THE ESTIMATION OF SILICA IN TISSUES.

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The estimation of silica in animal organs by the gravimetric method is attended with considerable difficulty. Large amounts of tissue are necessary, and ashing by the method of Stolte (1), or even with the aid of an electric muffle furnace is a tedious process requiring several hours for completion. Analysis of the ash for silica in the conventional way also takes considerable time, so that an estimation is a fairly lengthy process.

Isaacs (2) seemed to have supplied the need for a rapid and easily manipulated procedure in his colorimetric method for the estimation of silica in tissue. Discordant and inaccurate results, however, attended the use of Isaacs' method in this laboratory. The colors produced in both standard and test solutions were not proportional to the concentrations, and in many cases, even with the utmost care, duplicate estimations did not check. In the case of tissues high in calcium, there was invariably formed, when the test solutions were heated in the water bath, a heavy precipitate of calcium molybdate. This precipitate was a source of constant trouble, as the test solutions had to be filtered before they could be read, and in several instances it was thought that some of the blue color was being carried down by the calcium molybdate. At any rate the results obtained by this method did not agree with gravimetric analyses done as a check on the same tissues, and the discrepancy was largest where the precipitate in the solution had been heavy.

In the present paper a micro method is proposed which makes use of the intense yellow color formed when a silicate solution is treated with ammonium molybdate and sulfuric acid. The method is quick, and easily applied, and the proportionality of
color produced in test solutions prepared from sodium silicate and from ashed tissue holds over a wide range of concentration.

The production of yellow silicomolybdic acid formed the basis for Dienert and Wandenbulcke's (3) colorimetric method for estimating small amounts of silica in water, and was used by Atkins (4) and by Thresh (5) in their studies of the silica content of natural waters.

The yellow color is of the same tint as that of a dilute solution of picric acid, so that an artificial standard is possible. An artificial standard is preferable to a sodium silicate standard since the latter tends to deposit silica, and is difficult of standardization. The picric acid standard remains unchanged for at least 2 months, and can be made from any good brand of c.p. picric acid without recrystallization.

Phosphate, reckoned as $P_2O_5$, gives less than a tenth of the color given by an equal weight of $SiO_2$. In some cases where the phosphorus content is low a fairly reliable estimate of the silica present may be got without precipitation of the phosphate. But for its accurate determination, the removal of phosphate is necessary, and is accomplished by adding magnesia mixture to the solution of the ash, and filtering off the magnesium ammonium phosphate.

That the silica is not partially precipitated along with the phosphate, but is quantitatively estimated by the method, is shown by the following experiments.

**Experiment I.**

The silica of a mixed silicate phosphate solution is recovered in the filtrate after precipitation of the phosphate.

Solution 1.—10 cc. of sodium silicate solution containing 1 mg. of $SiO_2$, were diluted to 40 cc. with water, treated with 2 cc. of 10 per cent ammonium molybdic and 4 drops of 50 per cent (by volume) sulfuric acid, and made to 50 cc.

Solution 2.—1 mg. of $SiO_2$ and 2 mg. of P as $K_2HPO_4$ in 35 cc. of water were treated with magnesia mixture and ammonia.

1 Isaacs used silicate solutions made of highly diluted water glass, which he standardized against a solution containing a known weight of $SiO_2$, which had been fused with sodium carbonate and dissolved in water.
After being filtered and washed, the solution was neutralized, treated with ammonium molybdate and sulfuric acid, and made to volume.

Solution S.—1 mg. of SiO$_2$ and 5 mg. of P were treated in the same way.

These solutions had the same depth of yellow color.

**Experiment II.**

Silica added to tissue is quantitatively recovered as shown by difference. Several different tissues were analyzed according to the method before and after the addition of silica.

Lung 1 gave 11.40 mg. of SiO$_2$ per gm. of dried tissue, and 16.40 mg. after the addition of 5 mg. of SiO$_2$ per gm.; difference 5 mg.

Lung 2 gave 6.80 mg. of SiO$_2$ per gm. of dried tissue, and 11.76 mg. after the addition of 5 mg. of SiO$_2$ per gm.; difference 4.96 mg.

Liver 1 gave 0.80 mg. of SiO$_2$ per gm. of dried tissue, and 1.28 mg. after the addition of 0.5 mg. of SiO$_2$ per gm.; difference 0.48 mg.

