The determination of catechol, phenol, and hydroquinone in urine

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(Received for publication, September 22, 1945)

There have been many methods devised for the determination of urinary phenols based on colorimetric, gravimetric, and titrimetric procedures, but few of them include quantitative determinations of hydroquinone and catechol. These two phenols are produced in considerable amounts in animals exposed to benzene (1) and since we wished to study this problem further a convenient method of analysis had to be devised.

The present paper describes a relatively simple method for the determination of catechol, phenol, and hydroquinone in urine. All titrations are made with a single solution and only one primary standard, potassium bromate, is required.

Reagents—

2 M pyridine-acetate buffer. 160 ml. of pyridine and 10 ml. of glacial acetic acid diluted to 1 liter; pH 6.5.

Lead acetate solution, 37.9 gm. of Pb(CH$_3$COO)$_2$·3H$_2$O per liter. Add 1 ml. of glacial acetic acid.

Potassium iodate solution, saturated.

Sodium hydroxide solution, 30 per cent.

0.2 N bromate-bromide solution. 5.568 gm. of KBrO$_3$ and 20 gm. of KBr per liter.

0.2 N sodium sulfite solution. 12.6 gm. of anhydrous Na$_2$SO$_3$ per liter protected from air by CO$_2$ and standardized frequently against the bromate solution.

Ethyl alcohol, 95 per cent.

Ether, washed with sodium hydroxide-potassium permanganate solution until alcohol and aldehyde are absent, and then distilled.

Sodium bicarbonate, 10 grain tablets and powder.

Hydrolysis of Phenolic Esters—Hydrolysis of the esters is carried out in the extraction tube in the inverted position, A, Fig. 1.

Measure 25 ml. of centrifuged urine$^1$ into an extraction tube and add 8 drops of concentrated sulfuric acid. Insert a rubber stopper and tip the tube in a horizontal position with the side arm up until the CO$_2$ has

$^1$ With acid urines containing no precipitated carbonates no centrifuging is required.
The pH is now about 1.0, which is adequate for hydrolysis of the esters in 2 hours at 100°.

The adapter is connected to the side arm and to the condenser with the extraction tube immersed in boiling water.

**Fig. 1.** Apparatus for the hydrolysis and extraction of urine and concentration of the extract. The letters represent the respective positions.

*Extraction of Phenols at pH 7.0*—After hydrolysis of the esters at pH 1.0, the extraction tube is removed from the bath and the urine is saturated with sodium sulfite. This neutralizes sulfuric and phenolic acids and brings the pH up to 7. The mono- and diphenols may, however, be quan-
titatively extracted and the extract does not need further washing. Sulfite has the advantage over bicarbonate in furnishing SO₂ for reduction of any quinones which may have formed.

The urine is cooled and the apparatus is assembled for extraction as in B, Fig. 1. The glass wool plug on the funnel tube aids in the separation of ether from the urine. A few small pieces of pumice (40 mesh) are added to the ether boiler to prevent bumping. The water bath is kept at 55° and extraction is continued for 4 hours.

The rate of extraction may be considerably increased by wrapping a strip of lead (not shown) in a spiral around the lower third of the funnel tube. This facilitates saturation of each drop of ether which passes through the urine. By this means extraction is complete in about 3 hours, provided the rate of ether vaporization is maximal. This can be assured by keeping the boiling tubes filled about two-thirds full of ether. It has been shown that the rate of ether vaporization in the long narrow tubes with pumice present is dependent on the height of the ether column. This follows from the fact that the whole column is full of bubbles and therefore the effective surface for evaporation of ether is greatly in excess of the cross-sectional area of the tube.

Concentration of Extract and Collection of Ether—When extraction is completed, the extract is concentrated to dryness by inserting the collection flask as shown in position C, Fig. 1, first with the funnel tube in place and second with the funnel tube removed. Water is added to the urine to displace nearly all the ether from the extraction tube into the ether boiler. Concentration of the extract is then completed.

The ether may be used over again without further treatment.

Analysis of Extract

Separation of Catechol—After extraction and concentration of the extract, catechol is separated as the lead salt at pH 6.5.

A battery of small sintered glass funnels, of medium porosity, mounted for suction filtration is used for the separations to follow.

About 2.0 ml. of 0.1 M lead acetate solution are placed in one of the funnels. 3 ml. of 2 M pyridine-acetate buffer, pH 6.5, are added to the concentrated extract in the ether boiler and this is rinsed into the funnel with a few ml. of water. The mixture is stirred and catechol is immediately and quantitatively precipitated as a lead salt. After a minute it settles out, and is filtered with suction. The filtrate containing phenol and hydroquinone is received in a 100 ml. wide mouth, screw cap bottle.

