The filtrate from above, when adjusted to a *p*H of 5 with 10% sodium hydroxide, deposited a brown gum. This dissolved in 95% alcohol, precipitated by the addition of several volumes of ether, dried at 120 for three hours and then *in vacuo* over concd. sulfuric acid. Qualitative tests showed it contained chlorine. It did not melt below 250°; yield of 2 g. (4%).

Anal. Calcd. for $C_9H_{13}O_4NClAs$: As, 24.2. Found: As, 23.9.

Reaction of I with β-Chloro-β'-hydroxydiethyl Ether.—To 5 g. of I dissolved in 25 ml. of *N* sodium hydroxide was added 4.1 g. of β-chloro-β'-hydroxydiethyl ether and the mixture boiled under reflux for 5 hours. The cooled mixture was made acid to congo red with concd. hydrochloric acid and then evaporated to dryness on a steam-bath. The residue was extracted with two 50-ml. portions of boiling 95% alcohol, the extract charcoaled and filtered. To the filtrate was added 200 ml. of ether, precipitating a brown oily gum. The gum was dissolved in 10 ml. of 2.5 *N* sodium hydroxide and 100 ml. of cold 95% alcohol added, precipitating a white solid. This was washed with cold alcohol, dried at 120° for 2 hours and then *in vacuo* over calcium chloride; yield of 3.2 g. (43%).

Anal. Calcd. for $C_{10}H_{15}O_5NNaAs$: As, 22.9. Found: As, 23.1.

Reaction of I with Trimethylene Dibromide.—To 21.7 g. of I dissolved in 100 ml. of *N* sodium hydroxide was added 13 g. of trimethylene dibromide and the mixture boiled under reflux for three hours, a white solid gradually separating. The mixture was made acid to congo red with concd. hydrochloric acid, the white solid filtered off and washed with 95% alcohol and water. This product was redissolved in 5% sodium hydroxide, reprecipitated by addition of concd. hydrochloric acid, washed with 95% alcohol, hot water, and dried at 120°. This compound does not melt below 250°; yield of 6 g. (25%).

Anal. Calcd. for $C_{15}H_{20}O_6As_2$: As, 31.6. Found: As, 31.3.

The sodium salt was formed in the usual manner by dissolving in sodium hydroxide solution and precipitating by addition of alcohol.

Anal. Calcd. for $C_{15}H_{18}O_6N_2Na_2As_2$: As, 29.0. Found: As, 29.0.

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Water Absorption of Proteins. VI. Effect of Guanidino Groups in Casein[1]

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The vapor phase water absorption isotherms[2] of the amino groups in casein and of the peptide groups of some long polyglycine molecules show that these polar groups are specific sorptive sites over the entire range of relative humidities. The basic nature of the guanidino group indicated that it might also be a specific site for water absorption. Therefore, guanidino groups have been introduced into casein by the reaction of S-methylisothiourea with the free amino groups in alkaline solution. Several casein derivatives containing various amounts of substituted guanidino groups have been prepared in this manner.

The vapor phase water absorption isotherms of these samples were obtained as in the previously described studies.[2] The absorptions were calculated for a 1000-g. content of casein to eliminate the dilution effect due to the varying weight of guanidino group in each sample. The absorption of the substituted samples were subtracted from the control sample and the results listed in Table I.

TABLE I

DECREASE IN ABSORPTION DUE TO SUBSTITUTION

Relative humidity, %	Sorption of control, g./1000 g.	Decreased absorption, g./1000 g. casein, by samples 1	2	2A[a]
11.8	35	0	0	9
31.4	69	1	−1	16
50.9	97	0	0	23
75.1	146	2	1	32
83.6	174	6	5	40

[a] Calculated values for decreased absorptions due to the removal of the same number of amino groups as were substituted in sample 2.

The data listed for sample 2A are the calculated water absorption values for the same number of free amino groups[2] in casein as have been substituted in derivative 2. The absorption of the control sample was equal to the absorption of the same original casein which had not been treated with the alkali. This shows that there was no significant change in the casein due to this treatment. These data show that the vapor phase water absorption of the guanidino groups produced from the amino groups is not significantly different from the absorption of the free amino groups themselves.

