THE COLORIMETRIC DETERMINATION OF PHENOLS IN THE BLOOD.

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(Received for publication, August 20, 1918.)

In 1915, Folin and Denis\textsuperscript{1} described a method for the determination of free and conjugated phenols in the urine, using a phosphotungstic-phosphomolybdic reagent, which, in the presence of sodium carbonate, gives a color reaction with phenols. The formula for the color reagent was published in 1912,\textsuperscript{2} at the same time as the uric acid reagent. Later, Folin and Denis\textsuperscript{3} applied the method to phenols in the feces. Little has been done since in phenol determinations, except by Dubin, who published papers on the formation and elimination of phenols under normal and pathological conditions,\textsuperscript{4} and on the effect of feeding inositol on the elimination of phenols.\textsuperscript{5}

In connection with some work which we have been doing on the chemical analysis of blood in carcinomatous patients, we attempted to apply the color reaction to the determination of phenols in the blood. A method has finally been worked out, which we believe to be reasonably simple and accurate.

In brief, our method provides for the determination of (a) uric acid in one sample of the blood, and (b) of uric acid and phenols in a second sample of the same blood. The phenol content of the blood is then obtained by difference. At present this procedure seems preferable to one based upon any attempt to separate the uric acid from the phenol, and to determine the

\textsuperscript{1} Folin, O., and Denis, W., \textit{J. Biol. Chem.}, 1915, xxii, 305.
\textsuperscript{2} Folin and Denis, \textit{J. Biol. Chem.}, 1912, xii, 239.
\textsuperscript{3} Folin and Denis, \textit{J. Biol. Chem.}, 1916, xxvi, 507.
\textsuperscript{4} Dubin, H., \textit{J. Biol. Chem.}, 1916, xxvi, 69.
\textsuperscript{5} Dubin, \textit{J. Biol. Chem.}, 1916-17, xxviii, 429.
latter separately. Since, according to our technique, uric acid gives only about one-third as much color as does an equal weight of phenol, the error involved in correcting for the uric acid present is, we believe, negligible.

Blood filtrates freed from protein and treated with phenol reagent and 20 per cent sodium carbonate give solutions of a muddy green color, which are hard to read in the colorimeter and not very stable or satisfactory to work with. The solutions precipitate out easily. Dubin also reports this green color in working with urines.

The "stabilizing" action of sulfites upon the reduction of photographic plates by phenols is a matter of common knowledge. This fact led us to test the influence of sulfite upon the development of color by the phenol reagent. The effect is quite remarkable. The dirty green colorations change instantly to a clear and brilliant blue upon the addition of the sulfite. The sulfite is added after the carbonate, and an excess of carbonate is always used. The final solutions match the standards perfectly in the colorimeter, and are remarkably stable. The range of accuracy of the reaction depends on the particular sample of sodium tungstate used in the reagent. The standard solutions never precipitate out, and the unknown, only when a large excess of potassium oxalate has been added to the blood to prevent clotting. In that case they can easily be centrifuged and read. The maximum color is reached in 20 minutes, and the color is so stable that a standard may be used for determinations all day; in fact, some standards have been kept 5 days without altering. Some unknown solutions were left for several days, then read against a standard simultaneously prepared. The results were almost identical, as the following tabulation shows. The figures indicate mg. per 100 cc. of blood.

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<th>1 Day.</th>
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<td>4.90</td>
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Uric acid gives a blue color with the phenol reagent, but the color is only about one-third as intense as the phenol. As each fresh reagent is prepared, a known uric acid solution should be
compared with the phenol standard to determine the percentage of color produced by uric acid. The uric acid should be determined on the same specimen of blood, according to Benedict,\(^6\) calculated in terms of phenol, and subtracted from the total phenol color.

Folin and Denis\(^5\) use as standard a strong solution of phenol in 0.1 \(\text{N} \) HCl, and dilute from that. Phenol cannot be weighed accurately, and must be titrated with iodine and sodium thiosulfate. The dilute solutions keep only for a few days.\(^4\) Therefore, a phenol which can be weighed directly would be more desirable. Pure resorcinol gives 86 per cent of the color given by phenol. We use as a standard a solution of resorcinol (0.581 mg. of resorcinol in 5 cc.) in 0.1 \(\text{N} \) HCl, which corresponds in color to 0.5 mg. of phenol in 5 cc. A solution five times this strength in hydrochloric acid has been kept without altering for 4 months, and the dilution has been made from that each week.

The method as used by us is as follows: Precipitate the proteins from 5 cc. of defibrinated blood with five times the volume of boiling 0.01 \(\text{N} \) of acetic acid. Add an equal volume of boiling water and 10 cc. of aluminum cream and filter while hot, washing out the casserole with boiling water several times. Boil the filtrate to about one-half volume and precipitate again with aluminum cream, or boil to smaller volume and precipitate with colloidal iron. Boil this filtrate to about 10 cc. and transfer quantitatively to a graduated cylinder. To this add 8 cc. of phenol reagent (made by boiling under a reflux condenser for 2 hours, 100 gm. of sodium tungstate, 20 gm. of phosphomolybdic acid, 50 cc. of 80 per cent phosphoric, and 750 cc. of water, cooled and diluted to 1 liter) and 3 cc. of 20 per cent sodium bisulfite. Mix immediately and make up with distilled water to from 50 to 100 cc., according to amount of color produced. Compare in a colorimeter after 20 minutes against a simultaneously prepared standard of resorcinol (0.581 mg. in 5 cc.) similarly treated and made up to 100 cc. The colorimeter is conveniently set at 20 mm. Calculate in terms of mg. per 100 cc. of blood.

Determine uric acid on the same specimen and calculate in terms of phenol. Subtract this from the total color with phenol.

reagent, and the result represents mg. of phenol per 100 cc. of blood.

We have added known quantities of resorcinol to blood samples and have recovered about 85 per cent by the above method. Phenol itself added to blood disappears completely during the boiling of the blood filtrates. We have, however, assured ourselves of the practical absence of volatile phenols from blood by testing the distillate from the blood filtrates with the phenol reagent, with a negative result.
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J. Biol. Chem. 1918, 36:95-98.

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