lytic activity of the corresponding ammonium salts upon the ammonolysis of esters in liquid ammonia. Like ammonia, butylamine is a strongly basic solvent in which ionization of weak acids is presumably enhanced. On the other hand, however, the low dielectric constant ($\epsilon = 5.4$)[7] of the solvent undoubtedly promotes formation of inactive ion pairs, or even larger aggregates, with the consequence that stoichiometric concentrations give little or no insight into the concentrations of either the butylammonium ions or the negative ions in solution.

It also should be pointed out that the presence of a fairly large mole fraction of ester in the solutions under investigation undoubtedly influences the ionization of the butylammonium salts. Actually, very concentrated solutions of ester in amine were used in all experiments.

It is also apparent from a comparison of the experimental series 4, 9 and 10, that the ratio of amine to ester affects the rate of the reaction very appreciably. In the first eight series the molar ratios of amine, ester and catalyst are given approximately by the numbers 380:200:7. As the mole ratio of amine to ester is increased, the value for the specific reaction rate constant also becomes larger. It is obvious, therefore, that the reaction is not even pseudo-bimolecular in character, except for specified conditions. To obtain more rapid reaction it is advisable to use quantities of amine considerably in excess of those required stoichiometrically.

The tremendous effect of the addition of the relatively small quantity of butylammonium salt is emphasized by a comparison of the half-life times of the first ten series, with that for the uncatalyzed reaction. These findings again emphasize our contention[5] that the mechanism of all solvolytic reactions, whether they be hydrolytic, ammonolytic or aminolytic, is fundamentally the same as illustrated by the reaction

$$\left.\begin{matrix}\mathrm{HOH}\\ \mathrm{HNH_2}\\ \mathrm{R''NH_2}\end{matrix}\right\} + \mathrm{RCOOR'} \xrightarrow{\mathrm{S{\cdot}H^+}} \mathrm{RCO}\left\{\begin{matrix}\mathrm{OH}\\ \mathrm{NH_2}\\ \mathrm{NHR''}\end{matrix}\right. + \mathrm{R'OH}$$

(7) Schlundt, *J. Phys. Chem.*, **5**, 503 (1901).

Summary

1. The aminolysis of ethyl phenylacetate in *n*-butylamine to yield the N-butylamide of phenylacetic acid is catalyzed by butylammonium salts.

2. The catalytic effect of equimolar concentrations of various butylammonium salts decreases with the anion in the following order: $Cl^- > C_2H_3O_2^- > C_6H_5COO^- > Br^- > NO_3^- > CNS^- > I^- > ClO_4^-$.

URBANA, ILLINOIS RECEIVED JUNE 21, 1939

[CONTRIBUTION FROM THE SAMSON LABORATORIES]

The Relation of Cuprous Creatinine to Tests for Sugar in Urine[1]

BY MEYER SAMSON

It is almost a hundred years since Trommer[2] published the first practical directions for a copper reduction test for sugar. Of the hundreds of modifications[3] subsequently proposed, only the methods of Trommer, Fehling[4] and Benedict are largely used today.

Most of the proposed modifications were designed to avoid interference. Creatinine soon was recognized as the principal source of trouble, which arose from its own reducing powers, as well as from its property of holding in solution the cuprous oxide formed in the presence of glucose.[5-7]

Benedict,[8] in reviving the forgotten idea of Possoz[9] of using carbonate in place of hydroxide as alkali, believed he had found a copper solution on which creatinine was without appreciable effect.[10] When he later proposed the copper carbonate–citrate mixture in the solution[11] known by his name, he claimed it to be more sensitive to glucose either in pure solution or in urine than is Fehling's fluid. This is not correct, since

(1) Presented before the Division of Biological Chemistry, 96th meeting, American Chemical Society, Milwaukee, Wis., September 5–9, 1938.

(2) Trommer, *Ann.*, **39**, 360 (1841).

(3) Dehn, Jackson and Ballard, *Ind. Eng. Chem., Anal. Ed.*, **4**, 413 (1932).

(4) Fehling, *Ann.*, **72**, 106 (1849).

(5) Von Babo and Meissner, *Z. Rat. Med.*, **3**, 329 (1858).

(6) Winogradoff, *Arch. Path. Anat.*, **27**, 533 (1863).

(7) MacLean, *Biochem. J.*, **2**, 156 (1907).

(8) Benedict, *J. Biol. Chem.*, **3**, 101 (1907).

(9) Possoz, *Compt. rend.*, **75**, 1836 (1872).

(10) Benedict, *J. Am. Med. Assoc.*, **57**, 1193 (1911).

