CREATININE ESTIMATION IN BLOOD SERUM

A NEW METHOD

by

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For the estimation of the plasma creatinine level various modifications of the Jaffé reaction have been generally used as it is the easiest to perform. Nonetheless, papers continue to appear dealing with this point and showing that the last word has not yet been spoken. Recently developed laborious microbiological and condensation methods are also an expression of the feeling of dissatisfaction and uncertainty. Some authors even totally denied the existence of creatinine in serum; they contended that the Jaffé reaction is given by other compounds, and that creatinine is either absent from serum or only present in minute amounts. Notwithstanding this confusion, a function test indicating the glomerular filtration rate and the amount of reabsorbed water has been elaborated, based on the concentration performance of the kidney for the Jaffé-positive substances. The Jaffé reaction was thus accepted with minor modifications until KOŠTÍŘ AND RÁBEK[1] unexpectedly showed that part of the Jaffé reaction of serum can be accounted for by the presence of pyruvic acid. Subsequently, KOŠTÍŘ, BAUEROVÁ, AND SLAVÍK[2] found that pyruvic acid can be oxidized safely by ceric sulfate without lowering the creatinine content. Basing on these two findings we have elaborated a technique suitable for the routine estimation of creatinine in serum. A further task consisted in establishing the real value of serum creatinine and its relation to the complete Jaffé-positive chromogen. At the same time we checked the validity of the results of both papers mentioned here.

METHOD

Principle

Deproteinization is performed according to FOLIN AND WU[3], creatinine is estimated by the Jaffé reaction.

Reagents

I. A 5% aqueous solution of sodium wolframate $Na_2WO_4 \cdot 2H_2O$.
II. 0.66 *N* H_2SO_4.
III. A saturated solution of ceric sulfate $Ce(SO_4)_2 \cdot 4H_2O$ in *N* H_2SO_4, corresponding to 0.019 *M* $Ce(SO_4)_2$.
IV. A 10% solution of NaOH.
V. A saturated solution of picric acid, recrystallized according to BENEDICT.

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Procedure

4 ml fresh serum or plasma are mixed with 4 ml H_2O and 4 ml wolframate (I), 4 ml sulfuric acid (II) are added dropwise under continuous shaking. A fine pulpy suspension results. After 10 minutes it is centrifuged at 3000 r.p.m. for 10 minutes, 2 ml ceric sulfate (III) are added to the clear supernatant, and after a further 10 minutes, 2 ml NaOH (IV) are added and mixed. The contents of the test tube become turbid and after 1 to 2 min the pinkish precipitate is removed by centrifugation at 3000 r.p.m. The colourless clear supernatant is carefully decanted and 1 ml of the picric acid solution (V) is added to the supernatant.

After 30 min the yellow solution is assayed photometrically at wavelength 6030 Å. A cuvette containing 6 ml H_2O, 2 ml ceric sulfate (III), 2 ml NaOH (IV), and 1 ml picric acid (V) is placed in front of the compensatory photocell of the Hilger Spekker photometer.

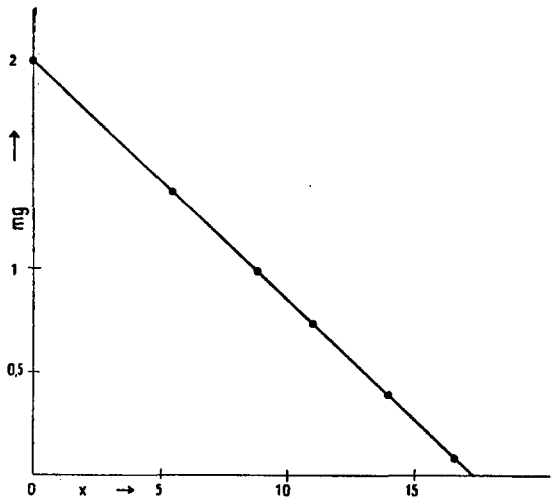

Fig. 1. Calibration curve for the determination of creatinine in blood serum. Ordinate: creatinine in mg/100 ml solution. Abscissa: Reading of the Hilger Spekker photometer taking 2 mg per cent concentration for zero (logarithmic scale).

