THE DETERMINATION OF CREATININE AND CREATINE IN BLOOD.

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The method of Folin1 for the determination of creatinine and creatine in blood, which has gained extensive use, particularly in connection with clinical material, has, during the past 2 years been the subject of critical study by Wilson and Plass,2 by Hunter and Campbell,3 and more recently by Greenwald and McGuire.4 The conclusions reached by these three groups of investigators have been in the main identical; i.e., that the results obtained for preformed creatinine and for total creatinine (creatine plus creatinine) are much too high; in fact, according to Hunter and Campbell, the figures for creatine in whole blood may be “four times as high as the amount [of this substance] actually present.”

A method for the determination of creatine and creatinine in blood has been described by Wilson and Plass, which according to these investigators gives more accurate results than the Folin procedure. This method consists essentially in the precipitation of the blood proteins with dilute acetic acid and aluminium hydroxide, determination of the preformed creatinine in the filtrate (after appropriate concentration) and total creatinine in the same after heating with hydrochloric acid.

Greenwald and McGuire have more recently described two methods for the determination of creatine and creatinine in blood. In one, precipitation is accomplished by means of dilute acetic acid and “dialyzed iron,” in the second procedure trichloracetic acid is used to remove the proteins. In addition these investigators

1 Folin, O., J. Biol. Chem., 1914, xvii, 475.
have introduced the use of kaolin which they have found to remove creatinine quantitatively from solution but to be without effect on creatine.

Until a sufficient time has elapsed to allow these new processes to be given a thorough trial, it would perhaps appear superfluous to publish further methods or modifications. It has, however, seemed worth while to describe a procedure for the determination of creatine and creatinine in blood which has been used in this laboratory for some time, because, while it is in all probability in no way superior to the trichloroacetic acid method of Greenwald and McGuire, it is considerably less time consuming than the methods which involve coagulation with boiling acetic acid, and is, we find, extremely convenient to use where it is desirable to determine both non-protein nitrogen and creatinine in the same sample of blood.

The method of precipitation is essentially the same as that described by Folin and Denis for the determination of non-protein nitrogen in blood by direct Nesslerization.

Blood is collected in bottles containing powdered potassium oxalate (0.10 gm. of oxalate to 10 to 15 cc. of blood). 10 cc. of blood or plasma are pipetted into a 50 cc. volumetric flask containing an approximately equal quantity of distilled water, and 6 cc. of a 20 per cent solution of m-phosphoric acid are then added. The flask is shaken, allowed to stand for at least 1 hour, then made up to volume with water, and the contents are filtered. Two 10 cc. portions of the filtrate are measured out, one into a 25 cc. volumetric flask, the other into a similar flask of 50 cc. capacity.

For the determination of preformed creatinine add to the first portion (contained in the 25 cc. flask) 10 cc. of saturated picric acid solution, and 2 cc. of 10 per cent sodium hydroxide solution; allow the flask to stand for 10 minutes, make up to volume, and read against an appropriate standard.

For normal blood, standards containing 0.01 and 0.02 mg. of creatinine are usually all that are required. For work on pathological material, however, it has been found necessary to use standards containing 0.03, 0.04, 0.05, 0.08, and 0.10 mg.

It has been found most convenient to retain the principle described in connection with Folin's original method of making up

\[6\] Folin, O., and Denis, W., *J. Biol. Chem.*, 1916, xxvi, 491.
these stock standard solutions with saturated picric acid,⁶ as in
this media the creatinine appears to keep indefinitely; in all,
seven standard solutions are provided, made up in saturated
picric acid to contain from 0.01 to 0.10 mg. of creatinine in 10 cc.
To prepare a standard for any given determination, place 10
cc. of one of the above standard solutions in a 25 cc. volumetic
flask, add 10 cc. of distilled water and 1.5 cc. of 10 per cent so-
dium hydroxide solution, let stand 10 minutes, then make up to
volume with distilled water.
To determine total creatininc (creatine plus creatinine) heat 10
cc. of the original m-phosphoric acid filtrate for half an hour in
an autoclave at 120°C. or add about 75 cc. of distilled water
and heat to boiling on a hot plate for 3 hours. When cool, add
to the solution (which should have a volume of approximately
10 cc.) 10 cc. of saturated picric acid solution, 2 cc. of 10 per
cent sodium hydroxide, and let stand for 10 minutes; then make
up to volume (50 cc.) and read against an appropriate standard.
The standards most useful in work on normal material are those
containing 0.08 or 0.10 mg. of creatine; for pathological blood
standards containing 0.15, 0.20, and 0.30 mg. are sometimes
necessary.
The standards are prepared in the same general manner as de-
scribed for the determination of preformed creatinine; i.e., 10 cc.
of the appropriate picric acid standard solution, 10 cc. of distilled
water, and 1.5 cc. of 10 per cent sodium hydroxide.
Blood filtrates prepared by means of m-phosphoric acid, when
treated with alkali and picric acid as described above, reach their
maximum color in 8 minutes, the continued development of color
after 10 minutes standing which is observed in the blood filtrates
obtained by means of the picric acid precipitation being entirely
absent.
In Table I are presented a few of the results obtained by the
use of the above method. As will be seen the figures obtained
for preformed creatininc are slightly lower than those secured by

⁶ All the picric acid used in this work had been purified according to the
method of Folin and Doisy (J. Biol. Chem., 1916-17, xxviii, 349). Our
“saturated” picric acid solutions had been made up to contain 1.2 per
cent piceric acid.
A Duboscq calorimeter was used for the color comparisons, the standard
solution being set at 20 mm.
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following Folin's method, while in the case of the results on total creatinine the discrepancy becomes relatively large. That these low results are not due to mechanical losses of creatinine or creatine or to chemical decomposition of these bodies by the reagents used is proved by the fact that I have repeatedly carried small quantities of pure solutions through the process as above outlined and obtained quantitative recovery; in the light of our present knowledge it must therefore be concluded that the method of protein precipitation used has eliminated, to a certain extent at least, the reducing bodies other than creatinine to the presence of which has been attributed the high results obtained by the picric acid precipitation.

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