per reagent;[10] 20% sodium hydroxide solution; acid molybdate reagent;[11] creatine standard: dissolve 100 mg. of creatine in water and dilute to 100 cc.

Technique.—Place 1.5 g. of phosphotungstic acid in a large Pyrex tube, 30- to 50-cc. capacity, and dissolve in 6 cc. of water. With the aid of a pipet add 4 cc. of urine sample and centrifuge until clear. Add 4 cc. of the basic lead acetate solution and centrifuge again.[12] When clear, add 6 cc. of 5% sulfuric acid and recentrifuge. Transfer 2 cc. of the resultant, clear filtrate to a Folin–Wu sugar tube. Add 2 cc. of ammoniacal copper solution and 1 cc. of 20% sodium hydroxide. Place in a vigorously boiling water-bath for ten minutes, cool, add 2 cc. of acid molybdate reagent, dilute to mark and compare immediately with a 1-mg. creatine standard similarly treated.

Observations

The phosphotungstic acid precipitates the proteins and some of the other interfering substances while the addition of lead acetate removes the excess phosphotungstic acid and the remaining interfering substances. The subsequent use of sulfuric acid precipitates the excess lead completely.

The optimum alkalinity of the medium required for the creatine to exert its most efficient reducing action necessarily detracts from the non-sugar reducing properties of the copper solution.

Normally, urine contains traces of glucose which are so minute that the error introduced by such amounts is negligible. However, when sugar is present in any appreciable amounts it must either be removed by the brief fermentation process described by Folin and Svedberg[6] and designed for use on filtrates, or the quantitative sugar determinations of Folin–Wu[11] run in conjunction with the determination of creatine. This latter method is preferred as the fermentation procedure must be regulated rather too exactly, otherwise the reducing powers are increased rather than decreased.

Recovery of Creatine Added to Urine.—Urine that had no reducing action on the copper solution or at best produced only a slight coloration which was impossible to read colorimetrically was selected and varying amounts of a standard creatine solution added.

Filtrates of these various specimens were then prepared as described above, and the determination proper performed on the filtrates.

From a series of twenty-eight different determinations recoveries ranging from 90 to 110% were obtained in contrast to recoveries of 82 to 92% employing the method of Folin.

The accompanying protocol shows a comparison of the values for creatine recovery, obtained by both the Folin and the suggested colorimetric method, when added to urine.

PROTOCOL

COMPARISON OF CREATINE RECOVERIES AFTER THE ADDITION OF A DEFINITE AMOUNT TO URINE

Urine no.	Creatine present, mg. per cc.	Recovery Folin method, mg. per cc.	Percentage error	Recovery Colorimetric method, mg. per cc.	Percentage error
1	2.6	2.5	− 3.9	2.6	0
2	3.6	3.2	−11.2	3.4	−5
3	4.6	3.8	−17.4	4.45	−3
4	1.0	0.92	− 8.0	0.97	−3
5	2.0	1.83	− 8.4	2.1	+5
6	3.0	2.57	−14.3	3.12	+4
7	1.0	0.88	−12.0	0.97	−3
8	2.0	1.74	−13.1	1.92	−4.2
9	3.0	2.38	−20.7	3.13	+4.5
10	0.6	0.49	−17.7	0.6	0

Conclusion

A preliminary report on a direct method for the determination of creatine in urine is presented. The full report is to be submitted on completion of the work.

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[10] Folin, *J. Biol. Chem.*, **82**, 88 (1929).

[11] Folin and Wu, *ibid.*, **41**, 367 (1920).

[12] Prior to the addition of the basic lead acetate, Larson recommends refrigerator incubation of the tube for one-half hour to minimize the time required for complete phosphotungstate precipitation. This procedure may be omitted as the results obtained, on final analysis of the filtrate, show very little variation.

The Common Basis of Intramolecular Rearrangements. IV.[1] A Correction: The Benzilic Acid Rearrangement

BY FRANK C. WHITMORE

Since the formulation of the working hypothesis used in this Laboratory, no facts have been found contrary to the assumption of the basis of molecular rearrangement as due to a carbon with an open sextet of electrons. However, the extreme extension of the hypothesis to include the benzilic acid rearrangement involving the addition of a proton to benzil to form a positive ion, cannot be supported. Ingold[2] has proposed the existence of an intermediate negative ion produced by the addition of hydroxyl ion to benzil and indi-

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[1] Whitmore, THIS JOURNAL, **54**, 3274 (1932); Whitmore and Stahly, *ibid.*, **55**, 4153 (1933); Whitmore and Fleming, *J. Chem. Soc.*, 1269 (1934); Wallis and Whitmore, THIS JOURNAL, **56**, 1427 (1934).