(11) Benedict, *J. Biol. Chem.*, **5**, 484 (1908).

aqueous glucose solutions which yield typical Trommer or Fehling tests give a barely perceptible red haze by Benedict's method. For urinary work, however, Benedict's reagent is unexcelled, since it gives bulky opacities with glucose concentrations which fail to precipitate with other reagents, and clear tests with urines containing no glucose but which give reductions with the Trommer or Fehling tests. The possibility that the increased delicacy of the Benedict test on urine might be due to a urinary ingredient was the origin of the work reported in this paper.

Creatinine as Opacity Promoter in the Benedict Test.—It is easy to show that an ingredient of urine promotes the typical Benedict test opacity with minimal amounts of glucose. Normal urine if shaken with blood charcoal yields a water-white filtrate which on addition of 0.1% of glucose gives in the Benedict test the barely perceptible red haze of cuprous oxide characteristic of aqueous glucose. A similar concentration of glucose added to the original urine gives the usual opaque test, showing that the urine contains a promoter which is removed by the charcoal. Lloyd's alkaloidal reagent, a type of fuller's earth used by Folin and Berglund,[12] has a similar action. Of the substances known to be removed from urine by Folin and Berglund's method, creatinine alone was found to be an opacity promoter. Solutions made of glucose with a proper amount of creatinine react in the Benedict test exactly as does glucose in urine.

Experimental.—To 37.5 cc. of urine were added 12.5 cc. of 0.1 *N* sulfuric acid and 4 g. of Lloyd's earth. After shaking for two minutes, the mixture was filtered, the filtrate neutralized and glucose added to 0.1%. The solution reacted to the Benedict test like aqueous glucose. The opacity promoter is removed readily from the Lloyd's earth by elution with dilute carbonate solution. The elution plus 0.1% glucose gives an opaque Benedict test.

Cuprous Creatinine.—The knowledge that creatinine is the sensitizer or opacity promoter directs attention to a possible mechanism. Reduction experiments on Benedict solution with glucose and creatinine at lower temperatures than the usual boiling, give at 60° a bulky, slightly yellowish precipitate, at 50° a voluminous white one which agglutinates readily on shaking and filters well. The same reaction goes on at room temperature, slower but complete overnight. Further experiment showed the precipitate to be a compound of creatinine and cuprous oxide, the existence of which has been reported previously, in incomplete form, by several investigators, whose work will be discussed a little later.

(12) Folin and Berglund, *J. Biol. Chem.*, **51**, 209 (1922).

Experimental.—A mixture of 60 cc. of 0.5 *M* creatinine (5.65%) in *N* hydrochloric acid and 25 cc. of 3 *M* glucose was added to 1200 cc. of Benedict reagent and allowed to stand overnight. The bulky white precipitate was filtered off on a Büchner funnel and washed free from cupric ion with 15% sodium carbonate solution. The white solid was removed from the filter and cautiously neutralized with 20% acetic acid, atmospheric oxidation producing some blue color. The heavy neutral suspension was transferred to centrifuge tubes and washed free from cupric ion by repeated settling and resuspension in water. Air oxidation of the moist precipitate may be hindered by storing it in practically full, tightly stoppered tubes. Solubility data for the substance are given in Table I; the aqueous solution shows *p*H 6.5. It is soluble in acids, without effervescence.

TABLE I

SOLUBILITY DATA OF CUPROUS CREATININE, $C_4H_7ON_3 \cdot CuOH$

Solvent	Soly., g./100 cc.
Pure water	1.1
Sodium carbonate, 7.5%	1.0
Sodium carbonate, 8%	0.87
Sodium carbonate, 9%	.14
Sodium carbonate, 10%	.054
Sodium carbonate, 15%	.018
Sodium carbonate, 20%	.016
Sodium carbonate, 30%	.008
Benedict solution at 25°	.019
Benedict solution at 100°	.09

The presence of creatinine in the precipitate may be shown by the picric acid or nitroprusside test, while cuprous copper is proved by oxidation and by precipitation with sulfide, cyanide, ferrocyanide, ferricyanide, or thiocyanate, the last three showing the precipitates characteristic of cuprous ion. Neutral chlorides have no effect but dilute hydrochloric and hydrobromic acids give a white precipitate, soluble in excess.

A saturated aqueous solution of the complex on addition of carbonate gives a bulky and voluminous precipitate rather than the creamy and chalk-like original neutral suspension. High concentrations of citrate and sulfate cause a clouding of the saturated aqueous solution, while tartrate, pyrophosphate, oxalate, acetate, salicylate and sulfite have no visible effect.