2 Pyrex, catalogue No. 33730.
3 A. H. Thomas 4 ounce bottle, No. 6284; plastic caps, No. 2849. Remove the cork liner and pour in a layer of paraffin to protect the cap from bromine fumes.
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precipitate is washed free of soluble lead salt with a minimum of water (about 12 ml.) which is added to the filtrate and treated as described in the following section.

The precipitate of lead catecholate is dissolved with 2 ml. of glacial acetic acid and washed into a 150 ml. beaker with water. An excess of saturated potassium iodate solution is added and the mixture heated on a hot-plate until lead iodate settles out. (Catechol is oxidized by iodate to a soluble red substance the color of which gives a useful index of the amount of catechol present.)

Lead iodate is filtered cold and washed, the filtrate discarded, and the original beaker in which precipitation occurred is returned as the receiver. Lead iodate is dissolved with 2 ml. of 30 per cent sodium hydroxide and washed into the beaker. 1.0 ml. of concentrated sulfuric acid and 0.5 gm. of potassium iodide are added, and the liberated iodine titrated with 0.2 N sodium sulfite solution, a platinum-platinum polarized electrode being used as indicator.

While it is true that thiosulfate and starch indicator may be used in this titration, sulfite must be used in the phenol determination and the electrode must be used in the hydroquinone determination. All three titrations may be made with sulfite and the electrode indicator.

Sulfite must be protected from oxidation by the air, preferably by storing it under an inert gas such as carbon dioxide. When properly protected the solution is stable indefinitely. We have found the following arrangement perfectly satisfactory: A 5.0 ml. micro burette graduated in hundredths of a ml. is fitted with a 3-way stop-cock and is filled by siphon from a 4 liter stock bottle. The latter is connected to a Kipp generator for CO₂ and the top of the burette is connected to the CO₂ space by means of a glass tube. Short rubber connections must be used throughout.

Calculation—

\[
\text{Mg. catechol} = \frac{(S - B) \times N \times 110.1}{12}
\]

\(S\) = ml. of sulfite used for the sample, \(B\) = ml. of sulfite used for the blank, \(N\) = the normality of the sulfite.

The blank for benzene-exposed animals is a sample of urine taken before exposure and treated exactly as the sample after exposure is treated.

For other purposes 25 ml. of water may be substituted for urine. These blanks are usually very small and for some purposes entirely negligible.

Determination of Phenol—The filtrate from lead catecholate is acidified with 1.0 ml. of concentrated sulfuric acid and 5.0 ml. of 0.2 N bromate-bromide mixture are added. This is sufficient to brominate about 8.0 mg.

\(\text{Before the funnels are used again they must be washed with acid and water.}\)
of phenol under these conditions. A small vial containing about 1.0 gm. of potassium iodide is placed in the bottle and the cap is screwed on.  

After about 30 minutes, when bromination is complete, the vial is tipped over and the excess bromine liberates an equivalent quantity of iodine from the iodide. The bottle is opened and the vial removed and washed carefully with alcohol, the washings being added to the mixture in the bottle. Alcohol is used in order to bring any precipitated iodine into solution and to prevent the precipitation of oxidized hydroquinone in the next step of the analysis.

The iodine is titrated with 0.2 N sulfite solution, the electrode being used as indicator.

Calculation—

\[
\text{Mg. phenol} = \frac{(B - S) \times N \times 94.11}{6}
\]

\(B, S,\) and \(N\) have the same significance as the corresponding symbols in the catechol determination.

It will be observed that this procedure is an adaptation of the Koppeschaar method (2) for phenol. The change from thiosulfate to sulfite was necessary because hydroquinone is to be determined in the same mixture.

It was shown that tetrathionate resulting from the oxidation of thiosulfate was further oxidized by iodine after the acid was neutralized by bicarbonate. Sulfite, on the other hand, is oxidized to sulfate, which is stable in bicarbonate buffer.

Determination of Hydroquinone—In our early experiments we used acetate buffer to control pH in the catechol precipitation. The buffer

\[
\begin{align*}
&\text{I}^- \\
&\text{O} \\
&\text{N} \\
&\text{O} \\
&\text{N} \\
&\text{I}^-
\end{align*}
\]

1,1',1''-(2,5-Dihydro-3-hydroxy-2,5-dioxo-\(p\)-phenylene)-tripyridinium betaine diiodide

A convenient indication that an excess of bromine is present is the color of the potassium iodide in the vial. If it is brown on top, sufficient bromate was added. If after about 10 minutes the potassium iodide is not brown, the bottle may be opened and another 5.0 ml. of bromate solution added. When only 0.5 ml. of sulfuric acid was used, the bromine uptake was too high and the subsequent hydroquinone recovery was low.
appeared with phenol and hydroquinone in the filtrate. Under these conditions hydroquinone consumed 2 equivalents of iodine per mole and quinone was formed.