These introduced guanidino groups differ from the naturally occurring guanidino groups in proteins only in that they are one methylene group further removed from the peptide chain of which they are side groups. This, however, would not be expected to cause any great difference in the polarity or water absorbing capacity of these groups. The 4.1 g. of arginine naturally present in 100 g. of casein[3] contains 0.0235 mole of guanidino groups. Therefore, the guanidino groups naturally present in casein will account for an average of 8.7% of the total water absorption of casein between 30 and 85% relative humidity and may account for as high as 12% at lower humidities.

The almost constant fractions of the total water absorption at all relative humidities which have been found to be due to the amino, peptide and guanidino groups show that these polar groups which comprise only a few per cent. of the total protein must be considered in any theoretical analysis of the sorption phenomena. It is consistent with the polarization theory of Bradley which we have shown fits the data for the water absorption of proteins and other high polymers.[4]

Experimental

Preparation of Guanidinated Caseins.[5]—Twenty-five grams of high nitrogen casein was swelled for one hour in 100 ml. of water. Then 200 ml. of water and 210 ml. of concd. ammonium hydroxide were added, and the mixture was stirred until the casein dissolved. S-Methylisothiourea sulfate (0.0, 4.0 or 12.0 g.)[6] in a small amount of water was added and the mixture was stored at 5° for three days. The solution was then transferred to a viscose tube and dialyzed

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[1] Presented before the 117th Meeting of the American Chemical Society at Philadelphia, April, 1950. Article not copyrighted.

[2] E. F. Mellon, A. H. Korn and S. R. Hoover, THIS JOURNAL, **69**, 827 (1947); *ibid.*, **70**, 3040 (1948).

[3] W. G. Gordon, W. S. Semmett, R. S. Cable and M. Morris, *ibid.*, **71**, 3293 (1949).

[4] S. R. Hoover and E. F. Mellon, *ibid.*, **72**, 2562 (1950).

[5] E. Schütte, *Z. physiol. Chem.*, **279**, 59 (1943).

[6] Control and samples 1 and 2, respectively.

against running distilled water until the dialysate was free of ammonia when tested with Nessler reagent. The solution was brought to *p*H 4.5 with hydrochloric acid. The precipitate was centrifuged and washed as in the previously described method. These products were air-dried, and ground to pass a 60-mesh screen.

The products were analyzed for total nitrogen by the Kjeldahl method, and for amino nitrogen by the Doherty and Ogg[7] modification of the Van Slyke method. Arginine was determined by the Sakaguchi method on a total hydrolysate of the protein; the reagents and conditions developed by MacPherson[8] were used. The presence of the other amino acids in the hydrolysate results in high arginine values by this procedure but the values obtained on a more extended series are sufficient to show that we get a proportionate increase in guanidine groups as the free amino groups are substituted. These analyses for the control and samples 1 and 2, respectively, are: total nitrogen, 14.97, 15.66 and 16.38; amino nitrogen, 0.91, 0.37 and 0.06; and apparent arginine, 5.53, —, 18.97.

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[7] D. G. Doherty and C. L. Ogg, *Ind. Eng. Chem., Anal. Ed.*, **15**, 751 (1943).

[8] H. T. MacPherson, *Biochem. J.*, **40**, 470 (1946).

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Kinetics of the Decomposition of Solid Mercurous Formate

BY GEORGE A. MILLER AND GEORGE W. MURPHY

The decomposition of solid mercurous formate into mercury, formic acid and carbon dioxide has been known for more than a century,[1] although no rate studies have been reported to date. In a kinetics study of this reaction, the present authors have found some interesting features.

Experimental

Preparation of Sample.—Mercurous formate was prepared by precipitation from a mixture of solutions of mercurous nitrate and sodium formate. In order to obtain a product of optimum purity, a fairly detailed technique was developed, which involved precipitation at −10° with a saturated solution of mercurous nitrate, slightly acidified with nitric acid, and an aqueous solution of sodium formate. Sufficient ethanol was added to prevent freezing. Previous experience had shown that precipitates obtained at room temperature had a grayish tint, which indicated that some decomposition had occurred. No evidence of decomposition appeared when the precipitation was carried out at low temperatures.

