ON THE DETERMINATION OF CREATININE AND CREATINE IN URINE.

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Ever since the introduction of the colorimetric method for the determination of creatinine, potassium bichromate, in half normal solution, has been used as the standard for measuring the color obtained in the reaction of creatinine with picric acid and sodium hydroxide. That the bichromate has been serviceable for the purpose is evidenced by the fact that no other standard has ever been proposed. Potassium bichromate is, however, by no means ideal as a general standard of measure for the color comparisons involved in creatinine determinations. For determinations in ordinary urines containing 7-15 mgm. of creatinine in a volume of 5-15 cc. it may be regarded as satisfactory. For more dilute urines (or creatinine solutions) obtainable in unlimited quantities a fair degree of accuracy can be obtained with the bichromate standard by using the proportions of picric acid and alkali recommended by Shaffer.

The use of the rigid bichromate standard imposes distinct and wholly unnecessary limitations on the applications of a remarkably flexible analytical method, and now that pure creatinine compounds can be prepared from urine with very little work it is, I think, a mistake to continue the use of the bichromate except perhaps for purely routine determinations in the course of ordinary urine analysis. The technique described in this paper is based on the use of 1 mgm. of creatinine as a standard in the making of the color comparisons.
Creatinine and Creatine Estimation in Urine

The determination of creatinine in urine.

With the substitution of a creatinine solution for the half normal bichromate solution as a standard in the colorimetric determination of creatinine certain additional modifications in the procedure have become necessary or desirable. The fading of the color produced when picric acid and an excess of alkali are added in the usual manner to the creatinine solution proved at first a distinct drawback to the use of the creatinine standard, for in order to avoid errors due to this fading it was necessary that the reaction should be made practically simultaneously in the standard and in the unknown. It is however possible to so make the reaction that the color does not fade in the course of twenty-four hours. If anything the color obtained is a shade stronger at the end of that time. This fact, discovered independently and simultaneously by Mr. Morris and myself, is, I think, important for it permits the use of a single standard for the whole day, and it obviates the necessity of being in any hurry in the making of the colorimetric readings.

One milligram of creatinine plus 20 cc. of saturated picric acid solution plus 1.5 cc. of 10 per cent sodium hydrate solution (added from a burette) diluted after ten minutes' standing to 100 cc. gives a highly colored, stable solution, one therefore eminently suitable for use as a standard in connection with all ordinary creatinine determinations. With the more common human urines containing from 0.5 to 1.5 mgm. of creatinine in 1 or 2 cc. the process of making the color comparison is so simple that it is hardly possible to make a mistake—provided only that the 10 per cent alkali is measured out with a burette so that the same amount within 0.1 or 0.2 cc. is added to both the standard and the unknown.

One cubic centimeter of the standard creatinine solution is measured into a 100-cc. volumetric flask and 1 cc. of the urine into another; 20 cc. of saturated picric acid solution (measured with a cylinder) are added to each and then the alkali, 1.5 cc. of 10 per cent solution. At the end of ten minutes the flasks are filled up to the mark with tap water and the color of the unknown is determined. It makes little difference whether the standard is set at 10, 15 or 20 mm., 10, 15 or 20 divided by the reading of the unknown gives in milligrams the amount of creatinine present in the volume of
urine taken. If the urine reads less than two-thirds or more than one and one-half that of the standard the determination should be repeated with more or with less urine.

The only special apparatus needed besides the colorimeter is accurate 1-cc. pipettes of the kind described in this Journal a short time ago. One precaution should perhaps be mentioned. Those not used to the small pipettes referred to will be apt to conclude that by diluting the standard creatinine solution so that 5 cc. contains 1 mgm. they can just as well use 5-cc. pipettes and then work with 5 cc. of urine and dilute the resultant colored solution to 500 cc. while the standard is diluted to 100. To do so would be to introduce a very considerable error. Those who wish to use 5- or 10-cc. pipettes had better work with diluted urines (1:4), or else they should take 5 or 10 mgm. of creatinine as the standard. In the latter case the amount of alkali taken must be increased to 5 cc. as in the original method, and a fresh standard must be used for each set of determinations because of the fading. It would doubtless be possible to find the proportions of picric acid and alkali which will give stable colored solutions with 5 and with 10 mgm. of creatinine but I have made no attempt to do so.

With dilute urines requiring more than 5 cc. to give 1 mgm. of creatinine the standard should also be diluted with a corresponding volume of water before adding the alkali.