Egg gave 0.2 mg. of SiO$_2$ per 10 gm. of white, and 0.69 mg. after the addition of 0.5 mg. of SiO$_2$; difference 0.49 mg.

**Experiment III.**

Values given by the colorimetric procedure check closely with those obtained by the gravimetric method. The results of several analyses are as follows:

*Comparison of SiO$_2$ Values Given by Colorimetric and Gravimetric Methods.*

Sample 1. 0.2 gm. of silicotic Lung 409 analyzed colorimetrically gave 63.0 mg. of SiO$_2$ per gm. of dried tissue.

5.0 gm. of the same ashed in an electric muffle furnace and analyzed gravimetrically gave 0.3180 gm. of SiO$_2$ or 63.6 mg. of SiO$_2$ per gm. of dried tissue.

Sample 2. 0.25 gm. of silicotic Lung 410 gave by the colorimetric method 60.0 mg. of SiO$_2$ per gm. of dried tissue.

5.0 gm. of the same gave by the gravimetric method 0.3035 gm. of SiO$_2$ or 60.7 mg. of SiO$_2$ per gm. of dried tissue.

Sample 3. 0.1 gm. of silicotic Lung 411 gave by the colorimetric method 29.6 mg. of SiO$_2$ per gm. of dried tissue.

8.5 gm. of the same gave by the gravimetric method 0.2468 gm. of SiO$_2$ or 29.0 mg. of SiO$_2$ per gm. of dried tissue.

Sample 4. 0.5 gm. of Liver 407 gave by the colorimetric method 1.33 mg. of SiO$_2$ per gm. of dried tissue.

50.0 gm. of the same gave by the gravimetric method 0.0560 gm. of SiO$_2$ or 1.32 mg. of SiO$_2$ per gm. of dried tissue.
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In Isaacs' method the presence of iron in the ash from those tissues containing considerable amounts of blood, such as liver and kidney, resulted in a greenish tint in the test solution. Isaacs diluted his standard with a solution of ferric alum till it matched the green color of the test, and made allowance for the dilution in his calculation. It has been found impossible in this laboratory to get even approximate matches in this way, and the dilution factor in the standard is an additional source of error. In the present method iron is precipitated along with the phosphate, so that the procedure is as applicable to those tissues containing blood, as it is in the case of lung tissue where the iron content is low.

Some results of analyses of animal organs are given in Table I. The values are of the same order as those given by Gonnermann (6) who used the gravimetric method.

### Table I.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Silica Content of Tissues.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SiO₂ per gm. dried tissue</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>0.78</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.48</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1.70</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.02</td>
</tr>
<tr>
<td>Dog</td>
<td>2.06</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.50</td>
</tr>
<tr>
<td>Human (normal)</td>
<td>1.40</td>
</tr>
<tr>
<td>&quot; (silicotic)</td>
<td>123.55</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>1.97</td>
</tr>
<tr>
<td>&quot;</td>
<td>1.70</td>
</tr>
<tr>
<td>Dog</td>
<td>1.12</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.80</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>1.90</td>
</tr>
<tr>
<td>&quot;</td>
<td>1.85</td>
</tr>
<tr>
<td>Dog</td>
<td>1.33</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.97</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>0.66</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.49</td>
</tr>
</tbody>
</table>
All reagents must be silica-free, and should be tested for its presence by adding ammonium molybdate and a few drops of sulfuric acid. Of the solutions used, only sodium hydroxide is likely to contain silica. It is therefore necessary to prepare this solution from metallic sodium, when a silica-free reagent will be obtained.

_Nitric acid_, concentrated.

_Ammonium nitrate_, 20 per cent solution.

_Boric acid_, saturated solution.

_Normal sodium hydroxide_, made by dissolving 2.3 gm. of metallic sodium in water in a nickel crucible, making to 100 cc., and keeping in a paraffined bottle.

_Sulfuric acid_, 50 per cent by volume.

_Magnesia mixture_, 5.5 gm. of magnesium chloride and 7 gm. of ammonium chloride dissolved in water and made to 100 cc.

_Ammonium Hydroxide 2.5 Normal._ Several samples of aqueous ammonia gave no test for silica. If a positive test is given, a silica-free solution should be prepared by distilling ammonia through a reflux condenser and bubbling the gas into distilled water contained in a paraffined bottle packed in ice. To prevent the water backing up the tube and into the condenser, a slow current of air is sucked, by means of a vacuum pump, through the entire apparatus. Specimens of the solution in the paraffined bottle may be titrated at intervals and the distillation stopped when the desired strength is reached.