Calibration curve

A series of standard solutions is prepared by diluting a 20 mg per cent stock solution of creatinine with water. 6 ml are then analysed under the same conditions as the deproteinized supernatant, *i.e.*, addition of 2 ml ceric sulfate solution (III) and 2 ml NaOH (IV), centrifugation and addition of 1 ml picric acid solution (V). A perfectly straight line results (Fig. 1) indicating the validity of BEER's law. For serum analyses, the highest value of the calibration curves was only 2 mg per cent.

RESULTS

In Table I results obtained by this method are compared with the values found according to BONSNES AND TAUSSKY[4], without treatment with Lloyds reagent. According to literature data, creatinine values in normal human serum exceed 1 mg per cent. The adsorption method with Lloyds reagent according to BONSNES AND TAUSSKY[4] gives normal values which are lower by approximately 10%, with an average of about 1 mg per cent. With the new method we find creatinine levels varying between 0.3 and 0.6%, as shown in Table I, which is certainly remarkable. These values, which are only half as high as those found by other methods, or even less, differ fundamentally from the generally accepted plasma creatinine levels.

Such a striking difference has prompted us to make further trials, thus checking both the observations of KOŠTÍŘ AND RÁBEK[1] and KOŠTÍŘ, BAUEROVÁ, AND SLAVÍK[2] and our present technique, with equally satisfactory results.

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TABLE I

A COMPARISON OF VALUES OBTAINED FOR CREATININE IN SERA OF VARIOUS PATIENTS BY THE METHOD OF BONSNES AND TAUSSKY (B.T.) AND BY THE NEW METHOD (K.S.)

Results expressed in mg creatinine per 100 ml serum
Q = ratio between values in columns 2 and 1

Diagnosis	B.T.	K.S.	Q	Difference B.T.–K.S. in %
Asthma bronch.	1.36	0.55	0.40	59.5
Card. decomp.	1.36	0.48	0.35	64.7
Hyperthyreosis	1.20	0.57	0.47	52.5
Hyperthyreosis	1.08	0.52	0.48	52
Anaemia sec.	1.44	0.59	0.41	59
Nephrosclerosis	1.48	0.76	0.51	49
Hypertensio essent.	1.10	0.58	0.53	47.3
Nephritis chronica	1.04	0.47	0.45	55
Nephropathia grav.	1.04	0.36	0.35	65.5
St. p. nephrectomiam	1.28	0.64	0.50	50
Hypertensio	1.20	0.64	0.53	46.8
Cystitis chronica	1.16	0.52	0.45	55
Nephritis chron.	1.80	0.84	0.47	53.5
Polyarthritis rh.	1.20	0.46	0.38	61.7
Cirrhosis hepatis	1.16	0.36	0.31	69
Thyreotoxicosis	1.12	0.34	0.30	69.6
Cirrhosis hepatis	1.12	0.31	0.28	72.4
Diabetes mellitus	1.00	0.30	0.30	70
Ulcus duodeni	1.08	0.33	0.31	69.5
Hypothyreosis	1.08	0.58	0.53	46
Arthrosis def.	1.00	0.44	0.44	60
Hyperthyreosis	1.00	0.44	0.44	60
Hyperthyreosis	0.92	0.34	0.37	63
Hyperthyreosis	0.96	0.42	0.44	56.3
Bronchiectasis	1.04	0.52	0.50	50
Hyperthyreosis	0.88	0.34	0.39	61.5
Acromegalia	1.00	0.40	0.40	60
Osteosarcoma	1.08	0.46	0.42	57.4
Hyperthyreosis	0.92	0.34	0.37	63
Hypopituitarismus	1.08	0.52	0.48	52
Pneumonia	0.92	0.32	0.35	65.3

1. We have confirmed that pyruvic acid is Jaffé positive. A solution of pyruvic acid of 1 mg/100 ml gives an extinction which is only slightly less than that of a 1 mg per cent solution of creatinine under the same analytical conditions.