Cuprous creatinine may be prepared rapidly in small quantities by heating the mixture to incipient boiling, and then cooling rapidly in running water. Aside from its pure white color, the resulting opacity is similar to that of a positive Benedict test.

The ratio of copper to creatinine in the complex was determined, using a preparation made from an alkaline copper mixture reduced by fructose instead of glucose, to speed up the reaction and avoid errors due to reducing action of creatinine or its entrainment by precipitation in uncombined form. An aliquot of the complex made in this way was analyzed for copper by the thiosulfate method[13] and for creatinine by the Folin method,[14] preliminary work with the latter having shown no interference by copper in the development of the picramic acid color.

The ratio of copper to creatinine in the aliquot taken was found to be 0.2624 g./0.4444 g., which varies only slightly from the calculated ratio 63.57/113 for one atom of copper to one molecule of creatinine. The complex probably is a simple addition compound, $C_4H_7ON_3 \cdot CuOH$. Such compounds with zinc chloride, silver nitrate or similar salts are well known. More recently it has been shown[15] that creatinine forms addition compounds even with alkali hydroxides.

Previous Work on Cuprous Creatinine.—The existence of this compound was first suggested by Maly,[16] investigating previous observations that Trommer's test is less sensitive to urinary glucose than to aqueous glucose. Maschke[17] prepared such a complex by saturating creatinine solutions with sodium carbonate and adding Fehling solution, but did not analyze it. The work of these investigators appears to have been overlooked in subsequent reports on the influence of creatinine on alkaline copper reactions.[7,18,19] In Merck's "Reagenzien-Verzeichnis"[20] as well as in "Beilstein," Maschke's finding is referred to as a test for creatinine detection, as the author emphasized, rather than as a complex important in copper tests for urinary glucose.

Cuprous Creatinine in Alkaline Copper Tests on Urine.—Aside from its susceptibility to oxidation, cuprous creatinine is a very stable compound, the bond being resistant to alkali, other than a slow conversion of creatinine to creatine which is slight in the time needed for a test. The complex is very soluble in alkali hydroxide, so that in a Trommer or Fehling test on glycosuric urine, containing also sufficient creatinine to combine with all the cuprous oxide formed, no visible precipitate results. In carbonate solution, on the other hand, the cuprous creatinine complex is quite insoluble (Table I), and very voluminous. These properties lead, in effect, to an enormous magnification of the volume of the precipitated cuprous oxide in a positive Benedict test.

The properties of cuprous creatinine, therefore, explain both the sensitivity of Benedict's test for urinary glucose, and the delay in precipitation in alkaline copper reagents employing hydroxide. The explanation of Benedict[10,11] that in the latter creatinine delays precipitation due to the fact that the normal reduction of the solution is inhibited for a period long enough to allow the strong alkali present to decompose the reducing substance formed from the sugar molecule into compounds which have no reducing power is not correct. It may be shown by following the reduction with phosphomolybdic acid as in blood sugar analysis, that any delay by creatinine on the reduction of copper by glucose is negligible. The delay in Trommer and Fehling tests reported by Von Babo and Meissner,[5] Winogradoff,[6] Worm-Müller,[18] Eury,[19] MacLean,[7] and Laird,[21] actually is a delay in the appearance of a precipitate and not a delay in the reduction of copper by glucose.

The Paradoxical Role of Creatinine in the Benedict Test.—It is well known that in addition to creatinine there are other substances either normally present in urine or resulting from pathological conditions or administration of medicaments, which disturb the normal course of alkaline copper reactions. Reduction by creatinine, uric acid, urochrome, etc., has a greater interfering effect in those tests which use a large proportion of urine to reagent (Trommer and most Fehling variations) than in the Benedict test, in which the urine comprises less than 10% of the liquid in the test. It is probable that this low ratio of urine is just as important in the Benedict test as is the use of carbonate as alkali and the stability of the reagent. Even in this restricted urinary proportion, however, creatinine still has interfering properties as well as the desirable sensitizing effect.

The opacity promoting property of creatinine is effective only in a range of concentrations from

(13) "Methods of Analysis," Assocn. Offic. Agr. Chem., 2nd ed., Washington, D. C., 1925, p. 191.
(14) Folin, *Z. physiol. Chem.*, **41**, 223 (1904).
(15) Bolliger, *J. Proc. Roy. Soc. N. S. Wales*, **71**, 40 (1937).
(16) Maly, *Z. anal. Chem.*, **10**, 382 (1871).
(17) Maschke, *ibid.*, **27**, 134 (1878).
(18) Worm-Müller, *Arch. ges. Physiol.*, **27**, 22 (1882).
(19) Eury, *Bull. soc. chim.*, [3] **23**, 41 (1900).
(20) "Merck's Reagenzien-Verzeichnis," 7th ed., Merck and Co., Darmstadt, 1932.
(21) Laird, *J. Path. Bact.*, **16**, 398 (1912).