_Ammonium molybdate_, 10 per cent solution.

_Standard Picric Acid._—A stock solution is made by dissolving 102.4 mg. of pure vacuum-dried picric acid in water and making to 100 cc. By diluting 1 part of the stock solution to 100 with water a solution is obtained, 50 cc. of which have the same quality and intensity of color as that developed in a solution containing 1 mg. of SiO₂ when treated with 2 cc. of ammonium molybdate and 4 drops of sulfuric acid and made to 50 cc.

_Method._

The tissue to be analyzed is dried to constant weight at 105° and finely pulverized in an iron mortar. Ashing is carried out in
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the wet way in a platinum crucible of 30 or 40 cc. capacity, though a smaller one may be used. An amount of the tissue (0.2 to 1.0 gm. is a convenient quantity) is treated with 3 cc. of nitric acid, 2 cc. of ammonium nitrate, and 1 cc. of boric acid. The boric acid prevents any loss of silica as fluoride. The mixture is heated on the water bath till frothing ceases and a clear solution is obtained and then to dryness on a hot plate or with a Bunsen flame. Care must be taken during the latter stage, or loss by spattering will occur. The crucible is then heated to redness over a Meker or Bunsen burner. The carbonaceous residue quickly oxidizes, leaving a white ash. Sometimes a second or even a third treatment with nitric acid and ammonium nitrate is necessary; this depends on the amount taken and on the type of tissue. Lung is easily ashed; liver and kidney usually require an additional treatment.

Solution of the silica in the ash is effected by adding 3 cc. of the N NaOH and several cc. of water and heating on the water bath. If the ash sticks to the bottom of the crucible and does not readily dissolve, the process is aided by rubbing it free with a rubber policeman. The crucible is now removed from the water bath, a drop of phenolphthalein added, and then dilute sulfuric acid till the pink color is almost discharged, leaving the solution faintly alkaline. Water is added to a volume of 25 or 35 cc., depending on the size of the crucible being used, and 1 cc. of magnesia mixture added for each 3 mg. of P present. (1 or 2 cc. are usually sufficient except in the case of tissue high in P such as brain.) 2 cc. of ammonia are added, a few minutes allowed for precipitation of the phosphate, and the solution then filtered through a small paper into a Pyrex test-tube graduated at 50 cc. The residue is thoroughly washed with distilled water, the washings being added to the filtrate. After the solution is neutralized, 2 cc. of ammonium molybdate and 4 drops of sulfuric acid are added, and water to 50 cc. The yellow color, which quickly develops, reaches a maximum in 5 minutes and remains constant for several hours.

Test solutions are conveniently matched in Hehner tubes against the picric acid standard, 50 cc. of which have a color equivalent to 1 mg. of SiO₂ in the test. Improvised tubes can be made by sealing a small glass stop-cock in the side near the bottom of one of two 50 cc. graduated cylinders which appear to be identi-
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cal as regards kind of glass, bore, height of graduation, etc. The standard solution is run out of the tube containing the stop-cock till it matches the test in the other tube. With a little practice, as accurate readings can be obtained with these tubes, when mounted on glass over white cardboard, as are got by the use of an expensive colorimeter. If Nessler tubes are used it is convenient to make several standards of different strength, by appropriate dilution of the stock solution of picric acid.

When the silica content of the tissue is abnormally high, as in silicotic lungs, it is advisable, after the sodium hydroxide is neutralized, to transfer the solution to a graduated cylinder, and use an aliquot portion for the estimation. While the 2 cc. of 10 per cent ammonium molybdate are sufficient for 3.5 mg. of SiO₂, it is desirable that the aliquot portion should contain about 1 mg. since the color of the test should fairly closely approximate that of the standard.

SUMMARY.

A micro method for the estimation of silica in animal tissues, which depends on the production of yellow silicomolybdic acid, has been described. Evidence is produced to show that the method gives accurate results.

The silica content of some tissues has been estimated; values are listed in tabular form.

BIBLIOGRAPHY.

1. Stolte, K., Biochem. Z., 1911, xxxv, 104.

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2 Dienert and Wandenbulcke found that 2 cc. of 10 per cent solution of ammonium molybdate were enough to react with not more than 3.5 mg. of SiO₂. This observation has been confirmed by the author.
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