A COLORIMETRIC METHOD FOR THE DETERMINATION
OF PHENOLS (AND PHENOL DERIVATIVES)
IN URINE.

BY OTTO FOLIN AND W. DENIS.

(From the Biochemical Laboratories of the Harvard Medical School and
of the Massachusetts General Hospital, Boston.)

(Received for publication, July 26, 1915.)

Since the appearance in 1882 of the pioneer researches of Bau-
mann on the urinary phenols, a large number of investigations
has been made on this subject. The method used for the quanti-
tative determinations of these bodies has been almost invariably
the iodometric titration variously modified and improved by
Kossler and Penny,¹ by Neuberg,² Mooser,³ and by Hensel.⁴ At
best the iodometric method has many disadvantages, being ex-
tremely time consuming and requiring the use of a comparatively
large amount (500 cc.) of urine. Further, as will be shown be-
low, if the directions given by the various investigators who have
worked on the technique of this method be followed a considera-
ble portion of the phenol contained in the urine may be lost.

The first step in the determination of phenols according to the
methods of Kossler and Penny, Neuberg, Mooser, etc., consists
in evaporating the faintly alkaline urine on a water bath to about
one-fifth of its original volume. We have found that during this
evaporation a considerable proportion of the phenol is oxidized,
thus producing results much below the truth.

Experiments Showing the Loss of Phenol When Heated in Alkaline Solutions.

1. 25 cc. of phenol solution required 16.80 cc. 37 iodine when titrated
according to the iodometric method.

25 cc. of the above phenol solution were treated with 0.5 cc. of 10 per
cent sodium carbonate and 100 cc. of water, after evaporation for one

² Neuberg, C., ibid., 1899, xxvii, 123.
³ Mooser, W., ibid., 1909, lxiii, 176.
⁴ Hensel, M., ibid., 1912, lxxviii, 373.
hour on the steam bath during which time it was reduced to a volume of 50 cc. It was found on titration to require only 16.00 cc. \(\frac{N}{10}\) iodine.

2. 25 cc. of the same phenol solution when treated like the above except that one drop of 10 per cent sodium hydrate was used instead of sodium carbonate, required when titrated only 6.00 cc. \(\frac{N}{10}\) iodine.

3. 25 cc. of a solution of para cresol required on titration 14.00 cc. of \(\frac{N}{10}\) iodine.

25 cc. of the same solution when evaporated on the steam bath for one hour after the addition of one drop of 10 per cent NaOH and 100 cc. of water required 4.00 cc. of \(\frac{N}{10}\) iodine.

Besides the uncertainty attending the preliminary evaporation in alkaline solution, the subsequent removal of the phenols from the urine by distillation with mineral acid, and the redistillation necessary to separate the phenols from acetone, formic acid, and other substances capable of reacting with iodine, make determination of phenols in urine a formidable task. By the use of the phosphotungstic phosphomolybdic reagent described by us\(^5\) some three years ago we are now able to make a colorimetric phenol determination in urine in ten to fifteen minutes and with the use of not more than 10 or 15 cc. of ordinary urine.

Our method is as follows: 10 cc. of ordinary, or 20 of very dilute, urine are placed in a 50 cc. volumetric flask. To this is added an acid silver lactate solution\(^6\) (from 2 to 20 cc.) until no more precipitate is obtained. A few drops of colloidal iron are then added, the flask is shaken, filled to the mark with distilled water, shaken again, and its contents are filtered. By means of this precipitation uric acid and traces of proteins, both of which also give a blue color with our reagent, are quantitatively removed. We have convinced ourselves by numerous experiments on a variety of phenols that these substances are not precipitated by the above procedure and may be quantitatively recovered in the filtrate. 25 cc. of the filtrate are transferred to a 50 cc. volumetric flask, and to it is added a sufficient quantity of saturated sodium chloride solution containing 10 cc. of strong hydrochloric acid per liter to precipitate all the silver. The flask is then filled to the mark and the contents are filtered.


\(^6\) This solution consists of a 3 per cent silver lactate solution in 3 per cent lactic acid.
To determine "free" (non-conjugated) phenols 20 cc. of this filtrate are placed in a 50 cc. flask and treated with 5 cc. of the phosphotungstic phosphomolybdic acid reagent\(^7\) and 15 cc. of saturated sodium carbonate solution. After diluting to volume with lukewarm water (30-35°C.) and allowing to stand for twenty minutes the deep blue solution is read in a Duboscq colorimeter against a standard solution of phenol.

To determine total (free and conjugated) phenols 20 cc. of the same filtrate are transferred to a large test-tube; to this are added ten drops of concentrated hydrochloric acid, and the test-tube is covered with a small funnel. This mixture is heated rapidly to boiling over a free flame and is then placed in a boiling water bath (we usually use for this a tall beaker) for ten minutes. At the end of this time the tube is removed, cooled, and the contents are transferred to a 100 cc. volumetric flask. 10 cc. of the phosphotungstic phosphomolybdic reagent and 25 cc. of saturated sodium carbonate solution are now added, and after making up to volume and shaking, the solution is read (after twenty minutes) against a standard solution of phenol. As a standard we use a solution of pure phenol in \(\frac{7}{10}\) HCl containing 1 mg. of the former substance in 10 cc. 5 cc. of this solution (equivalent to 0.5 mg. of phenol) when 10 cc. of saturated sodium carbonate solution are added and the whole is made up with water at about 30°C. to a volume of 100 cc., give, when set in the colorimeter at 20 mm., a convenient standard. As phenol is an exceedingly hygroscopic substance it is necessary to standardize the solution by means of the iodometric titration.

This titration is carried out as follows: Make a phenol solution in \(\frac{7}{10}\) HCl containing 1 mg. of crystallized phenol per cc. Transfer 25 cc. of the phenol solution to a 250 cc. flask, add 50 cc. \(\frac{7}{10}\) sodic hydrate, heat to 65°C., add 25 cc. \(\frac{7}{15}\) iodine solution, stopper the flask, and let stand at room temperature thirty to forty minutes. Add 5 cc. of concentrated hydrochloric acid.

---

\(^7\) This reagent is prepared by boiling together for two hours 100 gm. of sodium tungstate, 20 gm. of phosphomolybdic acid (or an equivalent amount of molybdic acid), 50 cc. of 85 per cent phosphoric acid, and 75 cc. of water. After the period of heating, cool, make up with water to a volume of 1 liter, and filter if necessary.
Phenols

acid and titrate excess of iodine with \( \frac{8}{15} \) sodium thiosulphate solution. \( ^8 \) 1 cc. of \( \frac{8}{15} \) iodine solution corresponds to 1.567 mg. of phenol. On the basis of the results dilute the phenol solution so that 10 cc. contain 1 mg. of phenol.

The above method as described is not directly applicable to the determination of phenols in blood, tissues, or feces. A modification suitable for work on this class of material will be published shortly.

\( ^8 \) Because of the red precipitate in the solution it is rather difficult to see the end-point of the titration. For those who have not had much experience it may be advisable to dilute the solution to a definite volume (after adding the hydrochloric acid), then to filter, and to titrate a portion of the filtrate as recommended by Sutton; with a little practice, however, the titration can be made without this procedure and we prefer not to use it.
A COLORIMETRIC METHOD FOR THE DETERMINATION OF PHENOLS (AND PHENOL DERIVATIVES) IN URINE
Otto Folin and W. Denis