In working with very small animals such as rats 0.5 or 0.2 mgm. of creatinine may be used as a standard as was done by J. L. Morris in our work on white rats. The one great advantage of using creatinine as the standard lies in the fact that the same technique is directly applicable to creatinine solutions of almost every degree of concentration provided only that the standard is varied accordingly.

In the making of colorimetric creatinine readings there is one other point which should be mentioned. It happens very often when the colorimeter prisms are immersed in the liquids to be compared that a bubble of air is caught under the prism. Unless this is prevented the readings obtained are, of course, erroneous. Moreover, in the making of an extended series of colorimetric comparisons it sometimes happens when the water is cold that a great many

1This Journal, xi, p. 494, 1912.
2Ibid., xiv, p. 510, 1913.
very minute air bubbles escape from the liquid and gradually accumulate under the stationary prism immersed in the standard solution. A considerable amount of light is thus cut off and the comparisons become correspondingly erroneous. The simplest effective way to avoid this error is to empty the cylinder as soon as any air bubbles are noticeable on the sides and refill it from the standard solution in the flask.

The determination of creatine in urine.

Much work has been done to find the most suitable condition for transforming creatine quantitatively into creatinine, but the only important modification of my original procedure is the use of higher temperatures by means of the autoclave, thus shortening the time of heating from three hours to about half an hour. This modification introduced by V. C. Myers in 1907 yields rather more certain results than are obtained by heating on the water bath for three hours. The choice of acid to be used is not particularly important. Hydrochloric acid is probably the least suitable because it produces the maximum amount of coloring matters, although the error due to coloring matters may be regarded as negligible in view of the fact that the heating with acids does not produce an increase of the creatinine readings in the case of most urines obtained from normal men. The phosphoric acid recently introduced by Rose is liable to give erroneous results. The anomalous results obtained by Rose with the urine of dogs are, however, probably due only to his not having added the right amount of alkali in making the color reaction. Phosphoric acid titrates (with phenolphthalein as indicator) as a dibasic acid, but in the presence of an excess of alkali it acts as a tribasic one, and unless due allowance is made for this fact the results obtained must necessarily be too low. The uncertain and unsatisfactory results obtained by Thompson and his associates with phosphoric acid may be similarly explained. It is not the acid but the temperature which is the important factor in the transformation of creatine into creatinine.

The substitution of the autoclave for the water bath in connection with the colorimetric determination of creatine is certainly

\[^{9}\text{Biochem. Journ., vii, p. 438, 1913.}\]
serviceable when it is a question of making a large number of such determinations at one time. But on the whole it does not seem altogether satisfactory to be dependent on it for so simple a determination. The time necessary for a creatine determination by the water-bath procedure can be materially shortened by boiling the solutions over a naked flame.

In using this procedure one can take advantage of the fact that by greatly diluting the creatine solution with water the process is hastened. Moreover, when working with about 1 mgm. of creatine and creatinine it is not necessary to add any mineral acid whatever. Instead of such acids I simply add in advance the picric which is to be used for the development of the color. The determination of creatine + creatinine in urine by this modification is as follows:

Enough urine to give 0.7–1.5 mgm. of creatinine is measured into a weighed Erlenmeyer Jena flask (cap. 200 cc.). Saturated picric acid solution (20 cc.), about 130 cc. of water and a few very small pebbles to promote even boiling are added and the mixture is gently boiled, preferably over a microburner for about one hour. At the end of this time the heat is increased and the solution is boiled down to rather less than 20 cc. The flask is transferred to the scales and enough water is added to make the total solution equal to 20–25 grams. The solution is cooled in running water, 1.5 cc. of 10 per cent sodium hydroxide are added, and the total creatinine is determined as in the preformed creatinine determination using 1 mgm. of creatinine as a standard. In my hands this procedure gives absolutely quantitative results every time with 1.32 mgm. of crystallized creatine, even in the presence of as much as 25 mgm. of urea nitrogen; and 50 mgm. of glucose, cane sugar, lactose, or levulose.

The autoclave method can also be used with picric acid as the only added acid provided that only enough urine is taken to give 0.7–1.5 mgm. of creatinine. It will not work with the old method of using 10 cc. of urine because of the large amount of ammonia set free during the heating.

In the presence of levulose, or sugars which on hydrolysis yield levulose, the autoclave process cannot be used.4

4Levulinic acid like acetoacetic ester gives a strong color with the alkaline picrate.
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