When pyridine was substituted for acetate, hydroquinone consumed 10 equivalents of iodine per mole and a new compound was formed having the probable structure (3) shown in the accompanying diagram.

The procedure is as follows: The mixture from the phenol determination still contains unchanged hydroquinone. Add 5.0 ml. of potassium bromate-bromide solution which liberates an excess of iodine, since KI is also present at this point. The bottle is covered with a watch-glass and the sulfuric acid is neutralized by adding three sodium bicarbonate tablets, one at a time. It was found impractical to use the powder at first because of the spraying resulting from the rapid release of carbon dioxide. After the tablets have dissolved, an excess of powdered sodium bicarbonate is added.

<table>
<thead>
<tr>
<th>Amount of each in mixture</th>
<th>Found without extraction</th>
<th>Found with extraction from urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Catechol</td>
<td>Phenol</td>
</tr>
<tr>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>2.2</td>
<td>2.15</td>
<td>2.19</td>
</tr>
<tr>
<td>4.4</td>
<td>4.44</td>
<td>4.36</td>
</tr>
<tr>
<td>6.6</td>
<td>6.62</td>
<td>6.30</td>
</tr>
<tr>
<td>8.8</td>
<td>8.96</td>
<td>8.48</td>
</tr>
</tbody>
</table>

The watch-glass is rinsed with alcohol and the mixture allowed to stand for 1 hour, after which it is titrated with sulfite.

During the oxidation of hydroquinone a red precipitate sometimes appears. This must be brought back into solution by the addition of a few ml. of alcohol. If it is allowed to remain separated, the oxidation will be incomplete and the end-point in the titration of excess iodine will be uncertain, since the precipitate carries down free iodine.

**Calculation**

\[ \text{Mg. hydroquinone} = \frac{(B - S) \times N \times 110.1}{10} \]

\( B, S, \) and \( N \) have same significance as above.

A few typical analyses are given in Table I. The first recoveries were made by analysis of a mixture of standard solutions of the three phenols without extraction; the second recoveries were made by adding the three standard solutions to 25 ml. of normal human urine and analyzing the mixture as described. A urine blank was also analyzed and the values found were subtracted from the totals to give those in Table I.
Fig. 2 is a typical set of curves showing the excretion of phenol, hydroquinone, and catechol by a rabbit during a series of injections of benzene in olive oil.

It will be observed that all curves are roughly parallel, which suggests a dependence of the secondary products of oxidation (hydroquinone and catechol) on the concentration of the primary product (phenol).

The peak occurred at the 9th day and, in spite of continued injections, the amount of urinary phenols declined sharply and the rabbit died on the 17th day.

The count of white blood cells declined from 11,000 on the 2nd day to less than 1000 on the 17th.

Further studies are in progress.

DISCUSSION

The specificity of these reactions is obviously not absolute. It is especially important that no trace of urine be allowed to pass into the ether boiler and with certain urines this has been a difficult problem. Contamination with urine is easily discovered during the precipitation of catechol, for a heavy rather insoluble precipitate of lead sulfate, phosphate,
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and phenolic acids is produced. These determinations must be discarded. "Phenol" determinations on such urines are also high. Acetone and other ketones will react like hydroquinone.

Our "phenol" fraction contains p-cresol and other substances such as alcohols, which can reduce bromine.

In spite of these interferences our results indicate that for urine of rabbits exposed to benzene, at least, the interpretations are usually uncomplicated and suitable controls can be devised.

SUMMARY

1. A system of analysis of urine for catechol, phenol, and hydroquinone has been described.
2. The extraction of phenols from urine has been improved by the use of sodium sulfite buffer which keeps diphenols reduced and holds back ether-soluble acids.
3. The precipitation of lead catecholate from the ether extract is controlled at pH 6.5 by the use of pyridine-acetate buffer and the separation of the lead as iodate instead of the usual chromate yields more iodine per atom of lead.
4. It has been shown that phenol may be brominated in the presence of hydroquinone, provided sufficient acid is present.
5. A new oxidation of hydroquinone is utilized in which 10 atoms of iodine are reduced per mole, resulting in the formation of a tripyridinium quinone betaine.
6. The methods are simple and fairly specific when the separations are successful. They have been applied to the analysis of rabbit urine during exposure to benzene and a typical set of curves is given.

BIBLIOGRAPHY

THE DETERMINATION OF CATECHOL, PHENOL, AND HYDROQUINONE IN URINE
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