The precipitates were dried in a vacuum desiccator over calcium chloride or anhydrous sodium sulfate at room temperature. It is likely that the dehydration achieved by this method was never quite complete; drying at elevated temperatures, however, was obviously out of the question.

Analyses.—Analyses were performed on both the reactant and products, in order to verify the equation of decomposition. The mercury in a sample of mercurous formate was determined gravimetrically as mercurous chloride. A substantial sample of reaction products was obtained by decomposing a 3.5-g. sample of mercurous formate. The gaseous product was analyzed for carbon monoxide and carbon dioxide by standard methods, using the Francis Auto-bubbler type pipet. The water-soluble residue was titrated with sodium hydroxide, and the residual mercury was weighed.

The results are in agreement with a molecular formula for the formate of $HCOOHg \cdot H_2O$, contrary to reports of earlier workers, who write the formula HCOOHg. Furthermore, it will be seen that the kinetic data support the water of crystallization formula. The analyses show that the overall reaction is probably exclusively

$$2HCOOHg \cdot H_2O = HCOOH + CO_2 + 2Hg + 2H_2O$$

The gaseous product was found to be entirely CO_2, and in the correct amount indicated by the stoichiometry. Keller and Körösy[2] found that both silver formate and cuprous formate decompose with the evolution of equal parts of hydrogen and carbon dioxide. These authors also noted that the salts were somewhat volatile. In mercurous formate we have found no evidence of either hydrogen evolution or volatility, although our method was not sensitive to the latter phenomenon.

Kinetics.—The progress of the reaction was followed by measuring the volume of gas evolved as a function of the time. A Pyrex sample tube of 2-ml. capacity was connected through ground-glass joints and capillary tubing to an ice-trap and thence through rubber tubing to a 10-ml. buret with attached leveling bulb. Mercury was used as the confining liquid. The sample was held at a constant temperature ±0.15° in a small mineral oil-bath equipped with a motor stirrer and a Fenwal "Thermoswitch" control. Early experiments above 100° resulted in minor explosions of the sample. Thereafter, the highest temperature tried was 94°, where the reaction proceeded smoothly. Most runs were made at 80°. Samples were normally about 0.15 g., so selected as to yield a total of about 10 ml. of gas for measurement.

Results of Kinetics Studies

A definite though short induction period was observed in all runs on the pure solid material. As soon as the induction period was over, the reaction followed first order kinetics (Fig. 1). Additions of small amounts of mercury and of formic acid to the starting material had little effect on either the induction period or the rate of decomposition. Strong irradiation of samples by an AH-4 mercury vapor lamp had no observable effect on the reaction. On the other hand, the addition of one drop of water completely removed the induction period, changed the order from first to zero, and greatly accelerated the decomposition.

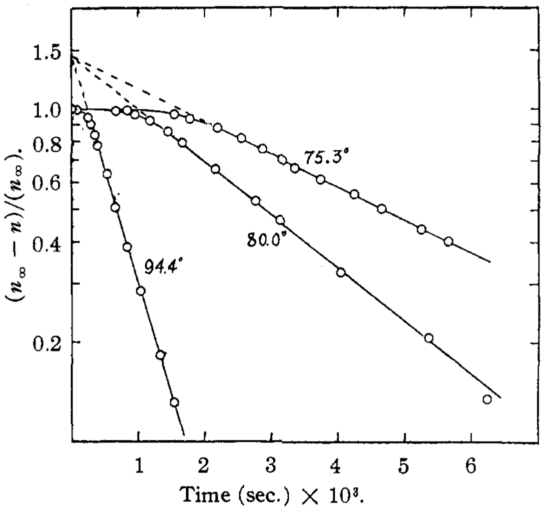

Fig. 1.—Rate data for decomposition of pure solid at three temperatures on semi-log plot.

We conclude tentatively from these results that water is the autocatalyst responsible for the induction period in the decomposition of the pure solid. The zero order kinetics observed with water is probably due to the limited solubility of mercurous formate. Apparently the chemically bound water in solid mercurous formate is not active in promoting decomposition. The reaction probably occurs at the interface between solid mercurous

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[1] Liebig, *Ann.*, **3**, 207 (1832).

[2] A. Keller and F. Körösy, *Nature*, **162**, 580 (1948).