0.03 to 0.15%. This is the range occurring in the majority of human urines. These concentrations sensitize the Benedict test for the very concentration range of glucose (0.05–0.3%) which yields doubtful results by other methods. A higher creatinine content, such as 0.2%, fortunately not common, in a urine containing 0.1% of glucose, delays the appearance of the opacity beyond the three-minute water-bath time recommended,[22] while the 0.4% creatinine level reported by Folin[24] for a starch and cream diet prevents the precipitation entirely, as it would in any other copper reduction test. On the other hand, creatinine concentrations below the optimum range, which can occur readily after copious water intake, cause the urine to react like an aqueous glucose, so that a 0.1–0.3% glycosuric urine with less than 0.03% creatinine gives in the Benedict test only a barely perceptible red haze, which would be considered negative by its author's instructions. In the Fehling or Trommer tests such a urine would give typical positive results.

It is evident, therefore, that at low glucose levels it is the accompanying content of creatinine which determines the sensitivity of a Benedict test. With this knowledge and the better understanding of the role of the various ingredients of alkaline copper solutions which has accumulated in the years since the appearance of Benedict's method, it may be possible to devise a more nearly infallible test and one sensitive to any significant concentration of glucose in urine. Progress of work on improved methods for the detection of sugar in urine is to be reported in a later paper.

(22) Benedict recommended one to two minutes of boiling over a flame. A three-minute period in a water-bath was shown by Folin and McEllroy (ref. 23) to be equivalent to the two minutes over a flame. The five-minute water-bath time, recommended in many texts, is an unwarranted extension and leads to many doubtful or false positive tests.

(23) Folin and McEllroy, *J. Biol. Chem.*, **33**, 513 (1918).

(24) Folin, *Am. J. Physiol.*, **13**, 66 (1905).

Summary

1. Benedict's test, in distinction to other alkaline copper tests used for the qualitative detection of sugar, is more sensitive to urinary glucose solutions than to aqueous ones.

2. Creatinine is the opacity promoting or sensitizing factor in urine which causes bulky opacity in positive Benedict tests with low concentrations of glucose.

3. The properties of cuprous creatinine account for the interfering effect of creatinine in the precipitation of cuprous oxide in Trommer's and Fehling's tests, and for its opacity promoting effect in the Benedict test.

4. The concentration of creatinine present in the majority of human urine samples has opacity promoting properties in the Benedict test on low glucose urines.

5. Exceptional concentrations of creatinine, higher or lower than the normal proportions, may lead to false results in the Benedict test.

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[CONTRIBUTION FROM THE PHYSIOLOGICAL LABORATORY, PRINCETON UNIVERSITY]

Sonoluminescence and Sonic Chemiluminescence

BY E. NEWTON HARVEY

Introduction

It is customary to designate a luminescence by the method of excitation, as electroluminescence, photoluminescence, triboluminescence, etc. Accordingly the luminescence which appears when sound waves pass through liquids has been called acoustic or sonic luminescence, for short, sonoluminescence. This type of light emission was first observed by Frenzel and Schultes[1] for supersonic and by Chambers[2] for audible frequencies. Using a magnetostriction oscillator of 8900 frequency, Chambers studied 36 pure liquids and found 14 of them to luminesce, the brightness varying inversely as the temperature and directly as the product of viscosity and dipole moment.

Frenzel and Schultes were led to look for luminescence accompanying supersonic waves in water from the formation of hydrogen peroxide which many observers (Schmitt, Johnson and Olson,[3] Beuthe,[4] Liu and Wu,[5] and Flosdorf, Chambers and Malisoff[6]) have found in fluids subjected to sonic treatment. Beuthe[4] believes

(1) Frenzel and Schultes, *Z. physik. Chem.*, **27**, 421–424 (1934).

(2) Chambers, *J. Chem. Phys.*, **5**, 290–292 (1937).

(3) Schmitt, Johnson and Olson, THIS JOURNAL, **51**, 370–375 (1929).

(4) Beuthe, *Z. physik. Chem.*, **163**, 161–171 (1929).

(5) Liu and Wu, THIS JOURNAL, **56**, 1005–1007 (1934).

(6) Flosdorf, Chambers and Malisoff, *ibid.*, **58**, 1069 (1936).