A DIRECT TURBIDIMETRIC METHOD FOR DETERMINING ETHEREAL SULFATES IN URINE*

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During the course of an investigation into the excretion of "detoxication products" in the chicken (1) the need arose for a reasonably simple and reliable method for the estimation of "ethereal sulfate" in quantities of 0.1 to 1 mg. of SO₄ in volumes of some ml. of chicken urine. As none of the methods tried seemed satisfactory in the form described, a modification was developed.

It appears that in all methods hitherto described ethereal sulfate is determined as the difference between the free and total sulfate. If the procedure used for the sulfate determination is not very accurate (which is undoubtedly the case with all existing methods when the amount determined is less than 1 mg.), the error inherent in the value for ethereal sulfate may well be considerable. This is also evident from an inspection of the values given in the literature, for instance by Morgulis and Hemphill (2) and Treon and Crutchfield (3). A direct method seems to be preferable.

It has been found that free sulfate (together with interfering substances) may be precipitated with barium chloride, and the resulting excess barium by the use of sodium carbonate, without apparent loss of ethereal sulfate.

For the determination of sulfate after hydrolysis the turbidimetric method is undoubtedly the most rapid (3), and its accuracy seems quite satisfactory and at any rate not inferior to the benzidine or chromate method. Treon and Crutchfield (3) pointed out that the barium chloride should be added as a solid, not in solution. The stability and reproducibility of the cloud are then very satisfactory. These authors followed the customary practice of using a relatively large quantity of hydrochloric acid for the hydrolysis and subsequently nearly neutralized the acidity of the solution. It is, however, possible to hydrolyze the ethereal sulfates at a relatively low acidity and develop the cloud without neutralization.

Reagents—

Acid barium chloride solution. Dissolve 1 gm. of barium chloride + 2H₂O in water, add 2 ml. of concentrated hydrochloric acid (sp. gr. 1.19), and dilute to 100 ml.

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Sodium carbonate solution. 5 per cent (weight by volume).
Dilute hydrochloric acid. 10 ml. of concentrated hydrochloric acid diluted to 100 ml.
Barium chloride, crystalline, of uniform size, preferably 30 to 50 mesh.

Method

5 ml. of chicken urine (undiluted when fluid, or suitably diluted) are mixed with 1 ml. of acid barium chloride solution in a centrifuge tube and centrifuged at 3000 R.P.M. for about 10 minutes. The centrifugate is drained into a clean centrifuge tube and mixed with 1 ml. of sodium carbonate solution, allowed to stand some minutes, and centrifuged 10 minutes or more. 3 ml. of this centrifugate are pipetted into a test-tube, and after addition of 1 ml. of dilute hydrochloric acid the test-tube is covered with a glass ball and heated in a boiling water bath for 30 minutes. After cooling, 0.1 ± 0.005 gm. of barium chloride is added. The test-tube is gently shaken until the substance has dissolved and the solution is transferred to a cuvette. The extinction has been measured with the aid of a Weca photoelectric colorimeter (according to Havemann), the 2 cm. micro cuvette being used.

Discussion

The removal of interfering substances is insured by the precipitation of free sulfate at a considerable acidity. Otherwise uric acid may interfere and cause considerable turbidity in the final stage (this seems to be due to the low solubility of the acid barium urate). This fact makes the difference method especially unsuited for chicken urine.

It is best to take chicken urine representing no more than 15 minutes excretion. Otherwise some slight turbidity may remain after the addition of sodium carbonate and following centrifugation.

The rate of hydrolysis has been tested for resorcinol sulfuric ester and phenol sulfuric ester (potassium salts were used). The results for the latter substances are as follows:

<table>
<thead>
<tr>
<th>Hydrolysis time, min.</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol sulfuric ester hydrolyzed, mg.</td>
<td>0.081</td>
<td>0.195</td>
<td>0.271</td>
<td>0.300</td>
<td>0.299</td>
<td>0.302</td>
<td>0.300</td>
<td>0.296</td>
</tr>
</tbody>
</table>

Similarly resorcinol sulfuric ester is completely hydrolyzed after 15 minutes. It is obvious that the concentration of HCl (about 0.25 N) is quite sufficient for hydrolysis of this type of ethereal sulfate in less than 30 minutes. It is also clear that care must be taken to avoid prolonged standing or heating of acid solutions containing ethereal sulfates, if hydrolysis is to be avoided. This is probably the reason why treatment with perhydrol...
in boiling acid solution for the removal of reducing substances, as recom-
mended by Morgulis and Hemphill (2), may result in heavy losses of
ethereal sulfate.

The density and stability of the cloud at different acidities are illustrated
by the figures in Table I. The density is not significantly influenced by
considerable change in acidity. The stability seems to be slightly better
at lower acidity. The difference does not seem to warrant the introduc-
tion of the additional operation of neutralization. The cloud appears very
rapidly, except at the lowest concentrations. When the cloud is just visible,
it is necessary to let the solution stand a few minutes before measuring the
extinction; otherwise a considerable error may be introduced.

The relation between the extinction and the concentration is a straight
line which meets the y axis slightly above the origin.

**Table I**

*Density and Stability at Different Acidities*

The samples contained 1 ml. of dilute HCl, 2 ml. of sulfate solution, and 1 ml. of
sodium acetate solution.

<table>
<thead>
<tr>
<th>Calorimeter readings at min.</th>
<th>Acetate concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>160</td>
</tr>
<tr>
<td>20</td>
<td>144</td>
</tr>
<tr>
<td>60</td>
<td>133</td>
</tr>
</tbody>
</table>

The standard deviation of a single determination is about 0.007 mg. of
SO₄ (calculated from eighteen pairs of duplicate determinations), and the
scatter seems to be fairly independent of the concentration. Thus the
relative accuracy increases with the concentration.

The recovery of phenol or resorcinol sulfuric esters added to chicken
urine is essentially complete (average recovery 97 per cent).

No light filters have been used in the present study for practical reasons
connected with the particular apparatus used. The extinction is, however,
higher when blue light is used.

The method seems to be applicable to other urines as well as to chicken
urine. It is then, of course, necessary to measure the extinction of the
urine before the final addition of barium chloride, and subtract the value
from the final extinction. Satisfactory results have been obtained with
rabbit urine.

**SUMMARY**

A turbidimetric method for the estimation of ethereal sulfate in urine is
described. Free sulfate and interfering substances are removed by barium
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chloride and the excess barium by sodium carbonate. Hydrolysis is then
effected in 30 minutes in approximately 0.25 N hydrochloric acid at 100º.
The resulting sulfate is then determined turbidimetrically after precipi-
tation as barium sulfate in the unneutralized solution. It is possible to
determine 0.1 to 1.0 mg. of SO₃ in 5 ml. samples. The standard deviation of
a single determination is about 0.007 mg. of SO₄.

BIBLIOGRAPHY

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A DIRECT TURBIDIMETRIC METHOD FOR DETERMINING ETHEREAL SULFATES IN URINE
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