[2] Ingold, "Ann. Repts. Chem. Soc. (London)," Vol. XXV, 1928, pp. 124–134.

cated the importance of an alkaline medium in the transformation.[3] The reaction rate study conducted by Westheimer[4] indicated the existence of a bimolecular reaction between benzil and hydroxyl ion which can best be explained by the formation of the same negative ion. Recent investigation by Roberts and Urey[5] on the oxygen interchange of benzil with water of a higher concentration of H_2O^{18} indicates a more rapid exchange in alkaline than in neutral solution which is explained by the rapid, reversible addition of hydroxyl ion to form a negative ion with benzil, followed by rearrangement.

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(3) Ingold, "Ann. Repts. Chem. Soc., (London)" Vol. XXX, 1933, p. 177.

(4) Westheimer, THIS JOURNAL, **58**, 2209 (1936).

(5) Roberts and Urey, *ibid.*, **60**, 880 (1938).

STATE COLLEGE, PENNA. RECEIVED JUNE 4, 1938

COMMUNICATIONS TO THE EDITOR

THE SALT EFFECT IN THE PARAMAGNETIC CONVERSION OF *p*-HYDROGEN

Sir:

In 1934 Sachsse [*Z. physik. Chem.*, **B34,** 429 (1934); *Z. Elektrochem.*, **40,** 531 (1934)] reported that the rate constants for the conversion of *p*-hydrogen by solutions of paramagnetic ions were independent of the concentrations of these ions if the change in solubility of hydrogen with changing ionic strength were taken into account. To this is now added the fact that these rate constants are also independent of the concentration of added diamagnetic salts. The measurements were made by shaking the solutions with p-H_2 at a shaking speed in the range at which the conversion rate was independent of the shaker speed. The ratio of gas volume to solution volume was known and kept constant. The apparent rate was independent of hydrogen pressure.

Some representative data are given in the following table for which the solution volume is always 100 cc., the gas pressure *ca.* 100 mm., the concentration of manganous chloride 0.02 *M*, at room temperature.

Expt.	Molality of diamagnetic salt	$k'_{0.02} \times 10^3$	l(Ostwald)	$k(l, m^{-1}, \text{min.}^{-1})$
1	0	3.03	0.0198	7.9
2	0.3 *M* $CaCl_2$	2.75	.0173	8.2
3	0	2.15	.0198	8.0
4	1 *M* $NaNO_3$	1.65	.0158	7.7
5	2 *M* $NaNO_3$	1.41	.0127	8.2
6	0.6 *M* $BaCl_2$	1.56	.0151	7.7

In experiments 1 and 2 the gas volume is 45.1 cc.; in expts. 3 to 6 the gas volume is 64 cc. k' is the observed first order rate constant calculated by $k'_{0.02} = \frac{1}{t} \log \frac{(p\text{-H}_2)_0}{(p\text{-H}_2)_t} - k'_w$ where t is the time in minutes, $(p\text{-H}_2)_0/(p\text{-H}_2)_t$ is the rate of the initial p-H_2 concentration to that at the time t and k'_w is the correction for the conversion by pure water under the same conditions and calculated in the same way. For expts. 1 and 2 $k'_w = 0.07 \times 10^{-3}$ and for expts. 3 to 6 $k'_w = 0.05 \times 10^{-3}$. k is the velocity constant reduced to unit Mn^{++} ion concentration and corrected for the gas not in solution by the relation $k = \frac{V_g \times 2.303}{V_s \times l \times M_{MnCl_2}} \times k'$ where V_g is the gas volume, V_s the solution volume, l the Ostwald solubility of H_2 (from Seidell's "Solubilities") and M_{MnCl_2} the molality of the manganous chloride.

Thus the rate of conversion of $p\text{-H}_2 \longrightarrow o\text{-H}_2$ by Mn^{++} can be expressed by

$$\frac{-d(p\text{-H}_2)}{dt} = k(\text{Mn}^{++})\,(p\text{-H}_2)$$

where (Mn^{++}) and $(p\text{-H}_2)$ are the concentrations of Mn^{++} ion and p-H_2, respectively. There is no need to introduce any activity factor of Brönsted $f_{Mn^{++}}f_{H_2}/f_{Mn^{++}\cdot H_2}$, although the concentration of the diamagnetic salts was carried as high as 4 *M* $NaNO_3$ and 2.4 *M* $CaCl_2$ in 0.02 *M* manganous chloride. If it is incorporated it must be a constant and this is not unreasonable since the two reactants completely retain their independent identities throughout the reaction.

The effectiveness of the inhomogeneous magnetic field of the ion is independent of the ionic environment of the paramagnetic ion. This is in agreement with the observation of Sachsse that solutions of manganous sulfate and manganous chloride give the same rate constant.

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