere at any conceivable physiologic concentration. The method has been tested on approximately 30 samples of serum with known sodium contents ranging from 103-175 mEq/L; the deviation from the flame photometer value averages approximately 3%.

REFERENCES