2. Within 1 minute, 2 ml ceric sulfate (saturated solution in N H_2SO_4) totally destroy the Jaffé-positive reaction of 6 ml of an aqueous solution of pyruvic acid containing 2 mg/100 ml.

3. In 20 minutes ceric sulfate, at the concentration mentioned above, does not cause a detectable impairment of creatinine in amounts equivalent to 2 mg per cent in serum.

4. Under the conditions mentioned above ceric sulfate is capable of oxidizing within one minute a 2 mg per cent surplus of pyruvic acid in a serum already containing normal amounts of this substance.

5. Recovery of a 2 mg per cent aqueous solution of creatinine added to serum before deproteinization is 95%.

6. 1 ml of a 10% NaOH solution suffices to precipitate 2 ml of the added ceric

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sulfate solution and to provide the alkaline medium necessary for the development of the Jaffé reaction.

These tests seem to exclude convincingly the possibility of errors. It would thus be necessary to take into account these low serum creatinine values. This finding has a special significance for hospital practice. As was stated initially, the method for measuring glomerular filtration rate and water reabsorption was based on the concentration performance of the kidneys for the Jaffé-positive chromogens. In spite of the objections against this method, determination of the so called endogenous creatinine clearance became indispensable for the examination of the uropoietic tract. Owing to its simple execution and mainly because it is not so time-consuming for the physician, nor so troublesome for the patients as the inuline or thiosulfate clearance and similar methods. If the normal serum creatinine value actually varies about 0.5 mg per cent, then with the usual analytical procedure for creatinine in urine, according to the clearance equation $\frac{U \cdot V'}{P}$ (U = creatinine in urine in mg per cent, V' = minute volume of the collection period, P = plasma creatinine mg per cent) glomerular filtration rates would be about 300 ml/min. These values would be twice those found by other methods. Hence it follows that creatinine is either also excreted by the tubules and is therefore unsuitable as a clearance substance for measuring glomerular filtration rates, or else the assumption that Jaffé-positive chromogens, not identical with creatinine, are not present in urine, is false.

SUMMARY

A method for the estimation of creatinine in serum or plasma has been elaborated, based on the observation that pyruvic acid is a Jaffé-positive substance and that it can be oxidized by ceric sulfate without destroying creatinine. Serum values found by the new method vary from 0.30 to 0.60 mg per cent with a mean value of 0.48 mg per cent. It will be necessary either to revise the creatinine clearance test and current conceptions on concentration mechanism, or to look for other possible Jaffé-positive chromogens in urine.

RÉSUMÉ

Nous avons établi une méthode permettant de déterminer la créatinine dans le sérum ou le plasma. Elle est basée sur le fait que l'acide pyruvique est une substance Jaffé-positive et qu'il peut être oxydé par le sulfate cérique sans destruction de créatinine. Les valeurs trouvées par cette nouvelle méthode pour le sérum varient de 0.3 à 0.6 mg % (moyenne 0.48 mg %). Les auteurs sont d'avis qu'il faudra revoir la méthode d'essai en usage ("créatinine clearance test") et les conceptions actuelles, ou bien chercher dans l'urine d'autres chromogènes Jaffé-positifs.

ZUSAMMENFASSUNG

Eine Methode zur Bestimmung von Kreatinin in Serum oder Plasma wurde ausgearbeitet. Sie gründet sich auf die Beobachtung, dass Brenztraubensäure eine Jaffé-positive Substanz ist und dass sie mit Hilfe von Cer (IV) Sulphat oxydiert werden kann, ohne dass Kreatinin zerstört wird. Mit Hilfe dieser neuen Methode wurden für Serum Werte von 0.3–0.6 mg % (Durchschnitt 0.48 mg %) gefunden. Diese Werte sind nicht im Einklang mit früher gefundenen Werten. Die Verfasser sind deshalb der Meinung, dass entweder der Kreatinin-Clearance-Test und die herrschenden Aussichten verändert, oder nach etwaigen neuen Jaffé-positiven Chromogenen im Harn gesucht werden muss.

REFERENCES

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Received February 9th, 1951